Effect of Concentration of Silver Nanoparticles on the Uptake of Silver from Silver Nanoparticles in Soil

Sara Pappas[1], Uday Turaga[2], Naveen Kumar[3], Seshadri Ramkumar[4], Ronald J. Kendall[5]

[1,2,3,5]The Wildlife Toxicology Laboratory, The Institute of Environmental and Human Health, Texas Tech University, Lubbock, TX, USA

[4]Nonwovens and Advanced Materials Laboratory, The Instutite of Environmental and Human Health, Texas Tech University, Lubbock, TX, USA

Abstract— *The bioavailability and uptake of silver from silver nanoparticles in soil was investigated. Two species of insects, Acheta domesticus and Tenebrio molitor, and two species of plants, Helianthus annuus and Sorghum vulgare, were exposed to a range of concentrations of silver nanoparticles in soil. Silver nanoparticles were charactrized by techniques including transmission electron microscopy, dynamic light scattering, and powder X-ray diffraction. The concentration of silver in insects and plants exposed to silver nanoparticles was measured using inductively coupled plasma-optical emission spectrometry. The results suggested an increase in the levels of silver in both insects and plants as a function of increasing concentrations of silver nanoparticles in soil. The translocation of silver to various parts of dicot plants such as stems and leaves was also observed. Such a result was not observed in the case of monocot plants. Results from this study suggests that silver nanoparticles would be available for uptake by insects and plants in terrestrial ecosystems.*

Keywords— Silver nanoparticles, Acheta domesticus, Tenebrio molitor, Helianthus annuus, Sorghum vulgare, inductively coupled plasma-optical emission spectrometry.

I. INTRODUCTION

The antimicrobial properties exhibited by silver and silver nanomaterials have propelled their widespread use in many consumer products that include detergents, textiles, home appliances, nutritional supplements, etc. In fact, silver based nanomaterials are one of the most common and most used among all nanomaterials [1]. According to a study by the Grand View Research, Inc. the global market for silver nanoparticles (Ag NPs) is projected to reach USD 2.45 billion by 2022 [2]. The widespread use of Ag NPs invariably raises questions and concerns about the risks and consequences resulting from their release into the environment.

Ag NPs are introduced into the terrestrial systems primarily through applications of sewage sludge to land [3-17]. Once present in an ecosystem, the environmental behavior, fate and ecotoxicity of metal-based nanoparticles are known to be influenced by their physical and chemical characteristics. Physical characteristics include size and shape of nanoparticles and chemical characteristics include acid-base character of the surface and aqueous solubility of the metal. The physical and chemical characteristics of nanoparticles influence their transformation phenomena such as aggregation, sorption to surfaces, and dissolution to metal ions. Additionally, surface coatings on metal based nanoparticles also influence their environmental behavior, fate and ecotoxicity [10]. For instance, the properties of Ag NPs that influence their uptake and toxicity to the earthworm *Lumbricus rubellus* in soil have been investigated by Makama et al. 2016 [18].

The physicochemical characteristics of soil influence the chemical form, mobility, bioavailability and toxicity of pollutants in terrestrial ecosystems. These physicochemical characteristics include pH, ionic composition, grain size, soil texture, organic matter content, temperature, solar radiation exposure, hydrostatic pressure, and cation exchange capacity. Thus, it is important to understand that the environmental behavior, fate, bioavailability and ecotoxicity of nanomaterials and other pollutants are influenced and determined by a combination of the physicochemical characteristics of the soil, the physicochemical characteristics of nanomaterials, and the physiological status of biota [19, 20].

Once present in a terrestrial environment, it has been shown that certain types of nanoparticles possess the ability to be taken up by insects [2 1] and plants [22-27], thereby entering the food web. The uptake of nanoparticles from soil depends on a large number of factors. One such factor is the concentration of nanoparticles present in soil. Larger amounts of nanoparticles may be prone to a phenomenon called aggregation, which causes the final size of the nanoparticles to be larger than they were originally. This could potentially affect the uptake of nanoparticles as the uptake of nanoparticles is

inversely proportional to their size. The small size of nanoparticles enables their easy uptake, dissolution and release of ions with increased surface area [28]. Conversely, the presence of a large amount of nanoparticles in a system may result in an increased uptake.

In the present study, the uptake of silver by insects and plants as a function of concentration of Ag NPs in soil was investigated. Studies to understand the uptake, kinetics and transformatoin of metal nanoparticles in terrestrial ecosystems usually include the terrestrial isopods [29-31]. Terrestrial ispods enable the study of uptake and transformation of metal nanoparticles because of their ability to uptake nanopartiles extensively through the oral route. Surface uptake of nanoparticles in terrestrial isopods was found to be negligible [32]. However, to comprehensively understand the effect of nanoparticles on terresrtial ecosystems, it is important to investigate their uptake in other components of terrestrial ecosystems that play a keyrole in terresrtial food webs.

Two species of insects, *Acheta domesticus* and *Tenebrio molitor*, and two species of plants, *Helianthus annuus* (a dicot) and *Sorghum vulgare* (a monocot) were used in this study. Insects constitute an important source of food to insectivorous birds. They serve as a crucual link in the metal-transport chains between trophic levels in food webs [33]. As they are a very good source of protein and other nutrients, insects and larvae serve as an important food source to birds especially during the breeding season [34]. Considering the signficance of insects in the food web of all insectivorous birds, it is important to understand if insects are able to uptake abd accumulate silver from Ag NPs in soil. Two different species of plants, a monocot and a dicot, were chosen for this study to understand if there is any difference in the uptake of silver from Ag NPs in soil by these plants. The objective was to examine if either of the monocot or dicot plant species uptake silver from Ag NPs in soil. Additionally, the possibility of translocation of silver to other tisseus of plant such as stem, seeds, etc was also evaluated. Considering seeds of plants also serve as an important food source for granivorous birds [35], such a study would help understand if these plants are playing a role in the metal-transport chain in the food web of granivorous birds.

