Ecological Indicators 75 (2017) 340–351

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Nematode-based biomarkers as critical risk indicators on assessing the impact of silver nanoparticles on soil ecosystems

Ying-Fei Yang, Yi-Hsien Cheng, Chung-Min Liao *

Department of Bioenvironmental Systems Engineering, National Taiwan University, Taipei 10617, Taiwan, ROC

ARTICLE INFO

Article history:
Received 24 August 2016
Received in revised form
23 December 2016
Accepted 30 December 2016
Available online 13 January 2017

Keywords:
Silver nanoparticles
Nematode
Caenhorhabditis elegans
Ecotoxicity
Soil ecosystems
Risk assessment

ABSTRACT

Soil contamination caused by silver nanoparticles (AgNPs) released from sewage treatment plants (STPs) is of great public concern. Understanding the relationships between the physicochemical properties of AgNPs and their toxicity is critical for environmental and health risk analysis. Here we presented an approach for rapidly screening and assessing the potential toxicity risk of AgNPs in general and sludge-treated soils based on the nematode *Caenhorhabditis elegans*-based probabilistic risk assessment framework. The soil environmental risks were estimated depending on the characteristics of AgNPs and geographic regions. We assessed the risk for soils exceeding a threshold of *C. elegans* neurotoxicity based on the statistical models. Our results indicated that locomotion inhibition of *C. elegans* was depending on surface properties, diameter, and exposure time of AgNPs. Here we showed that the overall sewage sludge-released AgNPs-associated soil contamination risk was very low among Europe, U.S., and Switzerland. However, large production and widespread use of AgNPs are highly likely to pose long-term ecotoxicity risk on general and sludge-treated soils, particularly for 26 nm citrate-coated AgNPs. Our approach of integrating probabilistic risk model and *C. elegans*-based ecological indicator provides an effective tool to rapidly screen and assess the impacts of STPs-released AgNPs on soil environment. We suggest that *C. elegans* as a proxy for estimating soil risk metrics can help develop methods of management for mitigating the metal NPs-induced toxicity on terrestrial ecosystems.

1. Introduction

Silver nanoparticles (AgNPs) are the most widespread metallic nanomaterials applied in numerous consumer products with the production up to 500 t yr^{-1} worldwide (Nanotech Project, 2016). Due to the antimicrobial effects of AgNPs, more than 400 consumer products are claimed to contain AgNPs with a concentration as high as 10,000 mg L^{-1} (Nanotech Project, 2016). However, AgNPs are found to be easily leached from various commercial products and most of the released AgNPs are discharged into the municipal sewer systems (Hagendorfer et al., 2010; Kaegi et al., 2010).

It was reported that approximately 60% of sewage sludge from sewage treatment plants (STPs) is applied to agricultural land as biosolids in U.S. and 50% is used for agriculture and soil amendment in Europe (European Environment, 2014; Johnson et al., 2011; USEPA, 1995). Gottschalk et al. (2009) estimated that AgNPs in treated sewage ranged from ~4 and ~6 mg kg^{-1} for Europe and U.S., respectively. USEPA (2009) further reported that there are nearly 73% samples of treated sewage containing AgNPs with a range of 1–20 mg kg^{-1}, whereas Ag concentrations in dry sludge or biosolids ranged from 1.94–856 mg kg^{-1} (Donner et al., 2015).

At present, only concentrations of several metals (As, Cd, Cr, Cu, Pb, Hg, Mo, Ni, Se, Zn) applied in agricultural and sludge-amended soils are legally regulated by European Union, U.S., and Netherlands (European Union, 2000; USEPA, 1990). However, concentrations of Ag and AgNPs are not yet regulated. Although environmental levels of AgNPs are considered to be very low (Gottschalk et al., 2009), the large quantity of AgNPs produced annually are highly likely to accumulate and to pose potential environmental risks in soil ecosystems (Massarsky et al., 2014). As a result, it is of importance to evaluate the risks of AgNPs-treated sludge soils for soil quality and ecotoxicity in soil ecosystems.

