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Influence of Silver and Copper Nanoparticles on the Enzymatic Activity of Soil-Borne Microorganisms

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Abstract:

Influence of metal nanoparticles synthesized by microorganisms upon soil-borne microscopic fungus *Aspergillus terreus* K-8 was studied. It was established that the metal nanoparticles synthesized by microorganisms affect the enzymatic activity of the studied culture. Silver nanoparticles lead to a decrease in cellulase activity and completely suppress the amylase activity of the fungus, while copper nanoparticles completely inhibit the activity of both the cellulase complex and amylase. The obtained results imply that the large-scale use of silver and copper nanoparticles may disrupt biological processes in the soil and cause change in the physiological and biochemical state of soil-borne microorganisms as well.

Keywords: Copper, Enzymatic activity, Microorganisms, Nanoparticles, Silver

Introduction:

Innovative application of metal nanoparticles in the different fields of industry stimulates considerably increasing interest towards their study and production[1,2]. Wide practical exploitation of nanoparticles is stipulated by a number of unique features. Nanoparticles have opened new horizons for design of the new materials and change of their properties by means of regulation of the particles size, morphology and distribution[3,4]. Advances in nanotechnology have been intensified with the study of the possibility of using nanoparticles as carriers and delivery of various drugs, as well as enzymes, while it was important to clarify their effect on enzymes[5,6]. It is well-known that enzymes are biocatalysts, which catalyze many chemical and biochemical reactions. The exceptional properties and possibilities for their application, as well as the change in their activity under the action of metal nanoparticles, are currently of significant interest. The interaction of enzymes with nanoparticles occurs by conjugation or internal capture, and these nanostructures are promising for use in many applications, including bioconversion, biosensing, and so on[7]. To date, the effect of metal nanoparticles on various biological objects has not been finally determined, though many functions have been determined. Thus, metal nanoparticles are characterized as stimulators of the growth, development and productivity of microorganisms[8]. In these regards, of great interest is a study in which the enzymatic activity, together with the microorganisms themselves, was exposed to the action of nanoparticles of various metals.

Currently, a great number of works is devoted studying the toxic effect of metal nanoparticles on soil, plants and microbiota[9,10]. Kolesnikov with colleagues[11] revealed that the nanopowders of Co_3O_4, CuO, NiO and ZnO exert the strongest effect on the microbiological parameters of chernozem in comparison with the “ordinary” forms of oxides. The absorption of nanoparticles by the soil may negatively affect the state of soil biota and plants, as its components, posing a serious risk to the environment. Soil contamination with metals in the nanoform has a pronounced negative character, which causes the disruption of the biocenosis, decrease in reproduction of its inhabitants and their death. In this case, the degree of negative impact is determined by the type of nanometal and the composition of the soil fauna. Thus, researchers reported an unfavorable dose-dependent effect of TiO, CuO, AL_2O_3 and other metal nanoparticles on plant root growth, seed germination, biomass growth, on microbial species diversity, antimicrobial and enzymatic activity of soil-borne microorganisms, as well as on various types of soil worms, including earthworms and red California worm (*Eisenia foetida*)[12].

The study of the ecosystem "soil-microbiota-plants", which is a sensitive indicator of microbial changes in soils, appears to be interesting as well as considerably important[13]. Therefore, the protection of soil microbial biomass and diversity is one of the main challenges in the field of sustainable use of resources. The study of the effect of silver nanoparticles revealed that small concentrations of silver are safe for mammalian cells, in particular for the human body. At the same time, it is a potent toxin for most bacteria and viruses. It was established that concentrations toxic to bacteria lay in the range of 10^{-5}-10^{-3} mol/L of silver, while to micromycetes in the range of 10^{-6}-10^{-3} mol/L of silver. Higher plants are cable to absorb and redistribute silver in the form of nanoparticles, which can though have a toxic effect on the plant organism in the long run[14]. The bactericidal properties of silver are associated with the release of silver ions, which suppress dangerous microflora[15]. A decrease in germination, slowdown in the formation of nodules (due to a decrease in the number of symbiont bacteria *Rhizobium leguminosarum*) and shoot growth, and a decrease in root length of *Vicia faba* L. was reported by the addition of silver nanoparticles to the nutrient medium[16].

