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Short Communication

Evidence for the inhibitory effects of silver nanoparticles on the activities of soil exoenzymes

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ABSTRACT

Silver nanoparticles (AgNPs) are well known to have antimicrobial ability, but very little is known about the effect of AgNPs on soil exoenzyme activities, which reflect the potential of a soil to support biochemical processes. This study provides evidence of the inhibitory effects of AgNPs on the activities of soil exoenzymes. Six exoenzymes related to nutrient cycles (urease, acid phosphatase, arylsulfatase, β-glucosidase) and the overall microbial activity (dehydrogenase, fluorescein diacetate hydrolase) were tested in soils treated with AgNPs (1, 10, 100 and 1000 μg g^{-1}) and silver ion (0.035, 0.175, 0.525, 1 and 1.5 μg g^{-1}). AgNPs were capable of inhibiting the activities of all the exoenzymes tested in this study. Especially, the urease and dehydrogenase activities were significantly related to the presence of AgNPs. The effects of silver ions dissolved from the AgNPs were not significant, indicating the adverse effects caused by AgNPs themselves. This study suggested that AgNPs negatively affect soil exoenzyme activities, with the urease activity especially sensitive to AgNPs.

1. Introduction

Silver nanoparticles (AgNPs) are one of the priority nanomaterials listed in the Organisation for Economic Co-operation and Development (OECD) (OECD, 2010). Due to their antimicrobial activity, they are being widely used in many nanoproducts and biological applications, including electronic products, medical appliances, tableware, clothing and cosmetics, etc. (Tolaymat et al., 2010). The wide application of AgNPs indicates a high potential of their inflow into the environment (Benn and Westerhoff, 2008), which may adversely affect the beneficial activities of natural microorganisms within the environment (Gajjar et al., 2009; Dimkpa et al., 2011).

Many studies have investigated the microbial toxicity of AgNPs (Gajjar et al., 2009; Gurunathan et al., 2009; Lee et al., 2009; Bae et al., 2010; El Badawy et al., 2011); however, much less is known about the exoenzyme activities in soils treated with nanoparticles. As shown in Table 1, Tong et al. (2007) observed negligible effects of fullerene (C_{60}) on the activities of β-glucosidase, acid phosphatase, urease and dehydrogenase. Du et al. (2011) performed a long-term field study, and reported the negative effects of TiO_2 and ZnO nanoparticles on the activities of soil protease, catalase and peroxidase, but no effect was observed on the urease activity. Cullen et al. (2011) found increased dehydrogenase activity, but a minimal effect of fluorescein diacetate hydrolase in soil treated with nano-zero valent iron (nZVI, 10 000 μg g^{-1} soil). The dehydrogenase, β-glucosidase and acid phosphatase activities were decreased in soil amended with 2000 μg g^{-1} of Zn and ZnO nanoparticles in a pot experiment with planted *Cucumis sativus* (Kim et al., 2011). Chung et al. (2011) reported inhibited activities of phosphatase, β-N-acetylglucosaminidase, β-glucosidase, cellobiohydrolase and xylosidase with 5000 μg g^{-1} of multi-walled carbon nanotubes (MWCNTs). Hänsch and Emmerling (2010) evaluated the effects of AgNPs on the activities of leucine-aminopeptidase, β-cellobiohydrolase, acid phosphatase, β-glucosidase, chitinase and xylosidase, and reported no effect of AgNPs at 0.32 μg g^{-1} for all the enzymes tested, with the exception of slight decrease of the leucione-aminopeptidase activity.

This study focused on evaluating the short-term effect of AgNPs on the activities of common exoenzymes found in soils. The changes in the activity of exocellular enzymes related to important nutrient cycles; nitrogen, phosphorus, sulfur and carbon, were measured using urease, acid phosphatase, arylsulfatase and β-glycosidase, respectively. The overall microbial activity was predicted by assessing the dehydrogenase and fluorescein diacetate hydrolase activities. The extent of inhibition of these enzymatic activities was used as an indicator of the toxic effect of AgNPs. In addition, the effects of dissolved silver ions from AgNPs on the soil exoenzyme activities were evaluated.

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Table 1
Nanotoxicity studies for soil exoenzyme activities.

Test NP	Enzyme activity	Result	Refs.
C_{60} (1000)[a]	Dehydrogenase acid phosphatase β-glucosidase urease	No effect	Tong et al. (2007)
Ag (0.32)	Acid phosphatase β-glucosidase β-cellobiohydrolase chitinase xylosidase leucione-aminopeptidase	No effect except slight decrease of leucione-aminopeptidase activity	Hänsch and Emmerling (2010)
TiO_2 and ZnO (not reported)	Protease catalase peroxidase urease	Negative effect except urease	Du et al. (2011)[b]
ZVI (10000)	Dehydrogenasefluorescein diacetate hydrolase	Positive effect for hydrogenase activity, but no effect for FDA	Cullen et al. (2011)
Zn and ZnO (2000)	Dehydrogenase acid phosphatase β-glucosidase	Negative effect	Kim et al. (2011)[c]
MWCNT (5000)	Phosphatase β-glucosidase β-N-acetylglucosaminidase cellobiohydrolase xylosidase	Negative effect	Chung et al. (2011)

[a] Values in parentheses are the maximum exposure concentration of nanoparticles as $\mu g\ g^{-1}$ as dry weight.
[b] About 22 months of field experiments with planted *Triticum aestivum.*
[c] 8 weeks pot experiment with planted *Cucumis sativus.*

