Effect of Silver Nanoparticles on the Rhizosphere Exoenzyme of *T. orientalis Presl*

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Abstract: Silver nanoparticles（AgNPs）were extensively used in medicine as an antimicrobial agent. The unavoidable and extensive usage of AgNPs leads to accumulating in environment. Rhizosphere microenvironments are the essential base of terrestrial ecosystems and are ready to be disturb by environmental pollutants. In the present study, we investigated effects of AgNPs in rhizosphere soils by measuring activities of the exoenzymes urease, dehydrogenase, acid phosphatase, neutral phosphatase, and alkaline phosphatase. The effect of the concentrations of AgNPs (0.024, 0.24, 4.80, 9.60 μg/g dry soil) on the wetland plant *Typha orientalis Presl* was studied. In general, AgNPs were capable of inhibiting the activities of all the exoenzymes tested in this study. Especially, the urease, dehydrogenase and phosphatase activities in rhizosphere soil were significantly inhibited in high AgNP level (4.80, 9.60 μg/g dry soil); however, the low concentration of AgNPs (0.024 μg/g dry soil) only had the major adverse effect to exoenzyme activities of rhizosphere soil of *Typha orientalis Presl*. This study suggested that high concentration AgNPs negatively affect rhizosphere soil exoenzyme activities.
Keywords: silver nanoparticles (AgNPs); rhizosphere soil; soil exoenzymes activities; wetland plant; *Typha orientalis Presl*

1. Introduction

The rapid growth of the nanotechnology has resulted in the increasing production and use of engineered nanoparticles (ENPs) with the huge applications in several fields (www. nanotechproject.org). There is an estimated inventory of 1814 consumer products of ENPs identified in October 2013, with 435 products of silver nanoparticles (AgNPs) (Marina *et al.* 2015). Compared with other ENPs, AgNPs has huge applications due to its antimicrobial activity in several commercial and medical products, including wound dressing, textiles, water filters, algicides, disinfectants, deodorants, toothpaste, and house appliances (Yehia and Erik 2012,

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Brend *et al.* 2011, Thabet *et al.* 2010). As a consequence, the wide application of silver nanoproducts results in the release of AgNPs from them, with their inflow into the environment (Fadri and Brend 2011). AgNPs may adversely affect the activities of microorganisms within the environment (Christian *et al.* 2011).

In recent years the effect of AgNPs to soil is more and more popular as a research project due to its presence in soil. AgNPs would more likely accumulate in soil of a constructed wetland. Microorganisms is the basis of a constructed wetland, which determine the running status and quality of outlet. Studies on the impact of AgNPs on soil microorganisms are an important project. Most studies have concluded that the Ag^+ released from the nanoparticles is the main chemical species contributing to toxicity (Levard *et al.* 2013, Xiu *et al.* 2012), while several studies have found the effects of nanoparticles themselves to microorganisms like oxidative stress (Arugete and Hocella 2010, Fabrega *et al.* 2009), and direct interaction and uptake by bacteria (Morones *et al.* 2005). Furthermore, there have been relatively few studies on the impact of AgNP performed directly on the terrestrial environment, which is a chemically and physically complex system. Many studies focus on nanoparticles toxicity to bacteria (Liang *et al.* 2010, Yuan *et al.* 2013, Luo *et al.* 2014). AgNPs may well directly affect to soil microorganisms and indirectly affect to soil enzymes because of the high affinity of silver for soil organic matter and thiol compounds (Jacobson *et al.* 2005). As a result, AgNP contamination in soils could potentially affect the numerous biogeochemical processes, including nutrient cycling and a large majority of microbially mediated processes.

