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Effects of Silver Nanoparticles on the Activities of Soil Enzymes Involved in Carbon and Nutrient Cycling

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ABSTRACT

Soils are continuously exposed to large amounts of engineered nanoparticles, especially silver nanoparticles (AgNPs), which can affect the activity, stability, and specificity of microbial enzymes. Therefore, the measurement of specific enzyme activity can be used to identify major changes in soil environments. Accordingly, the aim of the present study was to investigate the effects of AgNPs on soil enzymes that play critical roles in mineralizing carbon and nutrients in soil. Soil samples (silt loam and sandy loam) were collected from the surface layer (0–15 cm) of a field at the George Washington Carver Farm, Lincoln University of Missouri, USA. The soils were then treated with AgNP solutions at 0, 1 600, or 3 200 μg Ag kg^{-1} dry soil, using either 10- or 50-nm AgNPs and a randomized complete block design, with three replicates per treatment. The AgNP-treated soil samples were homogenized and incubated for one month, and soil acid phosphatase, β-glucosaminidase, β-glucosidase, and arylsulfatase activities were measured after one hour, one week, and one month of incubation. The activities of all four enzymes were reduced by AgNP treatment after one hour and one week. However, AgNP size had no effect. After one month of incubation, the AgNP treatments had mixed effects, which suggests that soil enzymes are only affected on a short-term basis. Further studies are required to determine the mechanisms by which AgNPs reduce soil enzyme activity.

Key Words: engineered nanoparticles, nanotechnology, nanoparticle concentration, nanoparticle size, soil environment

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INTRODUCTION

The field of nanotechnology is a rapidly growing industry that has the potential to have an enormous impact on the economy, society, and environment, owing to the large number of nanotechnology products being produced. Indeed, the demand for nano-based products has increased over the last few years, leading to growing concerns about their impact on the environment (Nowack and Bucheli, 2007). Nanoparticles can be engineered to have specific sizes, shapes, and properties, which consequently determine their optical, electrical, magnetic, and chemical characteristics; nanoparticles have a greater surface area per unit mass than larger particles, which generally makes them more reactive (Dinesh *et al.*, 2012).

The ecotoxicological properties and risks of nanoparticles have yet to be characterized fully. However, many nanoparticles have been reported to possess antibacterial properties and to directly affect microbial communities in soil (Vance *et al.*, 2015). Nanoparticles can affect soil microorganisms through i) direct effects (*i.e.*, toxicity), ii) changes in toxin or nutrient availability, iii) interactions with nontoxic organic compounds, or iv) interactions with toxic organic compounds (Aruguete and Hochella, 2010). Even though the toxicity mechanisms of most engineered nanoparticles remain unclear, putative underlying mechanisms include the disruption of membranes or membrane potential, protein oxidation, genotoxicity, the interruption of energy transduction, reactive oxygen species (ROS) formation, and the release of toxic constituents (Mishra and Kumar, 2009).

There is increasing evidence that nanoparticles released from industries can harm both aquatic and terrestrial habitats (Schlich and Hund-Rinke, 2015). Silver ions, for example, have been reported to harm bacteria and inhibit bacterial respiration. They can interact with sulfur (S)- and phosphorus (P)-containing enzymes in the bacterial cell wall and may disturb their activity (Morones *et al.*, 2005). Silver (Ag) ions may also enter bacterial cells as calcium (Ca) ions and may

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bind to S- and P-containing molecules, such as DNA. In addition, Ag ions may inhibit DNA replication (Feng *et al.*, 2000). Therefore, it is not surprising that Ag ions also inhibit the denitrification activity of bacteria (Throbäck *et al.*, 2007). Furthermore, silver nanoparticles (AgNPs) have been reported to affect earthworm reproduction, as well as enzyme activity of soils. The physiochemical characteristics of soil, such as pH, ionic composition, texture, organic matter content, and temperature, can affect the chemistry, mobility, bioavailability, and toxicity of nanoparticles (Schlich and Hund-Rinke, 2015).

Soil enzymes play important roles in regulating the cycles of carbon (C), nitrogen (N), S, P, and other nutrients by mediating biochemical reactions involved in the decomposition of organic matter. Soil enzyme activities are often related to the organic matter content, physical properties, microbial activity, and microbial biomass of soil, thereby serving as an early indicator of changes in soil health (Dick *et al.*, 1996; Bowles *et al.*, 2014).