It is known that copper nanoparticles slow down the growth of microorganisms and possess some antiviral properties as well[17]. A study of the effect of copper nanoparticles (50 nm) and microparticles (2.5 mm) on the microbiological activity of sandy loam soil and phytotoxicity revealed their persistent toxic effect on the cultures of *Cucumis sativus* and *Zea mays*, and on typical soil-borne microorganisms (*Bacillus*, *Pseudomonas*, *Azotobacter* and so on) as well[18]. Kim with colleagues have also established that the introduced copper nanoparticles inhibit the activity of soil enzymes and the growth of plant biomass[18]. Since the soil microflora plays crucial role in the development of the ecosystem, it is very important to study the effect of copper and silver nanoparticles on this component of the biocenosis. At the same time, many metals, including copper, are among the most common environmental pollutants. Considering rapidly increasing use of copper nanoparticles, it is necessary to determine its effect on the microbial community, which in this case may increase or decrease enzymatic activity. Due to their diverse application and the possibility of penetration into environmental objects, such as soil, copper nanoparticles are becoming the main direction in studies of the biological role and toxicity for the biosynthetic activity of microorganisms, including the enzymatic one[19]. The positive effect of nanoparticles on the biosynthesis of enzymes is also known; for example, nanoparticles ZnO/MgO (1:4), ZnO and Fe_3O_4 were reported as effective stimulators to increase the biosynthesis of proteases by micromycetes *Trichoderma koningii* and *Fusarium gibbosum*[20].

The aim of this work was to study the influence of silver and copper nanoparticles on the activity of extracellular enzymes and total protein of the soil-borne culture of *A. terreus* K-8.

Materials and Methods:

Nanoparticles of the metals were obtained by the addition of solutions of silver and copper salts (to final concentration 50 mg/L) to the cultural liquid of 2-3 day old *Pseudomonas* sp., active producers of the silver and copper nanoparticles[21,22]. Mixtures of cells and either silver or copper ions were incubated on the rotary shaker at 150 rpm and 28°C for a week. Formation of nanoparticles was observed visually: by staining of the solutions into colors characteristic for nanoparticles of silver and copper, and with use of UV-spectroscopy and AFM-methods. Efficiency of formation of the nanoparticles was determined by measuring the optical density of the received solutions by UV-spectroscopy within wavelength 200-700 nm[23]. After the formation of nanoparticles, the microbial biomass was removed by centrifugation (8000 rpm 15 min).

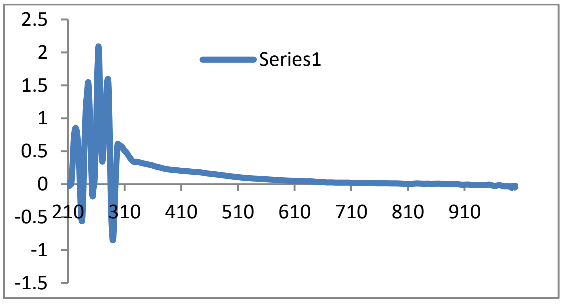
A strain of *A. terreus* K-8, preserved at the collection of industrially important cultures of microorganisms of the Institute of Microbiology (Tashkent, Uzbekistan) and possessing high level of enzymatic activity, and a strain of *Pseudomonas* sp., isolated from typical serozyom soil of the Tashkent region (Uzbekistan), were used as biological objects. Cultivation of producers with the addition of nanoparticles was conducted on 200 ml Mandels broth with addition of CMC in 1L Erlenmeyer flask (medium volume 200 ml) on rotary shaker (180-220 rpm) at 28-30°C. Afterwards, the biomass was separated by filtration and enzymatic activity was determined in supernatant.