The test species were exposed to concentrations of Ag NPs ranging from 0-625 mg/kg (ppm) in soil. Studies have suggested that sewage sludge can contain a wide range of concentrations of silver. Concentrations of silver as high as 960 ppm were reported in some sludge samples [12]. Thus, the concentration of Ag NPs used in this study is not outside the realms of possibility.

II. MATERIAL AND METHOD

2.1 Soil Collection and Preparation

All soil used during the insect and plant exposure experiments was collected 40 minutes south of Colorado City, Texas at an elevation of 684 m above sea level. Exact coordinates were as follows: Universal Transverse Mercator (UTM) 14 S 0319752 mE 3557792 mN. All soil was collected from the top 10 cm of soil, shoveled into clean plastic containers and transported back to The Institute of Environmental and Human Health (TIEHH) at Texas Tech University (TTU) in Lubbock, TX. Once at TIEHH the soil was processed for homogeneity. All large rocks, roots, living organisms, and other organic matter were removed first and large clumps of soil were crushed. The soil was then sifted through a 2 mm wire screen into another clean plastic storage container. Processed soil was covered and stored indoors until ready for use.

2.2 Soil Analysis

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

2.3 Characterization of Ag NPs

Uncoated Ag NPs (30-50 nm) were purchased from US Research Nanomaterials, Inc. (Houston, TX). According to the manufacturer, the Ag NPs contain 99.99% Ag (www.us-nano.com). The manufacturer also confirmed the size and spherical shape of each lot of nanoparticles.

2.4 Transmission Electron Microscopy

In order to confirm the size range and shape of the nanoparticles, transmission electron microscopy (TEM) was used. Each sample was prepared by dispersing the Ag NP powder in ethanol (EtOH). Each sample was 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.

2.5 Dynamic Light Scattering

Dynamic light scattering (DLS) was used as an additional method to confirm the size of the nanoparticles. Sample preparation was performed by placing approximately 10 mg of silver nanoparticle powder in 10 mL of reagent grade acetone (Fisher Chemical). Samples were sonicated until nanoparticles remained suspended in solution. Samples were analyzed using a Nanotrac NPA252 Combination (Microtrac Inc. Montgomery, PA) and Mictrotrac Flex Software (Version: 10.3.14). Method settings were customized for each sample of silver nanoparticles (absorbing particles) and acetone (refractive index: 1.36).

Samples were analyzed by running two consecutive 60 second scans. The average value of the two scans was recorded as the final result of each analysis. The particle size given at 50% was used as the average particle size of each sample.

2.6 Powder X-ray Diffraction

Powder x-ray diffraction (PXRD) was used to confirm the composition of the nanoparticles. 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 silver nanoparticles 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.

2.7 Insect Treatment with Ag NPs

Two replicates were prepared using 37.9 L terrariums (50.8 cm x 27.9 cm x 33.0 cm) for each insect species treatment group, including the control group. Before use, each terrarium was thoroughly cleaned using water, followed by a 10% bleach (calcium hypochlorite) solution to remove any remaining chemical residues. Exactly 2.5 kg of soil was weighed into each clean terrarium. An analytical balance was used to weigh out the necessary amount of Ag NPs for each treatment group: 0 (control), 1, 5, 25, 125, and 625 ppm. Nanoparticles for each treatment group were added to each terrarium and manually mixed for at least 60 seconds to ensure homogeneity. Insects used in the study were purchased from reptilefoods.com. Each terrarium received either 300 small crickets or 400 large mealworms. Insects were provided with fresh food and water as needed throughout the course of the study that lasted 28 days.

Once the 28 day exposure was complete, live insects were carefully extracted from the terrariums and placed in glass jars. The jars were then placed in a -80°C freezer until all the insects were deceased.

The insects were then freeze dried (FreeZone 2.5 Liter Freeze Dry System, Labconco, Corp. Kansas City, MO) 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.

2.8 Plant Treatment with Ag NPs

Commercially available 7.6 L plastic nursery containers were purchased and filled with approximately two inches of commercial pond pebbles to aid in proper drainage. Exactly 2.5 kg of soil was weighed out in a separate plastic container. An analytical balance was used to weigh out the necessary Ag NPs; these were added to the soil and mixed in for at least 60 seconds. The treated soil was then carefully transferred into each nursery container. Two replicates were prepared for each treatment group for each of the two plant species.

Seeds of each plant species were planted in their respective nursery containers and transported to the TTU greenhouse. The plants remained in the greenhouse until they reached maturity, approximately three months for *H. annuus* and six months for the *S. vulgare*. While in the greenhouse, plants received shaded sunlight and were maintained at or above 60°F. Once

plants reached maturity, the entire plant was harvested. The roots were separated from the remainder of the plant and rinsed using tap water for a full minute to remove all attached soil. The shoot system of the plant was separated into leaves, stems, and seeds. The plant tissue samples were stored in a freezer until they could be digested.

2.9 Processing of Samples for Analysis

Three identical samples were weighed out using the insect samples collected from each terrarium. For each plant treatment group, four samples were prepared from each nursery container: a root sample, a leaf sample, a stem sample, and a seed sample, if possible. For each sample, either plant or insect, approximately 1.0000 grams were weighed into a 100 mL beaker. It should be noted here that the weights are dry weight (dw) for the insects and wet weight (ww) for the plants. Exactly 10 mL of 70% reagent grade nitric acid (HNO_3) (Fisher Chemicals) was added to each beaker using an acid-washed 10 mL volumetric flask. A 10 mL aliquot of reagent grade 30% hydrogen peroxide (H_2O_2) (Fisher Chemicals) was carefully added to each beaker using a volumetric flask. A method blank was run with each set of samples by adding 10 mL of HNO_3 and 10 mL H_2O_2 to an empty beaker. All beakers were placed onto hot plates and covered with a Teflon watch glass and allowed to sit overnight before digesting. Samples were then slowly heated; the temperature was raised in 5°C increments until the solutions in the beakers began to gently reflux.

