The Influence of Soil pH on the Uptake of Silver Nanoparticles in a Terrestrial System

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Abstract

The production and use of silver nanoparticles (Ag NPs) are increasing in the United States and across the globe. The Ag NPs may enter the terrestrial ecosystem with the application of sewage sludge as a fertilizer on agricultural farms. The present study investigated the influence of soil pH on the uptake of Ag NPs by insects. Two insects *A. domesticus* and *T. molitor* were selected for the experiment. The insects were exposed to Ag NPs under different soil pH including: 5, 6, 7, 8 (control), and 9. The Ag NPs were characterized prior to the study with transmission electron microscopy, dynamic light scattering, and powder X-ray diffraction techniques. The concentration of silver in insects was measured with inductively coupled plasma-optical emission spectroscopy. Increased levels of silver in insects was observed in normal and alkaline soil as compared to acidic soil treatment groups. Additionally, there was no significant difference due to insect species in the silver uptake.

Keywords: Silver nanoparticles; Terrestrial system; Uptake; Soil pH

Introduction

As increasing amounts of silver nanoparticles (Ag NPs) are manufactured and used, the quantities of silver released into the environment will also increase [1-4]. The unique properties of Ag NPs include antimicrobial, antifungal, and partially antiviral factors. These properties are associated with the increasing production and this trend is expected to continue in the future [5-7]. As of 2007, the United States annually released over 13 Gg (14330 metric ton) of total (bulk and nano) silver into the environment, more than any other nation in the world [8,9]. According to Hendren et al. [10], the total production of Ag NPs in the United States was 2.8-20 metric ton/year and is growing. It is estimated that by 2025 the global Ag NPs market would be 800 metric ton [11]. There are many ways that Ag NPs can enter the environment, both intentionally and accidentally [12]. A potential route of entry of Ag NPs in the environment is waste water streams and effluent because the Ag NPs are retained in sewage sludge [13,14]. The sewage sludge is often used as a fertilizer in agriculture where it can move to the aquatic ecosystem through surface runoff or it can remain in the terrestrial environment [13,15]. Once Ag NPs are present in a system, their fate is uncertain since it is dependent on many interacting factors. The bioaccumulation and toxicity of heavy metals to soil inhabitants is a complex process which is determined by the soil characteristics, chemical form of metals, and physiology of organisms [16]. One of the driving factors in the terrestrial system could be the soil pH. As with other metals, soil pH plays a significant role in the availability of silver nanoparticles Janssen et al. [17,18]. Soils with lower pH have an increased amount of H^+ which compete with silver ions for binding sites causing a larger amount of silver ions to remain free in the soil [18].

The bioaccumulation and toxicity of Ag NPs has been reported for soil organisms such as *Eisenia fetida* [19], *Enchytraeus albidus* [20], *Porcellio scaber* [21], and *Folsomia candida* [22], however, the data is limited for the uptake of Ag NPs by insects. Nanoparticles can transfer from one trophic level to the next along the food chain. According to Unrine et al. [23] gold nanoparticles can transfer from soil to terrestrial organisms. The transfer of heavy metals such as cadmium, lead, etc. across the food web has been recognized previously [14,24-26]. Zhuang et al. [27] reported the transfer of lead from insect larvae to chickens (*Gallus gallus domesticus*). In the present study, two insect species (*Acheta domesticus* and *Tenebrio molitor*) were used as model organisms because these insects are soil dwelling and easy to culture [28-30]. Additionally, these insect species are native to the study area. Hence, the examination of the uptake of Ag NPs by these species would help us to understand the transfer of heavy metals along the food web in the native avian insectivore species such as Northern Bobwhite quail (*Colinus virginianus*). In this study, we investigated the influence of soil pH on the uptake of Ag NPs by insects.

Materials and Methods

Soil collection and preparation

All soil used during the insect exposure experiments was collected from the top 10 cm of soil from Colorado City (exact coordinates: 14 S 0319752 mE 3557792 mN), Texas and transferred to The Institute of Environmental and Human Health (TIEHH) at Texas Tech University (TTU) in Lubbock, TX. Before use, soil was processed for homogeneity and sieved through a 2-mm wire screen into a clean plastic storage container.