Determination of the reduced sugars was conducted according to Somogyi[24]. Determination of amylolytic activity was conducted by the colorimetric method with iodine according to the degree of hydrolysis of soluble starch to dextrin of various molecular weights[25]. Protein was determined according to Lowry et al.[26].

Results and Discussion:

Influence of biogenic silver and copper nanoparticles on enzymatic activity of *A. terreus* was studied.

Pseudomonas cultures capable of the production of metal nanoparticles[21,22] was selected for production of the copper and silver nanoparticles. Experiments revealed that the addition of 50mg/L either Ag^{+} or Cu^{2+} ions to *Pseudomonas* cultural liquid at selected conditions at pH 7 results in the formation of nanoparticles. It was established that the absorption bands appear in the wavelength ranging from 225 nm to 400 nm in the studied systems, which are associated with the formation of clusters and nanoparticles of either silver or copper (Fig. 1).



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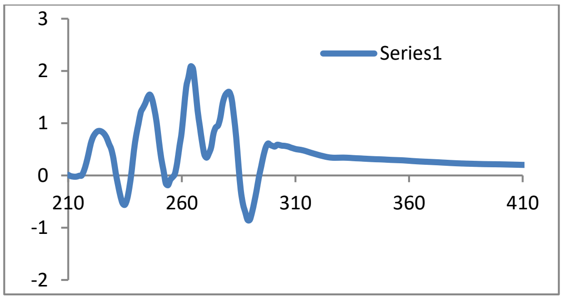

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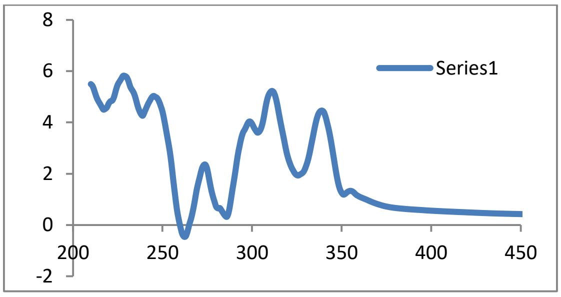


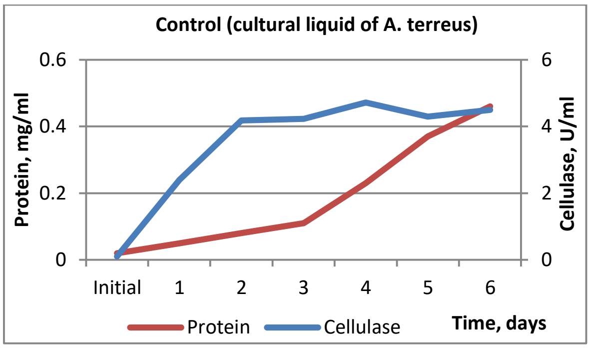
C

Figure 1. UV-spectra of cultural liquids: A – microorganisms + nutrient medium, B – microorganisms + nutrient medium + 50 mg/L Ag^{+}; C – microorganisms + nutrient medium + 50 mg/L Cu^{2+}

The results obtained indicate that when silver ions are added to the system, absorption bands are observed at a wavelength of 275 nm, characteristic for silver clusters with a size of 2-3 nm and at a wavelength of 400 nm, characteristic of nanoparticles with sizes from 5 nm to 20 nm as well, which correspond to a spherical shape. Analysis of copper nanoparticles revealed that the nutrient medium contains spherical copper nanoparticles in the form of spherulites with growth in the radial direction. After purification, both solutions contained 50 mg/L of silver and copper nanoparticles, respectively.

Further, the influence of the obtained silver and copper nanoparticles on enzymatic activity of *A. terreus* was studied. Studying the dependence of the dynamics of cellulase activity and total protein accumulation during cultivation of *A. terreus* K-8 in a liquid nutrient medium revealed that enzymatic activity of the cellulase complex of *A. terreus* K-8 in control (Fig. 2A) was quite high starting from the 2nd day and reached the maximum rate on the 4th day (4.72 U/ml), whereas considerable increase of the total protein accumulation started from the 4th day reaching 0.46 mg/ml by the 6th day.

