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Dose–response effects of silver nanoparticles and silver nitrate on microbial and enzyme activities in calcareous soils

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ABSTRACT

The extensive use of silver nanoparticles (AgNPs) in consumer and medical products leads inevitably to release of such particles into environment and soil resources. This study was conducted to provide evidences for biological effects of AgNPs in two calcareous soils with different textures and salinity levels. Basal respiration (BR) and substrate-induced respiration (SIR), as indicators of soil microbial activity and biomass, respectively, were determined in the calcareous soils spiked with a dilution series of AgNPs and $AgNO_3$. Urease and alkaline phosphatase activities were also measured in the spiked and control soils. Finally, dose–response approach was used to model the sensitivity of the soil biological properties to AgNPs and $AgNO_3$ contamination. The results revealed that the effects of AgNPs and $AgNO_3$ on the soil respiration and enzyme activities depended on Ag dose and soil type. For instance, soil respiration was not affected or even stimulated by low doses of AgNPs and $AgNO_3$, but negatively affected by high doses (>20 mg Ag kg^{-1}). Soil urease and phosphatase activities were generally inhibited in the presence of AgNPs and $AgNO_3$, though in low Ag concentrations there was no inhibition or even stimulation. Generally, the ecological dose (ED) values of AgNPs were smaller than those of $AgNO_3$, suggesting that AgNPs have more negative effects than $AgNO_3$ on the soil microbial and enzyme activities, at the same level of Ag dose. The results also revealed that suppression of microbial and enzyme activities by AgNPs and Ag ions are greater in the soil with lower clay content and ionic strength.

1. Introduction

The rapidly growing field of nanotechnology has posed new challenges regarding the potential environmental and human health effects of manufactured nanoparticles (MNPs) (Boxall et al. 2007). There are considerable evidences that many MNPs are toxic and potentially hazardous to environment and human health. Toxicity of MNPs is significantly associated with their unique physicochemical properties such as extremely small size and large surface area (Boxall et al., 2007).

Currently, silver nanoparticles (AgNPs) are the most widely commercialized MNPs that are used in various applications such as catalyst, jewelry, dentistry, photography, medicine, cosmetics, textile, and food package (Buzea et al., 2007). Worldwide production of AgNPs is over 400 tons per year, 30% of which are used in medical applications (Pourzahedi and Eckelman, 2014). During production, use, and disposal of AgNP-containing products, AgNPs are released into the environment. A large fraction of the released AgNPs eventually end up in agricultural soils, through improper disposal of household and medical wastes, sewage sludge and biosolid applications, wastewater irrigation, and application of AgNPs-constituted organic fertilizers/pesticides (Anjum et al., 2013). AgNPs can persist for a long time in soils or be taken up by biological organisms; hence, can act as ecotoxicological hazards or bio-accumulate in the food chain (Rico et al., 2011).

Due to the increasing prevalence of AgNPs in consumer products, there is an unprecedented worldwide effort underway to verify AgNPs hazards and to understand the mechanisms of action for antimicrobial effects. Although some studies have concluded that Ag^{+} released from AgNPs is the main chemical species contributing to toxicity (Morones et al., 2005), several other studies have attributed the toxic effects of AgNPs to direct interaction and uptake of the nanoparticles by cells, leading to membrane damage, oxidative stress, and significant mortality (Min et al., 2009). It has been suggested that the pronounced antibacterial activity of AgNPs is due to their large surface area to volume ratio providing better contact with the microorganisms and to their interaction with the functional groups on the microbial cell surface (Morones et al., 2005).

Our scientific knowledge of AgNPs toxicity is principally drawn from controlled laboratory experiments with single strains of bacteria or fungi grown in pure culture systems (Fabrega et al., 2009; Min et al.,

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2009). Although the mechanistic information gained from these laboratory exposure experiments is crucial to understand AgNPs impacts on organisms, extrapolating from single-species systems to multi-species communities in complex environments like soil is not practical (Colman et al., 2013). Moreover, physico-chemical behavior of AgNPs in soils largely varies depending on soil properties such as pH and salinity as well as organic matter and clay contents, which in turn can lead to altered AgNPs toxicity (Schlich and Hund-Rinke, 2015). For instance, Frenk et al. (2013) reported that microbial community structure and functions are less vulnerable to toxicity of MNPs in a soil with high organic matter and clay fraction and high native community richness and diversity.

In addition to antimicrobial activity, AgNPs have been shown to inhibit activities of some extracellular enzymes (Shin et al., 2012; Peyrot et al., 2014). Inhibitory effects of AgNPs may be related to interactions of released Ag^+ ions with thiol groups of enzymes (Liau et al., 1997). However, new findings suggest direct interaction of AgNPs with enzymes as the main reason for the enzyme inhibitions. Wigginton et al. (2010), for example, reported that strong binding of AgNPs to tryptophanase resulted in significant reduction of the enzymatic activity, probably because AgNPs binding changed the conformation or shields the active site of the enzyme. Shin et al. (2012) showed that AgNPs were capable of inhibiting the activities of six soil exoenzymes related to nutrient cycles. They revealed that the effects of silver ions dissolved from the AgNPs were not significant, indicating the adverse effects caused by AgNPs themselves. Peyrot et al. (2014) also reported that negative effects on soil enzyme activities were significant in the presence of AgNPs for phosphomonoesterase, β-D-glucosidase, arylsulfatase, and leucine-aminopeptidase.