Soil analysis

Soil samples were analyzed at Midwest Laboratories Inc. (Omaha, NE, USA) for texture, percent humic matter, percent organic matter, exchangeable cations (K^+, Mg^{2+}, Ca^{2+}), available phosphorus (P), pH, percent base saturation of cations (K^+, Mg^{2+}, Ca^{2+}, H^+), cation exchange capacity (CEC), and sulfur (S) content to characterize the soil.

Nanoparticle characterization

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All uncoated Ag NPs (30-50 nm) were purchased from US Research Nanomaterial, NC. (Houston, TX, USA). All Ag NPs were reported by US Research Nanomaterial, Inc. to consist of ≥ 99.99% Ag. The manufacturer also confirmed the size and spherical shape of each lot of nanoparticles. However, due to the tendency of nanoparticles to aggregate, the most significant properties, size and shape, were examined to confirm the manufacturer's specifications.

To confirm the size range and shape of the nanoparticles, transmission electron microscopy (TEM) was used. Each sample was prepared by dispersing the Ag NPs powder in ethanol (EtOH) and sonicated for 10 minutes before being drop cast onto a carbon coated copper grid. Samples were air dried before analysis. TEM (Hitachi H-8100 TEM) images were taken at 200 kV using a tungsten filament side-mounted camera.

Dynamic light scattering (DLS) was used as an additional method to confirm the nanoparticle size. Sample preparation was performed by placing approximately 10 mg of silver nanoparticle powder in 10 mL of reagent grade acetone (Fisher Chemical, MA, USA) and analyzed using a Nanotrac NPA252 Combination (Microtrac Inc. Montgomery, PA, USA) and Mictrotrac Flex Software (Version: 10.3.14). Powder x-ray diffraction (PXRD) was used to confirm nanoparticle composition. A Rigaku Ultima III X-Ray Diffractometer was used to analyze all samples. Samples were analyzed using Cu Kα radiation as x-ray source. The Ag NPs were analyzed using the following instrument parameters: parallel-beam geometry was used with a step width of 0.03° and a count time of one second; the divergence, scattering, and receiving slits were set at one. Once completed, the diffraction patterns were compared and matched to the phases in the International Center for Diffraction Data (ICDD) powder diffraction file (PDF) database.

Insect treatment groups

All insects were purchased from reptilefoods.com and randomly divided into treatment groups and control. The insects were transferred into a terrarium which had 2.5 kg of soil. Before each terrarium could be spiked with Ag NPs, the soil pH had to be adjusted. During the soil characterization, the control soil was found to have a pH of 8.01. Nitric acid (HNO_3) was used to reduce the soil pH from 8 to 5, 6, and 7 required for the treatment groups. It should be noted here that HNO_3 was chosen over sulfuric (H_2SO_4) and hydrochloric (HCl) acids because silver nitrate ($AgNO_3$) is soluble, unlike silver chloride (AgCl) and silver sulfide (Ag_2S). By using HNO_3 in place of H_2SO_4 or HCl, it was ensured that the Ag NPs would remain in solution rather than precipitating out. To adjust the pH of each treatment group, small volumes of HNO_3 were added to each experimental tank and dried overnight. The following day the soil was thoroughly mixed and allowed to sit for an additional day. A composite sample was then taken from each experimental tank and the pH was tested at 1: 1 soil: solution ratio with an Orion Star A211 pH Benchtop Meter (ThermoScientific).

As for the treatment group where the soil pH was equal to 9, commercially available garden lime (Espoma Organic Traditions Garden Lime) was used to increase the soil pH. The garden lime was mixed with equal amounts of distilled water and small volumes were added to each terrarium as described above. The treatment and control groups soil pH are summarized in Table 1.

All terrariums were spiked with 25 µg/g Ag NPs and thoroughly mixed to ensure homogeneity. Each terrarium received either 300 *A. domesticus* or 400 *T. molitor*. Insects were provided with fresh food (Fluker's Cricket Quencher®) ad libitum for the 28 day exposure period. Once the exposure was complete, all insects were euthanized at -80°C.

The insects were then freeze dried (FreeZone 2.5 Liter Freeze Dry System, Labconco, Corp. Kansas City, MO, USA) for at least 48 hours to ensure the removal of all moisture. Freeze dried insects were then crushed into a fine powder and stored in a freezer until they could be digested.