Enzymes found in soils have the biochemical and microbiological role and are “sensors” of soil health (Caldwell 2005). Urease, dehydrogenase and phosphatases are among the most frequently evaluated soil enzymes (Burns *et al.* 2013). Research in the effects of nanoparticles on soil enzyme is relatively few, but many researchs studied the effects of nanoparticles to pure soil were studied. Through long-term study, Du *et al.* (2011) found that TiO_2 and ZnO nanoparticles had affected the protease, catalase and peroxidase, but these nanoparticles had on effect on urease (Du *et al.* 2011). Shin et al evaluated the activities of 6 kinds of soil enzymes under stress of AgNPs, they found that AgNPs could restrain all enzyme activities, specially, could significantly inhibit the activities of dehydrogenase and urease (Shin *et al.* 2012). Raliya *et al.* (2015) explored the influence of TiO_2 nanoparticles on mung bean, they found that the activities of acid phosphatase, alkaline phosphatase and dehydrogenase with the rise of 67.3%, 72% and 108.7% respectively (Raliya *et al.* 2015). Soil enzyme assays have demonstrated potential for the early detection of anthropogenic or natural disturbances as well as a proven sensitivity for evaluating the effects of trace metals in contaminated soils (Chaperon and Sauve 2007, Zhang *et al.* 2010).

This study focused on evaluating the short-term effect of AgNPs on the activities of common exoenzymes found in rhizosphere soils of *Typha orientalis Presl*. The changes in the activities of exoenzymes related to removing abilities of constructed wetland to nitrogen and phosphorus, are measured using urease acid phosphatase, neutral phosphatase and alkaline phosphatase, respectively. The overall microbial activity is predicted by assessing the dehydrogenase activities. Thus, the study attempts to explore the influences of AgNP on exoenzymes in rhizosphere soil of the wetland plant *Typha orientalis Presl*, indirectly reflecting the degree of AgNP stress on rhizosphere. It also lays the foundation wetlands to understand the changes of decontamination ability under AgNP stress.

2. Materials and methods

2.1 Mesocosms

The mesocosms were circular shaped plastic drum and were kept indoors in the city of Nanjing, Jiangsu Province. Barrel mouth diameter and internal diameter at bottom of each mesocosm was 30 cm, 20 cm, respectively. Each mesocosm was 31 cm deep. At the bottom of mesocosms was covered with 5 cm thick gravel. 6 kilograms soils were added to provide a uniform 15 cm layer of soil over the gravel layer in the mesocosm. The soil used in the experiment had an average content of 17.02% moisture. These loam soils were taken from Siming mountain of Nanjing.

The species of wetland plant *Typha orientalis Presl* used in the study were purchased from a weland at the Muyang county, Jiangsu province, China on the November 2013. These plants were cultured in the same nutrient solution under common external environment. And then healthy, same growing plants were chosen as the supplied experimental plants. These selected plants were planted in the mesocosms on the January 2014, about 3 months prior to dosing with AgNPs.

2.2 AgNP stock suspensions

The Ag nanoparticles used in this study were purchased as monomer suspensions (Huzheng nano technology co., LTD, Shanghai, China). They were reported by the manufacturer to be roughly spherical and 15 nm in diameter with a PVP coating (2000 mg/L). The particles were polydisperse with particle sizes ranging from 10 to 40 nm in diameter based on TEM (JEM-2100, JEOL LTD.) and DLS measurements (z-average hydrodynamic diameter).

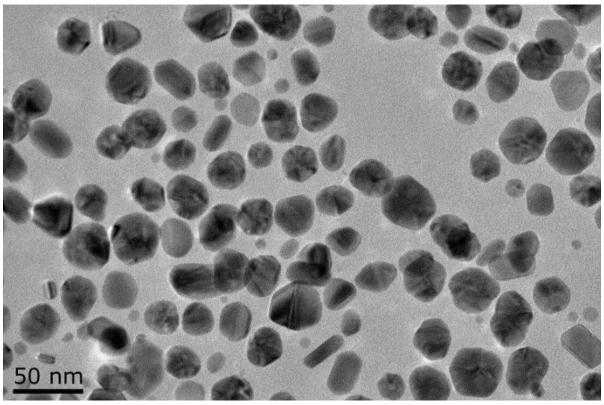

Fig. 1. TEM photograph of nanometer silver solution

2.3 Dosing AgNPs

AgNPs were dosed into the mesocosms on the April 1, 2014. During the test the ambient temperature was about 20-29℃ in day and about 12-22℃ at night. The experiment was