The beakers were diligently monitored to ensure that the mixtures did not boil over in order to prevent the loss of sample. Any samples that did boil over were removed and were rerun at a later time. Samples were periodically swirled during the reflux process if needed. The samples in the beakers were allowed to gently reflux until the volume had been reduced to roughly 5 mL.

Once a sample had reached the desired volume it was carefully removed from the hot plate and placed in an ice bath to cool. After the samples were cooled, the samples were filtered into 50 mL plastic centrifuge tubes (Corning CentriStar™) using acid-washed glass funnels and ashless filter paper (Whatman No. 41). By filtering each sample, any remaining solids and/or digested lipids were removed from the final sample. Exactly 10 mL of 5% HNO_3 was measured using a volumetric flask and poured into each empty sample beaker. The acid solution was swirled in each beaker and the contents were poured into the funnel. This process was repeated twice so the samples were diluted with a total of 20 mL of the 5% HNO_3 solution. Once filtering had been completed, the centrifuge tubes were stored at room temperature until analysis by inductively coupled plasma optical emission spectrometry (ICP-OES) could be performed.

2.10 Analysis of Samples on ICP-OES

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.

The three silver wavelengths were aligned next using a 10 ppm silver standard solution (SPEX CertiPrep, lot# CL7-09AGY). The instrument was calibrated using a range of silver standards from 0-20 ppm.

2.11 Statistical Analysis

The dilution factors were factored back into sample results by multiplying the analyzed concentration by the final sample weight and then dividing by the initial sample weight. These final calculated sample concentrations were then used to run statistical analyses.

All data was compared using a basic Kruskal-Wallis test in R [36] after being found to be non-parametric [37]. The Shapiro test was used to compare the normality of the data [38]. This was followed by a multiple comparison test after Kruskal-Wallis test to identify all significant differences among the treatment groups ($p<0.05$).

III. RESULTS AND DISCUSSION

3.1 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. 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/100g.

Other data from soil analysis is summarized in Table I. Additionally, control soil samples analyzed by ICP-OES were found to contain no detectable concentrations of silver.

TABLE 1
CHARACTERISTICS OF SOIL USED IN THE STUDY

Analysis	Results
Organic Matter	1.7%
Exchangeable Potassium	263 ppm
Exchangeable Magnesium	114 ppm
Exchangeable Calcium	3273 ppm
Soil pH	8.1
Cation Exchange Capacity (CEC)	18.0 meq/100g
Base Saturation, Potassium	3.7%
Base Saturation, Magnesium	5.3%
Base Saturation, Calcium	91.0%
Base Saturation, Hydrogen	0.0%
Sulfur Content	9 ppm
Humic Matter	0.01%
Sand Content	54%
Silt Content	36%
Clay Content	10%

3.2 Transmission Electron Microscopy

The 30-50 nm uncoated Ag NPs were found to be heavily aggregated after being dispersed in EtOH. However, the spherical shape of Ag NPs was confirmed by the TEM (Fig. 1).

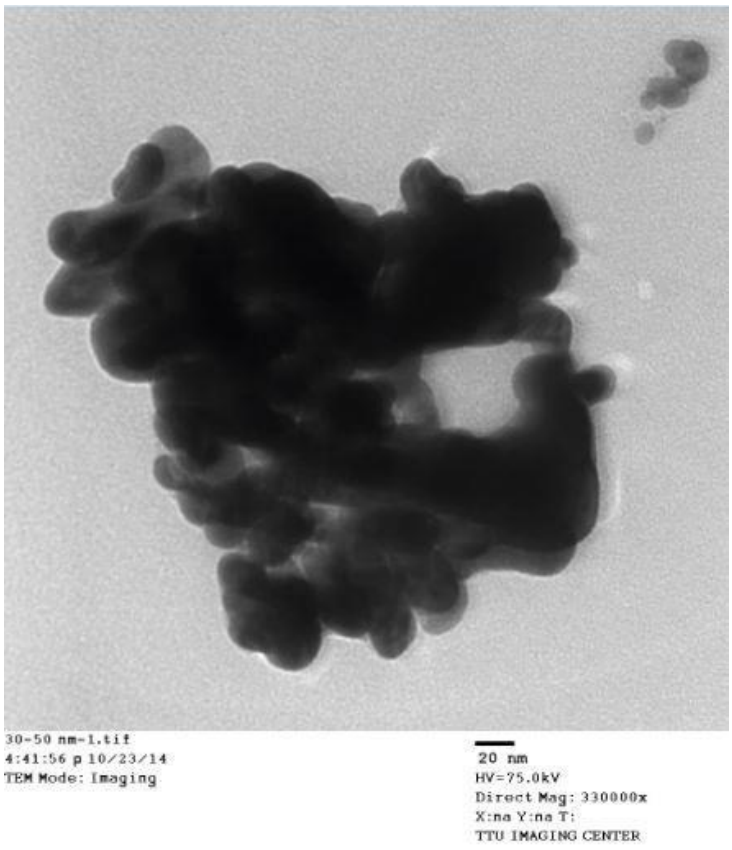

FIG. 1: TRANSMISSION ELECTRON MICROSCOPY IMAGE OF 30-50 nm UNCOATED Ag NPs.

3.3 Dynamic Light Scattering

Approximately 95% of the 30-50 nm Ag NPs were found to be between 30.70 to 52.90 nm. A representative size distribution pattern in presented in Fig. 2. The average size of the particles was 41.80 nm.