Sample digestions

Six samples were randomly collected from each terrarium, these weighed approximately one gram each and were placed into separate 100 mL beakers. It should be noted here that the weights are dry weight (dw) for the insects. The *A. domesticus* samples were calculated to be approximately 81% water; so, 1.00 g dry weight would correspond to roughly 5.25 g wet weight (ww) whereas, the *T. molitor* samples were found to be approximately 39% water; so, 1.00 g dw would correspond to 1.65 g ww.

Silver analysis

All samples were analyzed using a Teledyne Instruments (Hudson, New Hampshire) Prodigy High Dispersion Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). All samples were analyzed for silver at three wavelengths: 224.643, 328.068, and 338.289 nm. Ultimately, the data from 338.289 nm was chosen for statistical analysis. To ensure quality control throughout the run, the blank, 0.01, and 10 ppm standards were run at least once in each rack of 44 samples. Additionally, automatic check standard samples were run at various intervals throughout each run. The 0.05, 0.5, and 5.0 ppm standards were analyzed every 10 samples. And the 0.1 and 1.0 ppm standards were run every 20 samples. The standard samples were monitored throughout each run to ensure the instrument was constant in its analysis. Duplicate samples were also run at least once per batch to ensure consistency between runs.

Statistical analysis

The normality and distribution of variance of the data was examined before performing ANOVA. A two-way ANOVA was performed to compare the uptake of Ag from Ag NPs in soil at level α=0.05. Two factors were considered for the analysis including: soil pH and insect species. Soil pH had five levels including: 5, 6, 7, 8, and 9, whereas insect species were *A. domesticus* and *T. molitor*. An interaction between the soil pH and insect species was also added to the model. This was followed by a Tukey's pairwise comparison test. The soil pH and insect species were treated as fixed type factors in the model. All statistical analysis was performed using Minitab 17. The concentrations below 0.02 ppm were noted as zero during the data analysis.

Results

Soil characterization

The control soil was found to contain 54% sand, 36% silt, and 10% clay. This type of soil is classified as a sandy loam. The additional tests found the soil to contain 0.01% humic matter, 1.7% organic matter, and 9 ppm S. The pH of the control soil was slightly basic, 8.1 and the CEC of the soil was calculated to be 18.0 meq/100 g. Additionally, control soil samples analyzed by ICP-OES were found to contain no detectable concentrations of silver. The soil pH of treatment groups is summarized in the Table 1.

Transmission electron microscopy

The 30-50 nm uncoated silver nanoparticles were found to be heavily aggregated after being dispersed in EtOH. However, the TEM

could confirm the spherical shape of the nanoparticles (Figure 1). And most of the particles looked to be in 30-50 nm range, although there were outliers on either side of the range.

Dynamic light scattering

Approximately 95% of the 30-50 nm had a size between 30.70 to 52.90 nm (Figure 2). The average size of the particles was found to be 41.80 nm, well within the parameters set by the manufacturers.

Powder X-ray diffraction

The PXRD analysis of the silver nanoparticles confirmed their composition. The diffraction patterns matched both those in the ICDD and those provided by the manufacturer. A typical diffraction pattern can be seen below (Figure 3).

Silver analysis

All method blanks and control samples were not found to contain detectable amounts of silver. All insect samples were found to contain a quantifiable amount of silver (>0.02 ppm, Limit of Quantitation) except *A. domesticus* from treatment group soil pH 5. A lower amount of Ag was observed under acidic conditions in both insects i.e., *A. domesticus* and *T. molitor* as compared to normal and alkaline soil environment (Figure 4).

Data analysis

The normality test shows that the data was normally distributed (p=0.738) and has a homogeneous distribution of variance. A two-way ANOVA was run on 60 samples of insects to examine the effect of the soil pH and insect species on the uptake of Ag from AgNPs in soil. There was a significant interaction between the effect of the soil pH and insect species on the uptake of Ag in insects, $F_{(4,50)}=29.87$, p=0.000 (Figure 4). A simple main effect analysis showed that the effect of the soil pH on the uptake of Ag in insects was significantly different, $F_{(4,50)}=72.84$, p=0.000, but there was no significant difference due to the insect species (p=0.602). Post hoc comparisons indicated that the mean uptake of Ag from treatment groups with normal to alkaline soil (pH 7, 8, and 9) was significantly higher than acidic soils (Figure 4).