In the case of adding silver nanoparticles to the cultural liquid, the protein concentration in medium remains more or less low with its sharp increase on the 6th day (0.36 mg/L), whereas the highest cellulase activity was observed on the 3rd day (3.17 U/ml) with subsequent decrease (Fig. 2). The addition of copper nanoparticles to the cultural liquid of *A. terreus* K-8 resulted in total suppression of cellulase activity and low rate of protein accumulation (Fig. 2C). Obviously, there are some arguable questions in the case of studying the effect of copper nanoparticles on microorganisms' activity and there is a need for further detailed analysis. In particular, Shobha et al. mentioned the complex role of nanoparticles in both positive and negative influence on microorganisms[27].



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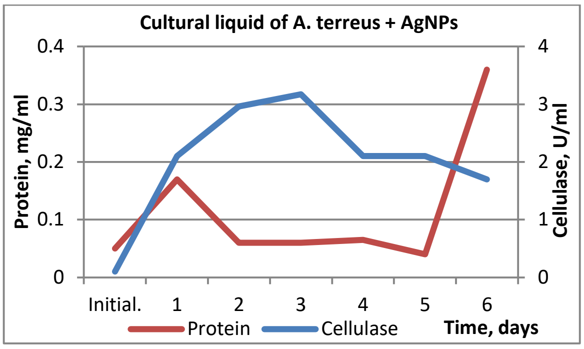

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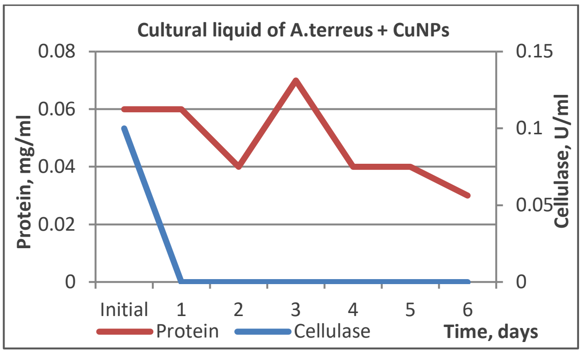

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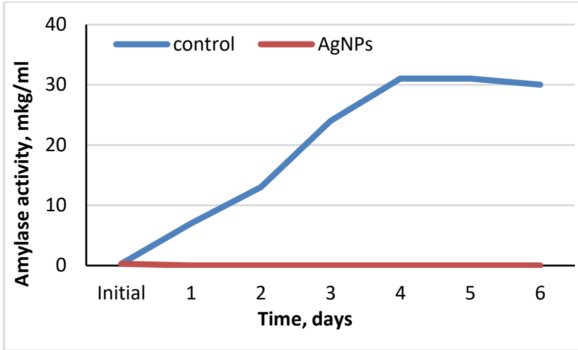
Figure 2. Dynamics of cellulase activity and accumulation of the total protein: A – *A. terreus* K-8 (submerged cultivation), B – *A. terreus* K-8 (submerged cultivation with AgNPs), C – *A. terreus* K-8 (submerged cultivation with CuNPs)

The obviousness of the change in cellulase activity under the influence of metal nanoparticles is beyond doubt. It was established that the introduction of silver nanoparticles leads to a decrease in cellulase activity and total protein content.

Studying the effect of metal nanoparticles on amylase activity of *A. terreus* K-8 revealed that both silver and copper nanoparticles totally inhibit its activity (Fig. 3). The obtained results are in correlation with some reports on inhibitory action of 10 mg/L ZnO/MgO and Fe_3O_4 nanoparticles on amylolytic activity in *Aspergillus niger*[16].

Thus, as a result of conducted studies, it has been revealed that metal nanoparticles synthesized by microorganisms effect on the enzymatic activity of the studied *A. terreus* K-8 strain. Silver nanoparticles caused a decrease in cellulase activity and completely suppressed amylase activity of the fungus, whereas copper nanoparticles completely inhibited the activity of both the cellulase complex and amylase. The obtained results imply that the large-scale use of silver and copper nanoparticles may disrupt biological processes in the soil and cause change in the physiological and biochemical state of soil-borne microorganisms as well.