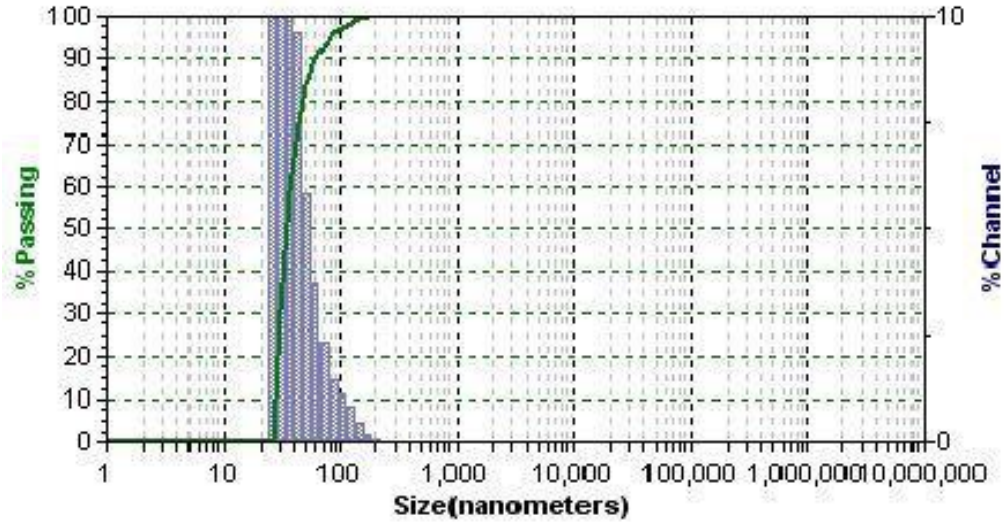

FIG. 2: SIZE DISTRIBUTION OF UNCOATED 30-50 nm Ag NPs AS DETERMINED BY DYNAMIC LIGHT SCATTERING.

3.4 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 is presented in Fig. 3.

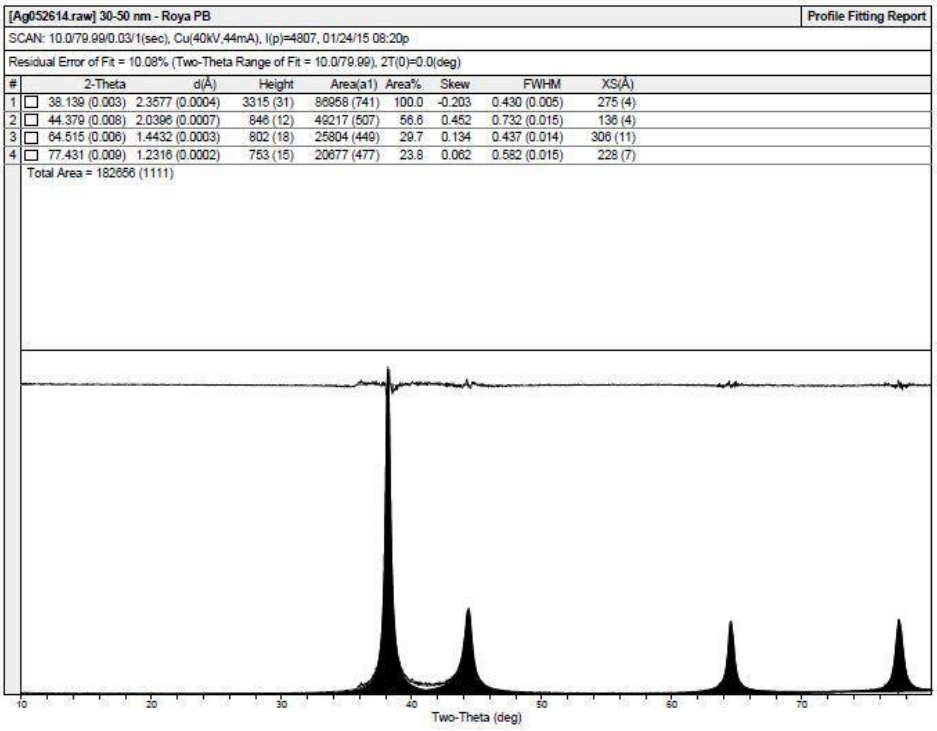

FIG. 3: DIFFRACTION PATTERN OF UNCOATED 30-50 nm Ag NPs aS DETERMINED BY POWDER X-RAY DIFFRACTION.

3.5 ICP-OES Results

Of all the three wavelengths considered for the analysis of silver in the present study, the data from 338.289 nm was chosen for subsequent analysis. Wavelength 224.643 nm was a double and deemed not usable. The wavelength at 328.068 nm was

bracketed by iron at 328.026 nm and 328.129 nm, which could have been a potential interference in both the insects and plant samples.

3.6 Uptake of Silver from Ag NPs in Soil by Insects

Previous studies in literature speculated that silver nanoparticles would be completely immobilized in a sewage sludge due to the formation of the insoluble silver sulfide (Ag_2S) [6, 20, 39, 16, 40]. It was theorized that the silver nanoparticles would remain immobile, and therefore unavailable for uptake, after the sludge was applied as a land amendment. However, the present study showed that Ag NPs would be available for uptake by both insects and plants at high enough concentrations.

It should be noted that this study used pristine nanoparticles rather than silver that had interacted with sewage sludge. However, the widespread use of Ag NPs in detergents, textiles, etc. pontetially serves as an anthropogenic source for the release of pristine silver nanoparticles into terrestrial ecosystems. Sewage sludge typocally contains 0.3-2.3 wt% of sulphur [41]. Hence, it is possible that there may not be enough sulphur in the sludge in the event of presence of high concentration of Ag NPs.

In the case of both the insects used in the study, a concentration dependent increase in the uptake of silver from Ag NPs in soil was observed (Fig. 4 & 5). Insects exposed to the highest concentrations of Ag NPs used in the study (125 and 625 ppm) were observed to contain quantifiable concentrations of silver (> 0.1 ppm). Levels of silver in insects exposed to the lowest concentrations of Ag NPs used in the study (0 and 1 ppm) could not be quantified (below instrument detection limits or < 0.005 ppm). Lastly, insects exposed to intermediate concentrations of Ag NPs used in the study (5 and 25 ppm) were found to contain at least trace amounts of silver. In the case of *A. domesticus*, the levels of silver found in the highest treatment groups (125 and 625 ppm) were found to be significantly higher than the levels of silver in the control and 1 ppm treatment groups ($p<0.05$). In the case of *T. molitor*, levels of silver in insects from the 625 ppm treatment group were found to be significantly higher than the levels of silver in insects exposed to 1 ppm Ag NPs in soil ($p<0.05$).