Pradas Del Real et al. (2016) found that application of AgNPs-treated sludge soils decreased the crop production. Chen et al. (2015) also reported that AgNPs-amended biosolilds inhibited nodulation and induced unique shifts in expression of gene profiles in *Medicago truncatula* A17. On the other hand, AgNPs were proved to pose reproductive toxicity and neurotoxicity to both the earthworm *Eisenia fetida* and the nematode *Caenhorhabditis elegans* (Heckmann et al., 2011; Kim et al., 2012; Lim et al., 2012; Roh et al.,

* Corresponding author.
E-mail address: cmliao@ntu.edu.tw (C.-M. Liao).

http://dx.doi.org/10.1016/j.ecolind.2016.12.051
1470-160X/

2009; Shoults-Wilson et al., 2011; Starnes et al., 2015). Formation of ROS, oxidative stress-related mitochondrial DNA damages, mortality, growth inhibition, shortened lifespan, and internalizations of AgNPs were also observed in *C. elegans* (Ahn et al., 2014; Bone et al., 2015; Chatterjee et al., 2014; Contreras et al., 2014; Ellegaard-Jensen et al., 2012; Eom et al., 2013; Lim et al., 2012; Luo et al., 2016b; Maurer et al., 2016; Meyer et al., 2010; Roh et al., 2012; Yang et al., 2012). A recent study also reported that AgNPs could be accumulated in *E. coli*, exhibiting toxicity to higher trophic level of *C. elegans* (Luo et al., 2016a).

The development of soil bioassays for assessing soil contamination and toxicity is increasingly demanded. Instead of the earthworm *E. fetida* that is currently used for standardized examination of soil contamination in both U.S. and Europe (ASTM, 1997; OECD, 1984), other alternative soil organisms such as *C. elegans* have also been used. The *C. elegans* are common nematodes that freely reside in soils and have been widely used for assessment of ecological risk in various ecosystems including pore water, soil, and sediments (Freeman et al., 2000; Hodgkin, 1998; Peredney and Williams, 2000; Roh et al., 2009).

In light of the advantages of *C. elegans* for their ease and short duration of testing, wealth of biological information, and high sensitivity to toxicants compared to other organisms in soils, they have been extensively used as a model organism to explore nanotoxicology in various studies (Jung et al., 2015; Maurer et al., 2015). We thus used *C. elegans* as an effective tool to rapidly screen and assess potential toxicity of AgNPs in general and sludge-treated soils. The *C. elegans* are efficient in evaluating environmental safety, toxicological study, and investigations of translocation of NPs (Wang, 2016; Zhao et al., 2013). Moreover, the motor behavior of *C. elegans* has been well described and established given the full map of the nervous system (White et al., 1986), it is worthwhile utilizing locomotion as endpoints for toxicant screening. We also addressed the issue of comparing the probabilistic risks of AgNPs in different coatings and sizes in *C. elegans* that have not been investigated in the existing studies (Gottschalk et al., 2009; Voelker et al., 2015).

The purpose of this study was fourfold: (1) to obtain dose-response profiles of AgNPs in different sizes and coatings based on the laboratory *C. elegans* exposure experiments, (2) to analyze acceptable levels of AgNPs based on data of concentrations yielding locomotion inhibition to *C. elegans*, (3) to estimate exceedance risks and risk quotients of AgNPs among different geographic regions, and (4) to implicate the potential risks of AgNPs in sludge-treated soils for agricultural applications.

2. Materials and methods

2.1. Problem formulation and AgNPs characterization

The main source of AgNPs in soils is through effluents of STPs and land application of sewage sludge (Supplementary Fig. S1). Fig. S1 illustrates the route that AgNPs-containing products taken from production plants to sewage treatment plants and to the terrestrial and aquatic environments.

In our experiment, the stocks of AgNPs in different coatings and sizes were provided by Dr. Y.-J. Wang with National Cheng Kung University, Tainan, Taiwan. AgNPs were synthesized from the reduction of $AgNO_3$ by $NaBH_4$ in the presence of sodium citrate or polyvinylpyrrolidone (PVP) (Lee et al., 2014; Sileikaite et al., 2009; Solomon et al., 2007). Detailed information of methods of AgNPs syntheses was provided in Supplementary texts. Results of AgNPs characterization such as chemical composition, zeta potential, polydispersity index (PDI), and specific surface area (SSA) were provided by Dr. Y.-J. Wang (Supplementary Table S1).