Discussion

The transfer of heavy metals from soil to organisms is a complicated process. The soil physiochemical properties (pH, cation exchange capacity, organic matter content, etc.), metal properties, nature of metal (essential vs non-essential), physiological state of organisms, and the route of exposure can affect the bioaccumulation/uptake of heavy metals [31]. It was expected that the samples from the treatment groups with lower soil pH values would contain more silver than those samples from the higher soil pH groups. The decreased pH of the lower treatment groups would have increased the amount of free silver ions present overall since metals dissolve in acidic environments. Additionally, soils with lower pH values have a decreased amount of OH⁻ groups which provide additional binding sites for cations. However, the data from this study differ from this theoretical concept. The soil organisms may be exposed to nanoparticles by dermal or oral routes. Diez Ortiz et al. [32] reported that the ingestion of soil is a major route of exposure to Ag NPs in earthworms (*Lumbricus rubellus*) and the inhibition of soil ingestion result in the reduction of Ag NPs uptake. The data from the present study shows that the uptake of Ag NPs by *A. domesticus* and *T. molitor* was higher under normal and alkaline soil treatment groups as compared to the acidic soil. Bengtsson et al. [5] reported that the

Soil Treatment Group	Measured soil pH
5	5.03 ± 0.03
6	5.99 ± 0.04
7	6.91 ± 0.02
8 (Control soil)	8.01 ± 0.00
9	8.45 ± 12

Table 1: Soil pH of insect treatment groups adjustment with HNO₃ and lime
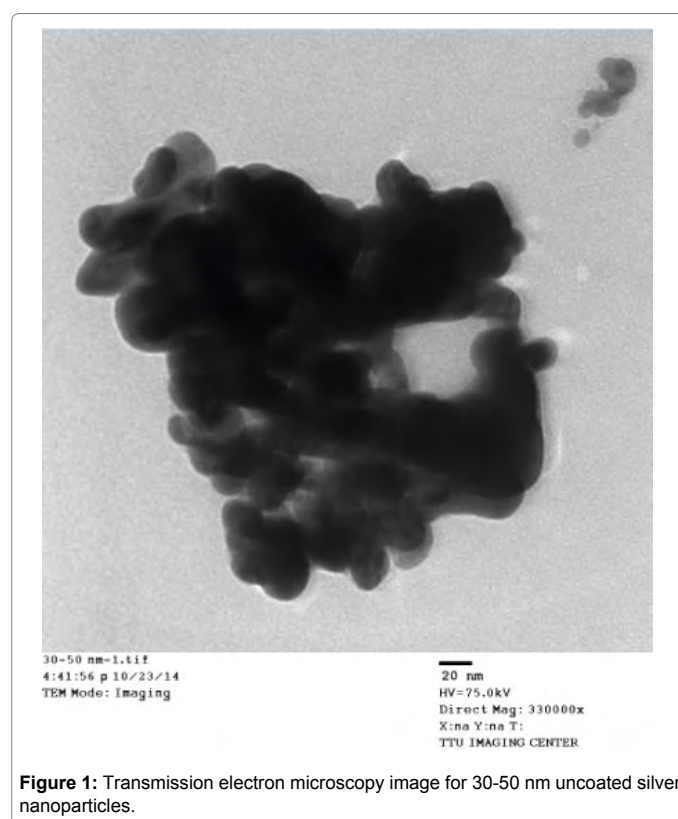

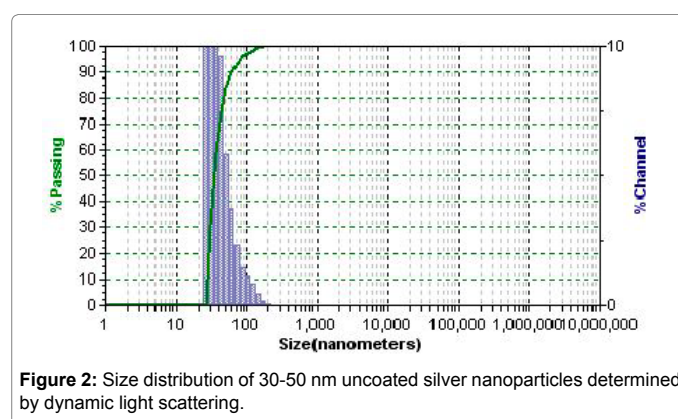**Figure 1:** Transmission electron microscopy image for 30-50 nm uncoated silver nanoparticles.