Past studies have found insects to uptake bulk metals from soil [42-44]. In the present study, an uptake of silver from Ag NPs as a function of concentration of Ag NPs in soil was observed. This result is in agreement with a previous study that has investigated the uptake of silver from Ag NPs in sandy loam soil. An analysis of the characteristics of soil used in this study has determined that the soil is sandy loam in nature. The low organic matter content, low organic carbon content, low clay content and low CEC of sandy loam soil usually result in more Ag ions being available for uptake. A similar result was observed in another study that has dealt with the uptake of silver ions from Ag NPs by earthworms [20].

The uptake of silver ions from Ag NPs can occur via ingestion or via endocytosis. Either one of these processes could be responsible for the uptake of Ag NPs in *A. domesticus* and *T. molitor*. Nanoparticles are also known to adhere to the cell walls or damaged cells, a phenomenon which was previously observed with quantum dots [45]. Another explanation could be the dermal exposure to silver ions by the test species. Studies have suggested that the uptake and accumulation whole particles is possible in cases of dermal exposure to nanoparticles [20].

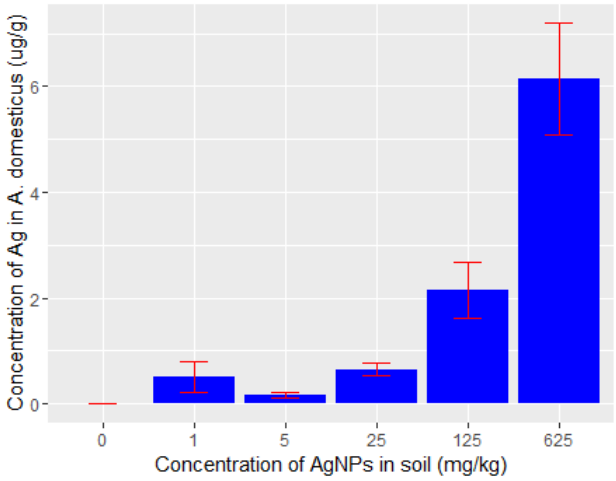

FIG. 4: UPTAKE OF Ag FROM Ag NPS IN SOIL BY *A. DOMESTICUS* (n=2).

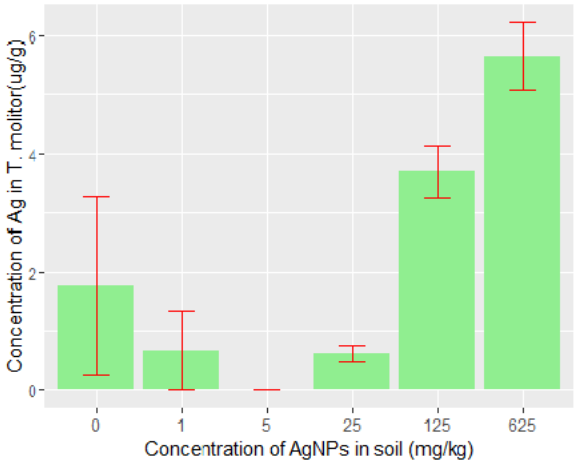

FIG. 5: UPTAKE OF Ag FROM Ag NPS IN SOIL BY *A. MOLITOR* (n=2).

3.7 Uptake of Silver from Ag NPs in Soil by Plants

All *S. vulgare* root samples were found to contain at least trace amounts of silver, regardless of treatment group. No other plant tissue samples were found to contain silver except the leaf samples from the 625 ppm treatment group, which contained trace amounts. An increase in root concentrations of Ag was observed as a function of increasing concentrations of Ag NPs in soil (Fig. 6).

The *H. annuus* control samples were found to contain silver concentrations below detection limits, except one stem sample which was found to contain trace amounts of silver. Such an observation could be likely due to contamination and sample preparation and processing errors. The 1 ppm treatment group was found to contain no silver. All of the root and leaf samples of the 5, 25, 125, and 625 ppm treatment groups were found to contain at least trace amounts of silver. The stem samples of the highest treatment groups (125 and 625 ppm) were also found to contain at least trace amounts of silver. The root samples showed an overall trend of increasing concentrations of silver as a function of increasing concentrations of Ag NPs in soil (Figure 7).

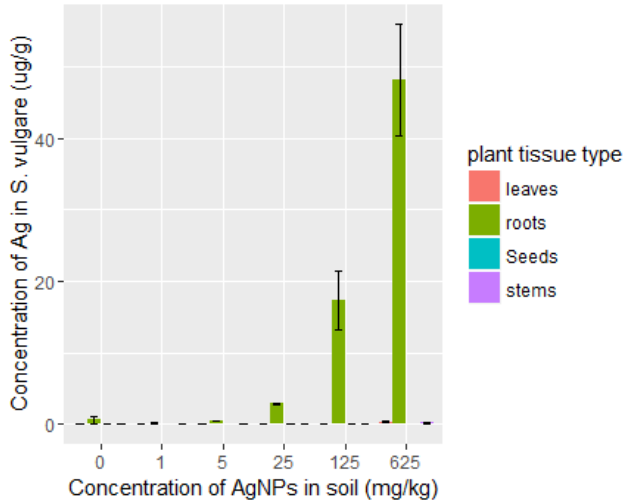

FIG. 6: UPTAKE OF Ag FROM Ag NPs IN SOIL BY *S. VULGARE* (n=2).