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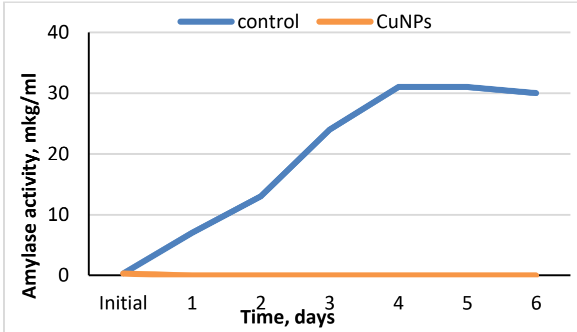

B

Figure 3. Influence of silver (A) and copper (B) nanoparticles on amylolytic activity of cultural liquid of *A.terreus* K-8

Conclusion:

Based on received results, it may be concluded that the influence of nanomaterials on the biosynthesis of enzymes and accumulation of protein depends both on the type of nanoparticles used and on the physiological and biochemical characteristics of the strain. Evaluation of the ability of metal nanoparticles to aggregate or interact with soil-borne microorganisms may be of special interest in assessing the resistance of soils both to nano contamination and to the tested enzymatic activity of soil-borne microorganisms. Such studies will promote to better understanding of the effect of silver and copper nanoparticles on the components of the soil environment and its enzymatic activity.

The problem of obtaining new knowledge about the mechanisms of the effect of nanoparticles on various objects and their role in the life of soil biota remains extremely urgent. The available information is often scattered and still does not allow to fully assess the risk from the entry of nanoparticles into the natural ecosystem. There is a need for further study to determine the maximum permissible concentrations of metal nanoparticles in various environments, to establish differences in the effect of nanoparticles on living organisms.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the

Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.

- Ethical Clearance: The project was approved by the local ethical committee in Institute of Microbiology, Uzbekistan.

Authors' contributions statement:

L.I.Z and S.I.K conceived of the presented idea, developed the theory, supervised the findings of this work, and drafted the manuscript. R.N.J, N.A.L and A.M.M conducted experiments, analyzed and interpreted received data. J.J supervised the findings of this work, drafted and revised the manuscript, translated and proofread the manuscript into English. All authors discussed the results and contributed to the final manuscript.

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الجسيمات النانوية الفضية والنحاسية على النشاط الإنزيمي للكائنات الدقيقة المحمولة بالتربة

لودميلا زينيتيدينوفا **روكسيلا جورافا** **جافلون تاشبولانوري** **سيفتلانا كوكانوفا**
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الخلاصة:
تمت دراسة تأثير الجسيمات النانوية المعدنية التي تم تصنيعها بواسطة الكائنات الحية الدقيقة على الفطريات المجهرية التي تنقلها التربة *Aspergillus terreus K-8*. ثبت أن الجسيمات النانوية المعدنية التي يتم تصنيعها بواسطة الكائنات الحية الدقيقة تؤثر على النشاط الإنزيمي للبيئة المدروسة. تؤدي الجسيمات النانوية الفضية إلى انخفاض في نشاط السليولاز وتثبط نشاط الأميليز تمامًا للفطريات ، بينما تثبط جزيئات النحاس النانوية تمامًا نشاط كل من مركب السليولاز والأميلاز. تشير النتائج التي تم الحصول عليها إلى أن الاستخدام الواسع النطاق لجزيئات الفضة والنحاس قد يعطل العمليات البيولوجية في التربة ويسبب تغيرًا في الحالة الفسيولوجية والكيميائية الحيوية للكائنات الحية الدقيقة التي تنقلها التربة أيضًا.

الكلمات المفتاحية: النحاس، النشاط الأنزيمي، الكائنات الدقيقة، الجسيمات النانوية، الفضة