2. Materials and methods

2.1. Chemicals

Citrate-coated silver nanoparticles (cAgNPs) were obtained in an aqueous colloidal state from ABC Nanotech (Daejeon, Korea). The cAgNP contained 1% of a capping agent, which allows for their stable dispersion in water. Dry particle characterization was provided via the manufacturer's certificate of analysis; the average particle diameter was 9.9 nm (CV 15.9%) from 129 particles measured using TEM (Tecnai G2-20S-Twin, FEI Company, USA) and the mean particle surface area was $3.18 \times 10^2\ nm^2\ particle^{-1}$. The hydrodynamic radius of the cAgNPs in deionized water was determined to be 20.08 nm ± 2.24, using a Dynamic Light Scattering (DLS, DynaPro Plate Reader, Wyatt Technology Corp, USA). The AgNPs were diluted with distilled water to obtain a range of exposure concentrations for the soil tests. For the silver ion toxicity test, silver nitrate ($AgNO_3$, Sigma–Aldrich, St. Louis, MO, USA, 99.0+% purity) was purchased from Sigma Aldrich Co. INT-violet (Iodonitrotetrazolium chloride, $C_{19}H_{13C}IIN_5O_2$), INTF (iodonitrotetrazolium formazan, $C_{19}H_{14}IN_5O_2$), TRIS buffer (1 M, pH 8.0), formaldehyde (37% solution, ACS reagent grade), THAM (Tris hydroxylmethyl amino methane), disodium *p*-nitrophenyl phosphate hexahydrate (PNP, $C_6H_4NO_6PNa_2{\cdot}6H_2O$ 97.0+% purity), potassium *p*-nitrophenyl sulfate (PNS, $C_5H_4NO_6S$), *p*-nitrophenyl-β-D-gluco(pyrano)side (PNG, $C_{12}H_{15}NO_8$) and fluorescein diacetate (FDA, $C_{24}H_{16}O_7$) were obtained from Sigma. *p*-nitrophenol ($C_6H_5NO_3$, 98% purity) was obtained from ACROS organics (New Jersey, USA). Urea (NH_2CONH_2) and tetrahydrofuran (THF, 99.5+% purity) were purchased from Duksan Pure Chemical Co., LTD., Korea. All chemicals were used as received.

2.2. Test soil

Surface soil (0–5 cm) was collected from the campus of Konkuk University (Seoul, Korea) and used for the exoenzyme assay. Soil samples were air-dried for 1 d, sieved (<1.4 mm), and stored in plastic bags at 4 °C until the experiment. Since the microbial activity is related to the physical and chemical properties of the soil, the main characteristics of the test soils were measured, and are listed in Table 2. The soil was composed of sand (93.3% sand, 6.0% silt, and 0.7% clay) with a pH of 7.0, 3.5 ± 0.4% organic matter and a gravimetric water holding capacity (WHC) of 0.60 ± 0.07 mL g^{-1} dry soil. The soil pH was measured in water (1:4, w/v extract) (Sparks et al., 1996). The sand, silt, and clay contents were measured using a soil texture kit (Model 1067, LaMotte Company, Chestertown, MD, USA). The percentage of organic matter in the test soil was determined using an organic matter soil test kit (Model ST-OR 5020, LaMotte Company, Chestertown, MD, USA). The gravimetric WHC was measured following saturation and free drainage for 2 h.

Table 2
Physicochemical properties of the test soil used in this study[a].

Parameter	Mean	SD[b]
Soil pH	7.0	0.0
Percent organic matter	3.54	0.41
WHC	0.60	0.07 mL g^{-1} dry soil
Sand	93.3%	
Silt	6.0%	
Clay	0.7%	
Texture class	Sand	

[a] Test soils are surface soils (0–5 cm).
[b] Values are the means and standard deviations of triplicate determinations.

2.3. Toxicity test for enzyme activities

The test unit was a flat bottomed glass jar (ID 26 mm, length 75 mm, volume 34 mL). AgNP solution was added to the test unit, which contained 10 g of soil as equivalent dry weight. The AgNP concentrations of 0, 1, 10, 100, and 1000 $\mu g\ g^{-1}$ were prepared. Unamended soil was used as the control. Two method blanks were prepared to correct the background absorbance. Soil treated with 3.5 mL of formaldehyde, for the inhibition of the microbial activity, was used as a method blank to correct the absorbance of soil extract. Soil amended with AgNPs in the absence of substrate was used as the second method blank to correct the absorbance due to AgNPs themselves. The second method blank was prepared for each concentration of AgNPs used in the present study. The moisture content was adjusted to about 60% of the water holding capacity, with the soils then incubated at 25 °C in the dark for 1 and 7 d. Each treatment was prepared in triplicate. The soil pH was measured for each exposure concentration and duration, but no significant differences were observed.

To determine whether Ag^+ dissolved from AgNPs may play a role in the enzyme activities, the dissolved Ag^+ concentrations were measured for the lowest and highest AgNPs exposure concentrations tested. After the addition of 10 mL of distilled water to the amended soil (5 g of dry soil), with thorough mixing using a vortex shaker, the soil and AgNPs were filtered through a Whatman No. 2 filter and a 0.2 μm nylon filter. The dissolved Ag^+ concentrations in each filtrate were then analyzed via inductively-coupled plasma atomic emission spectroscopy (ICP-AES; JY 138; Ultrace, Jobin Yvon, France). The Ag^+ toxicity test for enzymes was assayed using $AgNO_3$, and conducted in the same manner as the AgNPs toxicity test. The soil pH was measured at the beginning and end of the test, but no differences were observed.