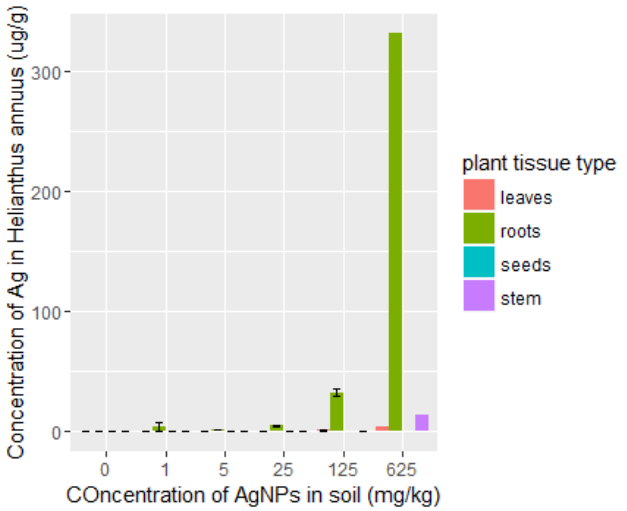

FIG. 7: UPTAKE OF Ag FROM Ag NPs IN SOIL BY *H. ANNUUS* (n=2), n = 1 FOR THE 625 PPM TREATMENT GROUP.

There were no significant differences among any of the plant tissue samples. Additionally, in both plant species, the roots were found to contain the highest concentrations of silver, followed by the leaves, the stems, and finally the seeds in the *H. annuus* tissue samples. A similar result was observed by Reddy and Dunn 1984 who have investigated the uptake of heavy metals like Cadmium, Nickle, and Chromium by soybeans grown on sludge-amended soils (Reddy and Dunn 1984). A major limitation that considerably affected the obseved inferences was the limited sample size (n =2). Such a small sample size has considerably affected the power of the statistical tools used in analysing the data.

Uptake of silver from Ag NPs in soil by the plants used in the study is observed at the highest soil treatment groups. The *H. annuus* samples were found to take up more than the *S. vulgare*, although these amounts were not found to be significantly higher. These results were not unexpected as dicots are better able to take up metals from soil than monocots due to the differences in root exudates [4 6] . The root exudates of dicots contain more organic acids than monocots. These organic acids include citric acid, maleic acid, ascorbic acid, and oxalic acid [47, 48]. The presence of organic acids in root exudates lowers the pH in the vicinity of the roots thereby solubilizing metals and enabling their uptake [49, 25, 50]. However, the uptake and accumulation of silver by *S. vulgare*, a monocot plant, may be explained by its root morphology. The presence of thin and numerous roots in monocots present a high surface area for nanoparticles that facilitates penetration and accumulation into the system [51]. Additionally, recent studies have suggested that the presence of elevated levels of chlorine (Cl) in soil, due to its salinity or when irrigated with water containing high amounts of Cl, may potentially increase the bioavailability and subsequent uptake of silver. This phenomenon could be possible explained due to the formation of $AgCl_x$ complexes in soluble or colloidal forms at elevated Cl concentrations. High Cl concentrations result in an increase in the mass transport of Ag and labile Ag, eventually resulting in the uptake of silver by plants [52].

Additionally, *H. annuus* was also found to translocate silver to the shoot portion of the plant whereas, *S. vulgare* did not. It has long been established that some plants are able to uptake metals from soil via the roots and distribute them throughout the shoot system [53, 23, 8, 54]. A possible explanation for the differences in translocation may be due to the differences in root exudates. If dicots are better at dissolving metals, a higher concentration of silver ions would be present around the roots; the ions would be able to travel further into a plant than a silver nanoparticle. Ions would also be able to diffuse across a membrane or be actively transported across the lipid bilayer through transport proteins [55] whereas solid nanoparticles would not.

IV. CONCLUSIONS

An uptake of silver from Ag NPs in soil by both insects and plants was observed at high concentrations (125 and 625 ppm). Both species of plants were found to accumulate silver in the roots. Not surprisingly, the dicot species were found to translocate more silver to the shoot system than the monocot species. Sewage sludge samples have been found to contain levels of silver as high as 960 ppm. Therefore, the possibility of bioaccumulation of silver and its eventual entry into the food chain of insectivorous and granivorous birds cannot be discounted.

ACKNOWLEDGEMENTS

The authors would like to thank Sematech, TX for their financial contribution to this project. We also would like to thank Dr. Melanie A Barnes, Department of Geosciences, Texas Tech University, Ms. Roya Baghi, Department of Chemistry & Biochemistry, Texas Tech University, and Michael T Abel, Trace Analysis, Inc., Lubbock, Texas, for all their valuable expertise, time and help with this project. Finally, we would like to extend our appreciation to the prospective Editors and Reviewers.

REFERENCES

[1] The Project on Emerging Nanotechnologies. Available from: www.nanotechproject.org [Accesesed 01 August 2016].

[2] Global Silver Nanoparticles Market By Application (Electronics & Electrical, Healthcare, Food & Beverages, Textiles) Is Expected To Reach USD 2.54 Billion By 2022. Available from: https://www.grandviewresearch.com/press-release/global-silver-nanoparticles-market [Accessed 01 August 2016].

[3] Nowack B, Bucheli TD. Occurrence, Behavior and Effects of Nanoparticles in the Environment. Environ Pollut. 2007; 150:5–22.

[4] Cornelis G, Kirby JK, Beak B, Chittleborough D, McLaughlin MJ. A Method for Determination of Retention of Silver and Cerium Oxide Manufactured Nanoparticles in Soils. Environ Chem. 2010; 7:298–308.

[5] Kim B, Park C-S, Murayama M, Hochella Jr MF. Discovery and Characterization of Silver Sulfide Nanoparticles in Final Sewage Sludge Products. Environ Sci Technol. 2010; 44: 7509–14.

[6] Unrine JM, Hunyadi SE, Tsyusko OV, Rao W, Shoults-Wilson WA, Bertsch PM. Evidence for Bioavailability of Au Nanoparticles from Soil and Biodistribution within Earthworms (Eisenia Fetida). Environ Sci Technol. 2010; 44:8308–8313.