Figure 2: Size distribution of 30-50 nm uncoated silver nanoparticles determined by dynamic light scattering.

highest heavy metal concentration in tissues was not associated with the lowest soil pH in earthworms (*Dendrobaena rubida*). Based on data from the current experiment, we believe that the soil pH is playing an important role to facilitate the uptake of Ag NPs by both insects. It appears that acidic conditions reduce the feeding activity. Similarly, reduced earthworm growth and activity was also reported in acidic soils [33]. The feeding activity of soil fauna can be significantly boosted
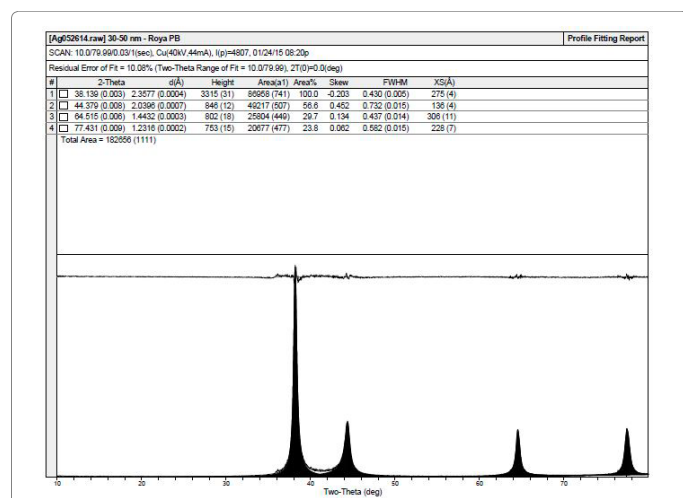
Figure 3: Diffraction pattern for 30-50 nm silver nanoparticles used in pH study as determined by powder x-ray diffraction.
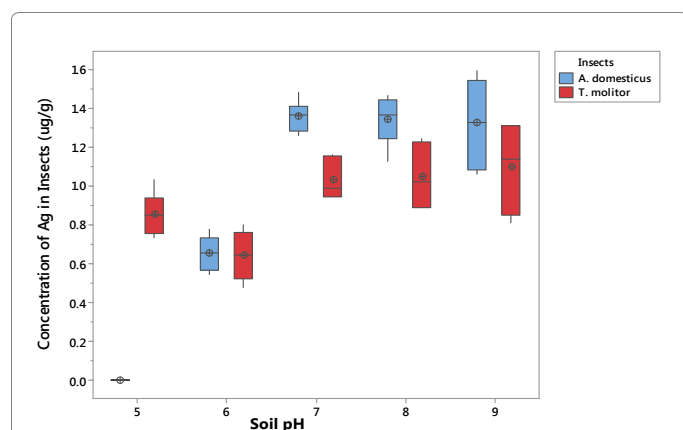

Figure 4: Influence of soil pH on uptake of Ag NPs by insects (*A. domesticus* and *T. molitor*).

with liming the soil [34]. The feed consumption ratio in isopods (*Porcellionides pruinosus*) was higher at soil pH 7.3 as compared to soil pH 6.2 and 4.5 [35].

The apparent decrease in feeding activity may have resulted in lower accumulation of Ag NPs in tissues. Furthermore, if the Ag NPs are simply adhering to the surface of the insects, the soil pH may not have had a large effect but we observed a difference in uptake due to soil pH. Hence, we can confirm that the dermal exposure is not a major contributing route for exposure in these insects. It appears that feeding/ingestion activity is affecting the uptake of Ag NPs by insects. However, the mechanism by which these insects accumulate less Ag in acidic soil as compared to higher soil pH is not fully understood and will need a further study.

Conclusion

The present study shows that the soil pH influences the uptake of Ag NPs by *A. domesticus* and *T. molitor*. The high amount of Ag in the control group insects (soil pH 8.01) as compared to acidic soil indicates that the Ag may transfer to the next trophic level (native avian insectivore species). Further studies will be needed to clarify exactly how these silver nanoparticles are bioavailable to terrestrial insects and

the role of soil pH.

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