Biological effects of nanoparticles in soil are considerably dependent on soil physico-chemical properties (El Badawy et al., 2010; Cornelis et al., 2014). Particularly, several studies have highlighted the major roles of clay content and ionic strength on nanoparticle availability and toxicity in soils (e.g., Frenk et al., 2013; Schlich and Hund-Rinke, 2015). It has been suggested that soils with higher clay content and ionic strength retain and immobilize higher amounts of AgNPs and Ag ions, and therefore, can further resist to biological Ag effects. In a preliminary study on nine different calcareous soils from the Isfahan region, we noted that the soils had a wide range of abilities to retain AgNPs and Ag ions (unpublished data). Moreover, we found that clay content and ionic strength were the most important factors determining retention of AgNPs and Ag ions in these soils. Hence, we hypothesized that negative effects of Ag on biological traits are greater in the soils with lower clay content and ionic strength. In this study, we monitored soil basal and substrate-induced respirations as well as urease and phosphatase activities following exposure to increasing concentrations of AgNPs or $AgNO_3$, in two soil types. The selected soil types were representative of two different soil textures and soluble salt concentrations, which are common in Isfahan region and many other regions in the world. Furthermore, we aimed to use dose–response approach to model the behavior of the soil biological properties and their sensitivity to Ag contamination.

2. Materials and methods

2.1. Synthesis of AgNPs

All the chemicals reagents used in our experiments were of analytical grade and were used as received without further purification. A sonochemical method was applied for preparing AgNPs in aqueous polyvinylpyrrolidone (PVP) solutions. In a typical preparation, 0.05 g of PVP was added to 100 mL of aqueous solution containing 0.1 g of $AgNO_3$. The PVP acts as both reducing and capping agent. The mixture was stirred for complete dissolution and agitated under sonication. Ultrasound irradiation was carried out with a multiwave ultrasonic generator (Sonicator 3000; Bandeline, MS 72, Germany), equipped with a converter/transducer and titanium oscillator (horn), 12.5 mm in diameter, operating at 20 kHz with a maximum power output of 60 W, was used for the ultrasonic irradiation. The operating condition was at 5 s pulse on and 5 s pulse off time with amplitude of 72% at 25 °C for 20 min.

2.2. Characterization of AgNPs

Transmission electron microscope (TEM) images of the synthesized AgNPs were obtained on a Philips CM30 instrument at an accelerating voltage of 150 kV. Average hydrodynamic diameter and surface charge characteristics of the AgNPs were determined with a Malvern ZEN 3600 Nano ZS Zetasizer (Malvern Instruments, UK). Zeta potential (ζ) was measured with the same equipment between −200 and + 200 mV. X-ray powder diffraction (XRD) pattern was recorded by a Philips X'Pert Pro diffractometer using CuK_α radiation to determine the crystallite size and identity of the AgNPs. The crystallite size (D) of AgNPs was calculated using Scherrer equation (Moore and Reynolds, 1997):

$$D = \frac{k\lambda}{\beta \cos\theta} \quad (1)$$

where, k is the so-called shape factor, and usually takes a value of about 0.9, λ depicts the wavelength of CuK_α radiation and β is the breadth of the observed diffraction line at half maximum (FWHM).

2.3. Soils

Surface soils (0–10 cm) were collected from two sites in Isfahan, Iran. A soil from the Campus of Isfahan University of Technology, Golshahr (GOL) soil series and a soil from Tiran region, Janatabad (JNT) soil series were sampled (Table 1). Soil samples were air-dried, sieved (<2 mm), and stored. Some properties of the studied soils were measured, and are summarized in Table 1. Soil pH and electrical conductivity (EC) were determined in 1:2 (soil:water) extracts with a glass electrode (Cyberscan 2100) and a conductivity-meter (Elmetron CC-501), respectively. Calcium carbonate equivalent (Loeppert and Suarez, 1996), organic carbon (Nelson and Sommers, 1996), total nitrogen contents (Bremner, 1996), and soluble cations (calcium and magnesium, sodium and potassium) (Rhoades, 1996) were also measured in the soils. Particle size distribution was determined by the pipette method as described in Gee and Bauder (1986) and soil water content was measured by gravimetric method. Soil cation exchange capacity (CEC) was determined with the ammonium acetate method at pH 8.2 (Chapman, 1965). Water holding capacity (WHC) of the soils was measured at matric suction of 33 kPa in the laboratory using a pressure plate apparatus (Cassel and Nielsen, 1986). Total silver concentration of the soils was determined by a Perkin Elmer AAnalyst200 atomic absorption spectrometer (AAS) after open vessel aqua regia (1:3 HNO_3:HCl) digestion at 140 °C (Cornelis et al., 2010).

2.4. Basal respiration (BR)

To evaluate the effect of AgNPs and $AgNO_3$ on total biological activity of the soils, BR was measured according to the alkali base trap technique. Subsamples from the soils were initially spiked with AgNPs or $AgNO_3$ at concentrations of 0, 0.01, 0.1, 0.5, 1, 5, 10, 20 and 50 mg Ag kg^{-1} soil. For this purpose, appropriate volumes of the stock AgNPs suspensions or $AgNO_3$ solutions supplemented by adequate distilled water were slowly added to the soil subsamples to reach the predefined soil Ag concentrations and water contents (60% of WHC; Shin et al., 2012). The AgNPs suspensions were sonicated for 30 s with a sonication probe (Hielscher UP200H) at 1800 WL^{-1} before adding to the soil samples. The spiked and control soil samples (80 g) were transported to screw bottles (Schott, 250 mL), in triplicates, and incubated for 60 days at 25 °C with 10 mL of 0.2 M NaOH traps. The control samples