[7] Oromieh, A. G., 2011. *Evaluating Solubility, Aggregation and Sorption of Nanosilver Particles and Silver Ions in Soils*. Thesis (MS). Department of Soil and Environment, Swedish University of Agricultural Sciences.

[8] Miralles P, Tamara L C, Andrew TH. Toxicity, Uptake, and Translocation of Engineered Nanomaterials in Vascular Plants. Environ Sci Technol. 2012; 46: 9224–39.

[9] Pan B, Xing B. Applications and Implications of Manufactured Nanoparticles in Soils: A Review. Eur J Soil Sci. 2012; 63:437–456.

[10] Tourinho PS, Van Gestel CA, Lofts S, Svendsen C, Soares AM, Loureiro S. Metal- based Nanoparticles in Soil: Fate, Behavior, and Effects on Soil Invertebrates. Environ Toxicol Chem. 2012; 31:1679–1692.

[11] Unrine JM, Shoults-Wilson WA, Zhurbich O, Bertsch PM, Tsyusko OV. Trophic Transfer of Au Nanoparticles from Soil along a Simulated Terrestrial Food Chain. Environ Sci Technol. 2012; 46:9753–9760.

[12] VandeVoort AR, Arai Y, Sparks DL (2012) Environmental Chemistry of Silver in Soils: Current and Historic Persective. *Advances in Agronomy* 114. Academic Press: 59–90.

[13] Anjum NA, Sarvajeet SG, Armando CD, Eduarda P, Iqbal A. Silver Nanoparticles in Soil–plant Systems. J Nanopart Res. 2013; 15:1–26.

[14] Hou W-Che, Westerhoff P, Posner JD. Biological Accumulation of Engineered Nanomaterials: A Review of Current Knowledge. Environ Sci.: Processes & Impacts. 2013; 15:103–22.

[15] Kaegi R, Voegelin A, Ort C, Sinnet B, Thalmann B,Krismer J, Hagendorfer H, Elumelu M, Mueller E. Fate and Transformation of Silver Nanoparticles in Urban Wastewater Systems. Water Res. 2013; 47:3866–3877.

[16] Reidy B, Haase A, Luch A, Dawson KA, Lynch I. Mechanisms of Silver Nanoparticle Release, Transformation and Toxicity: A Critical Review of Current Knowledge and Recommendations for Future Studies and Applications. Mater. 2013; 6:2295–2350.

[17] Yu S-J, Yin Y-G, Liu J-F. Silver Nanoparticles in the Environment. Environ Sci.: Processes & Impacts. 2013; 15:78–92.

[18] Makama S, Piella J, Undas A, Dimmers WJ, Peters R, Puntes VF, Van De Brink NW. Properties of silver nanoparticles influencing their uptake in and toxicity to the earthworm Lumbricus rubellus folowing exposure in soil. Environ Pollut. 2016; 218:870-878.

[19] Babich H, Stotzky G, Ehrlich HL. Environmental Factors that Influence the Toxicity of Heavy Metal and Gaseous Pollutants to Microorganisms. CRC Cr Rev Microbiol. 1980; 8:99-145.

[20] Shoults-Wilson WA, Reinsch BC, Tsyusko OV, Bertsch P M , Lowry GV, Unrine JM .Role of Particle Size and Soil Type in Toxicity of Silver Nanoparticles to Earthworms. Soil Sci Soc Am J. 2011; 75:365–377.

[21] Unrine JM, Tsyusko OV, Hunyadi SE, Judy JD, Bertsch PM. Effects of Particle Size on Chemical Speciation and Bioavailability of Copper to Earthworms (Eisenia Fetida) Exposed to Copper Nanoparticles. J Environ Qual. 2010; 39:1942–1953.

[22] Zhu H, Han J, Xiao JQ, Jin Y. Uptake, Translocation, and Accumulation of Manufactured Iron Oxide Nanoparticles by Pumpkin Plants. J Environ Monitor. 2008; 10:7

[23] Nair R, Varghese SH, Nair BG, Maekawa T, Yoshida Y, Kumar DS. Nanoparticulate Material Delivery to Plants. Plant Sci. 2010; 179:154–63.

[24] Rico CM, Majumdar S, Duarte-Gardea M, Peralta- Videa JR, Gardea-Torresdey JL. Interaction of Nanoparticles with Edible Plants and Their Possible Implications in the Food Chain. J Agr Food Chem. 2011; 59:3485–3498.

[25] Yin L, Cheng Y, Espinasse B, Colman BP, Auffan M, Wiesner M, Rose J, Liu J, Bernhardt ES. More than the Ions: The Effects of Silver Nanoparticles on Lolium Multiflorum. Environ Sci Technol. 2011; 45:2360–2367.

[26] Lee W-M, Kwak J, An Y-J. Effect of Silver Nanoparticles in Crop Plants Phaseolus radiatus and Sorghum bicolor: Media Effect on Phytotoxicity. Chemosphere. 2012; 86:491–499.

[27] Song U, Jun H, Waldman B, Roh J, Kim Y, Yi J, Lee EJ. Functional Analyses of Nanoparticle Toxicity: A Comparative Study of the Effects of TiO_2 and Ag on tomatoes (Lycopersicon esculentum). Ecotox Environ Safe. 2013; 93:60–67.

[28] Powers CM, Badireddy AR, Ryde IT, Seidler FJ, Slotkin TA. Silver nanoparticles compromise neurodevelopment in PC12 cells: critical contributions of silver Ion, particle size, coating, and composition. Environ Health Persp. 2011; 119:37–44.

[29] Romih T, Drasler B, Jemec A, Drobne B, Novak S, Golobic M, Makovec D, Susic R, Kogej K. Bioavailability of cobalt and iron from citric-acid-adsorbed $CoFe_2O_4$ nanoparticles in the terrestrial isopod *Porcellio scaber* Sci Total Environ. 2015; 508:76-84.