The present study investigated the effects of silver nanoparticles on soil enzymes that are known to play a critical role in the mineralization of C, N, P, and S in soil (Tabatabai, 1994) and are sensitive to changes in soil quality. Four enzymes were chosen in this study: acid phosphatase, β-glucosidase, β-glucosaminidase, and arylsulfatase. The activity of acid phosphatase was studied because it catalyzes the hydrolysis of various organic and inorganic phosphomonoesters and is, therefore, important in soil P mineralization and plant nutrition (Eivazi and Tabatabai, 1977). The activity of β-glucosidase, the most predominant glycosidase in soil, was studied because it is involved in the last limiting step of cellulose degradation (Eivazi and Tabatabai, 1988), and that of β-glucosaminidase, a key enzyme in the hydrolysis of *N*-acetyl-β-D-glucosamine residues from chitooligosaccharides (Parham and Deng, 2000), was studied because of its role in converting chitin into amino acids, which is considered important in soil C and N cycling. The activity of arylsulfatase was studied because it is generally used to investigate the mineralization of organic S in soil.

The overall objective of the present study was to determine the effects of different sizes and concentrations of AgNPs on the activities of soil enzymes over one-month incubation.

MATERIALS AND METHODS

Topsoil samples (0–15 cm) of two soils were collected from a field (38°31′34.3″ N 92°08′27.9″ W) at the George Washington Carver Farm, Lincoln University of Missouri, USA. The first soil was a Wrengart-Gatewood silt loam (fine-silty, mixed, active, mesic Fragic Oxyaquic Hapludalf), with a pH of 5.8, 3.4% organic matter, cation exchange capacity of 11.7 $cmol_c$ kg^{-1}, and 20% clay, whereas the second soil was an Elk sandy loam (Ultic Hapludalt), with a pH of 6.0, 2.1% organic matter, cation exchange capacity of 15.7 $cmol_c$ kg^{-1}, 7% clay, and 76% sand. The soil samples were thoroughly mixed, air dried, passed through a 2-mm sieve, and stored in sealed plastic bags for enzyme determination.

Two sizes (10 and 50 nm) of polyvinylpyrrolidone (PVP)-functionalized AgNPs (0.02 mg mL^{-1} in water) were obtained from Sigma Aldrich Co. (USA) and characterized using ultraviolet-visible (UV-vis) spectroscopy. The 10-nm AgNPs exhibited maximum absorption at 390 nm (molecular weight: 107.87; density: 0.996 g mL^{-1} at 25 °C), whereas the 50-nm AgNPs exhibited maximum absorption at 425 nm (molecular weight: 107.87; density: 1.001 g mL^{-1} at 25 °C).

The soils were treated with either 0 (control), 1 600, or 3 200 µg Ag kg^{-1} dry soil by applying 0, 240, or 480 µL of AgNP solutions (aqueous dispersion). Then, the treated soil samples were homogenized and incubated for one month. This was performed for each AgNP size, and the enzyme activities of each soil were measured after one hour, one week, and one month of incubation. The activities of three of the enzymes (acid phosphatase, β-glucosidase, and arylsulfatase) were measured, as described by Tabatabai (1994). Standardized protocols that included incubation at 37 °C for a fixed time period at a specific buffer pH range, as well as the inclusion of necessary cofactors, were followed for each of the enzyme assays (Eivazi and Tabatabai, 1977, 1988). Meanwhile, β-glucosaminidase activity was determined using a method developed by Parham and Deng (2000). Briefly, 2 mL 0.1 mol L^{-1} acetate buffer (pH 5.5) and 0.5 mL 10 mmol L^{-1} *p*-nitrophenyl *N*-acetyl-β-D-glucosaminidase (*p*NNAG) solution were added to 0.5 g soil. The mixtures were incubated at 37 °C for 1 h, and the reaction was stopped using 0.5 mL 0.5 mol L^{-1} calcium chloride ($CaCl_2$) and 2 mL 0.1 mol L^{-1} tris(hydroxymethyl)aminomethane (THAM) buffer (pH 12). The resulting solutions were filtered and measured for color intensity at 420 nm. The activities were measured in triplicate, along with a control. The absorbance of the products formed in each enzyme assay was measured with a Thermo Genesys spectrophotometer (Thermo Scientific, USA), and all substrates used in the assays were obtained from Sigma