Stocks of AgNPs in various concentrations were freshly prepared in EPA water. Morphologies of 1 mg L^{-1} 26 nm citrate-coated and 10 mg L^{-1} 83 nm citrate-coated, 23 and 70 nm PVP-coated AgNPs were captured by transmission electron microscopy (TEM) (TEM; JEM1200EXII; Jeol Ltd., Tokyo, Japan). Before analyzing the hydrodiameters of AgNPs, stocks were sonicated for 30 min before being analyzed by dynamic light scattering (DLS) machine (Delsa Nano C; Beckman Coulter, CA, USA). Numbers of nanoparticles in various sizes were analyzed as differential numbers (%) by the DLS machine (Supplementary Table S2). TableCurve 2D (Version 5.01, AISN Software, Mapleton, OR, USA) was used to perform model fittings of size distributions of AgNPs.

2.2. Examination of AgNPs bioaccumulations in C. elegans

To observe uptake of AgNPs in *C. elegans*, synchronized wildtype L1 larvae were exposed to 5 mg L^{-1} rhodamine-coated AgNPs in the EPA water with *E. coli*OP50 added as a food source (O.D. = 1.1) for 24 and 48 h (Supplementary Fig. S2). Subsequently, worms were washed with distilled water and transferred to nematode growth medium (NGM) plates. Randomly selected worms from each set of experiments were mounted onto microscope slides coated with 2% agarose, anesthetized with 1 M sodium azide, and capped with coverslips. The red fluorescence was captured with a Leica epifluorescence microscope (Leica, Wetzlar, Germany) with excitation at 552 nm and emission at 636 nm and a cooled charge-coupled device camera.

2.3. Experimental design

Synchronized L1 larvae of wildtype were exposed to various concentrations of AgNPs in different sizes and coatings. To avoid overcrowded and starvation of worms, numbers of L1 were controlled in the range of 600–800 worms in each treatment. The EPA water (0.096 g $NaHCO_3$, 0.06 g $CaSO_4{\cdot}2H_2O$, 0.06 g $MgSO_4{\cdot}7H_2O$, 0.004 g KCl in 1L distilled water) was used as the dosing medium according to previous studies (Peltier and Cornelius, 1985; Yang et al., 2012). Worms were exposed to 0.01, 0.1, 0.5, and 1 mg L^{-1} of 26 nm citrate-coated, 0.01, 0.1, 5, and 20 mg L^{-1} of 23 nm PVP-coated, 0.01, 0.1, 1, 5, and 20 mg L^{-1} of 83 nm citrate-coated, and 0.01, 1, 5, 10, and 20 mg L^{-1} of 70 nm PVP-coated AgNPs, respectively, for 65 h in the presence of *E. coli* OP50 (O.D. = 1.1) at 20 °C.

Before analyzing the locomotive behaviors and development, worms were washed with double distilled water for 3 times to remove adhering bacteria and residual AgNPs in the medium. Subsequently, young adult worms were transferred to nematode growth medium (NGM) plates to observe the changes in locomotion post AgNPs exposure.

Bioassay of locomotive behaviors was performed by counting body bends of worms that was adopted from a previous study (Tsalik and Hobert, 2003). After a recovery period of 1 min on NGM plates, the body bends of worms were counted in an interval of 20 s. A body bend was counted as a change in direction of the part of worm corresponding to the posterior bulb of the pharynx along *Y*-axis, with the assumption that the worm travelled along *X*-axis. At least 3 independent experiments were performed and approximately 15–20 worms were examined per condition in each trial.

On the other hand, head thrashing of worms was also employed as another endpoint to compare AgNPs toxicity in *C. elegans* (Supplementary Table S8). After a recovery period of 1 min on NGM plates, the head thrashing was counted in an interval of 1 min. A thrash was defined as a change in the direction of bending in the mid body (Tsalik and Hobert, 2003).