[30] Tourinho PS, Van Gestel CAM, Jurkschat K, Soares AM, Loureino S. Effects of soil and dietary exposure to Ag nanoparticles and $AgNO_3$ in the terrestrial isopod *Porcellionides pruinosus*. Environ Pollut. 2015; 205:170-177.

[31] Tourinho PS, Van Gestel CAM, Morgan AJ, Kille P, Svendsen C, Jurkschat K, Mosselmans JFW, Soares AMVM, Loureiro S. Toxicokinetics of Ag in the terrestrial isopod *Porcellionides pruinosus* exposed to Ag NPs and $AgNO_3$ via soil and food. Ecotoxicology. 2016; 25:267-278.

[32] Vijver MG, Wolterbeek HT, Vink JP, Van Gestel CAM (2005) Surface adsoprtion of metals onto the eartheorm *Lumbricus rubellus* and the isopod *Porcellio scaber* is negligible compared to absorption on the body. Sci Total Environ 340:271-280.

[33] Dauwe T, Janssens E, Bervoets L, Blust R, Eens M. Relationships betwee metal concentration in great tit nestlings and their environment and food. Environ Pollut. 20014; 131:373-380.

[34] Winter S, Streit B. Organichlorine compounds in a three-step terrestrial food chain. Chemosphere. 1992; 24:1765-1774.

[35] Shuman TW, Robel RJ, Zimmerman JL, Kemp KE. Variance in Digestve Efficinces of Four Sympatric Avian Granivores. The Auk. 1989; 106:324-326.

[36] Rstudio Team (2015). Rstudio: Integrated Development for R. Rstudio Inc., Boston, MA. http://www.rstudio.com/.

[37] McKillup, S., 2011. Statistics Explained: An Introductory Guide for Life Scientists. Cambridge University Press.

[38] Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). Biometrika. 1965; 52:591-611.

[39] Levard C, Hotze EM, Lowry GV, Brown Jr GE. Environmental Transformations of Silver Nanoparticles: Impact on Stability and Toxicity. Environ Sci Technol. 2012; 46:6900–6914.

[40] Whitley AR, Levard C, Oostveen E, Bertsch PM, Matocha CJ, Kammer FVD, Unrine JM. Behavior of Ag Nanoparticles in Soil: Effects of Particle Surface Coating, Aging and Sewage Sludge Amendment. Environ Pollut. 2013; 182:141–149.

[41] Dewil R, Baeyens J, Roels J, De Steene BV. Environ Eng Sci. 2008; 25: 879-886.

[42] Migliorini M, Pigino G, Bianchi N, Bernini F, Leonzio C. The Effects of Heavy Metal Contamination on the Soil Arthropod Community of a Shooting Range. Environ Pollut. 2004; 129: 331–340.

[43] Suthar S, Singh S. Bioconcentrations of Metals (Fe, Cu, Zn, Pb) in Earthworms (Eisenia Fetida), Inoculated in Municipal Sewage Sludge: Do Earthworms Pose a Possible Risk of Terrestrial Food Chain Contamination? Environ Toxicol. 2009; 24:25–32.

[44] Del Toro I, Floyd K, Gardea-Torresdey J, Borrok D. Heavy Metal Distribution and Bioaccumulation in Chihuahuan Desert Rough Harvester Ant (Pogonomyrmex rugosus) Populations. Environ Pollut. 2010; 158:1281–1287.

[45] Al-Salim N, Barraclough E, Burgess E, Clothier B, Deurer M, Green S, Malone L, Weir G. Quantum Dot Transport in Soil, Plants, and Insects. Sci Total Environ. 2011; 409:3237–3248.

[46] Sauerbeck DR. Plant Element and Soil Properties Governing Uptake and Availability of Heavy Metals Derived from Sewage Sludge. Water Air Soil Poll. 1991; 57:227–37.

[47] Wang H, Inukai Y, Yamauch A. Root Development and Nutrient Uptake. CRC Cr Rev Plant Sci. 2006; 25:279–301.

[48] Koo B-J, Chang AC, Crowley DE, Page AL, Taylor A. Availability and Plant Uptake of Biosolid-Borne Metals. Appl Environ Soil Sci. 2013; Article ID 892036, 10 pages.

[49] Raskin I, Nanda Kumar PBA, Dushenkov S, Salt DE. Bioconcentration of Heavy Metals by Plants. Curr Opin Biotech. 1994; 5:285–290.

Reddy MR, Dunn SJ. Accumulation of Heavy Metals by Soybean from Sludge-Amended Soil. Environ Pollut B. 1984; 7:281–295.

[50] Lee W-M, An Y-J, Yoon H, kweon H-S. Toxicity and bioavailability of copper nanoparticles to the terrestrial plants mung bean

(Phaseolus radiates) and wheat (Triticum aestivum): Plant agar test for water insoluble nanoparticles. Environ Toxicol Chem. 2008; 27:1915-1921.

[51] Wang P, Menzies NW, Dennis PG, Guo J, Forstner C, Sekine R, Lombi E, Kappen P, Bertsch PM, Kopittke PM. Silver nanoparticles entering soils via the wastewater-sludge-soil pathway pose low risk to plants but elevated Cl concentrations increase Ag bioavailability. Environ SciTechnol. 2016; 50:8274-8281.

[52] Ma X, Geiser-Lee J, Deng Y, Kolmakov A. Interactions between Engineered Nanoparticles (ENPs) and Plants: Phytotoxicity, Uptake and Accumulation. *S*ci Total Environ. 2010; 408:3053–3061.

[53] Zhao L, Peralta-Videa JR, Ren M, Varela-Ramirez A, Li C, Hernandez-Viezcas JA, Aguilera RJ, Gardea-Torresdey JL. Transport of Zn in a Sandy Loam Soil Treated with ZnO NPs and Uptake by Corn Plants: Electron Microprobe and Confocal Microscopy Studies. Chem Eng J. 2012; 184:1–8.

[54] Campbell NA, and J. B. Reece. 2005. "Biology. 7th." *Ed Pearson Benjamin Cummings. Cape Town* (2005).