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

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Abstract— *The uptake of silver from silver nanoparticles in soil was investigated in the presence of increasing concentrations of soil organic matter. Especially, the effect of Humus component of soil organic matter on the uptake of Ag from silver nanoparticles was studied. Two insect species, Acheta domesticus and Tenebrio molitor, and two plant species, Helianthus annuus and Sorghum vulgare, were exposed to silver nanoparticles(25 ppm in the presence of increasing concentrations of Humus (0, 1, 5, 10, 15, and 20% Humus) in soil (by weight). The techniques of transmission electron microscopy, dynamic light scattering, and powder X-ray diffraction were used to characterize the silver nanoparticles used in the study. An inductively coupled plasma-optical emission spectrometer was used to measure the levels of silver in test samples. Increasing concentrations of Humus in soil has resulted in an increase in the sulfur content and cation exchange capacity of the soil. A general decrease in the concentrations of silver was observed in Acheta domesticus and both the plant species, as a function of increasing concentrations of Humus in soil. In the case of plant species, the accumulation of silver nanoparticles was predominantly observed in the root tissue. Additionally, the translocation of silver from the roots to other plant tissues was observed in the case of Helianthus annuus. Results from this study suggest that the presence of Humus in soil could possibly decrease the uptake of silver from silver nanoparticles by insect and plant species.*

Keywords— ***Silver nanoparticles, Acheta domesticus, Tenebrio molitor, Helianthus annus, Sorghum vulgare, inductively coupled plasma-optical emission spectrometer, Soil organic matter, Humus.***

I. INTRODUCTION

Silver nanoparticles (Ag NPs) are among the most commonly used metal nanoparticles today with an estimated global market projected to be worth USD 2.45 billion by 2022 (https://www.grandviewresearch.com/press-release/global-silver-nanoparticles-market) [1]. The range of applications of Ag NPs includes consumer products such as detergents, textiles, home appliances, nutritional supplements, etc (www.nanotechproject.org) [2]. Such widespread use of Ag NPs may inadvertently facilitate their entry into various ecosystems. Predominantly, Ag NPs often find their way in to terrestrial ecosystems through the application of sewage sludge to land [3-5].

Metals in soil are found to be present in eight different fractions: 1) free metal cations; 2) inorganic complexes; 3) organo-metal complexes; 4) organo-complex chelates; 5) in association with high molecular weight organic materials; 6) bound as diverse colloids; 7) adsorbed to colloids and 8) within the soil particles [6]. Spurgeon and Hopkin 1996 [7] observed that metals in the first four fractions remain in soil solution and subsequent uptake [7]. The fate and toxicity of metal nanoparticles in soil is governed by the physicochemical properties of both the metals and soil. The size and shape of nanoparticles, the aqueous solubility and acid-base character of nanoparticles, and the presence of any surface coatings on nanoparticles are some of the factors that influence their fate in a terrestrial system [8]. On the other hand, the many different properties of soil such as pH, texture of the soil, cation exchange capacity and the soil organic matter (SOM) govern the mobility, bioavailability and toxicity of metals in a terrestrial system [9,10].

Decomposing plant material predominantly serves as a source of organic matter in soil [11]. SOM is composed of humic substances (often referred to as humus) and non-humic substances. Humus is comprised of humic and fulvic acid (Foth 1978, cited in [12]). SOM plays a key role in determining the mobility, fate and toxicity of metals in terrestrial systems [11,12]. Complexation and adsorption are the phenomenon involved in the retention of metals by SOM [13]. Additionally, SOM influences many difference functional properties of soil that include: 1) water holding capacity; 2) aggregate stability; 3) compaction characteristics and friability; 4) soil erodibility; 5) nutrient cycling; 6) buffering capacity to acidification and 7) cation exchange capacity[14] . SOM and clay content of soil play a major role in decreasing the availability and subsequent uptake of metals because of the negative charges and these negative charges are observed to be temporary in the case of SOM (Wild 1993 cited in [7]). The negatively charged functional groups in SOM include phenols, carboxyl(-ate), and amino groups. It was also observed that the negative functional groups increase in number during the process of humification of organic matter (Foth 1978, cited in [12]). However, SOM is also known to chelate metals [15], a phenomenon that may enhance their availability and uptake in soil.

This study attempted to measure the effects of Humus on the uptake of Ag from Ag NPs in soil by insect and plant species in a terrestrial system. The chelating ability of SOM may increase the bioavailability to plants; conversely, the increased S content and CEC could decrease the overall bioavailability.

The interaction between nanoparticles and SOM was thoroughly reviewed by Grillo et al. 2015 [16]. The present study investigated the uptake of Ag from Ag NPs in a terrestrial system using two insect and two plant species. The insect species used in the study include *Acheta domesticus* and *Tenebrio molitor*. The plant species employed in the study include *Helianthus annuus* (a dicot plant) and *Sorghum vulgare* (a monocot plant). All insect and plant species employed in the study are native to the region where the soil was collected. Insects serve a crucial role in the food chain of insectivorous birds, especially during the breeding season [17,18]. Similarly, plants serve as an important food source for grainivorous birds [19]. Results from this study would enable the understanding of the uptake of Ag from Ag NPs in soil by the insect and plant species under consideration and their subsequent role in bioaccumulation and bioconcentration of Ag NPs.

II. MATERIAL AND METHOD

2.1 Soil collection and preparation

All soil used during the insect and a plant exposure experiment was collected from the Colorado City, Texas at the following coordinates: Universal Transverse Mercator (UTM) 14 S 0319752 mE 3557792 mN. The soil was collected from the top 10 cm in to clean plastic containers and and transported back to The Institute of Environmental and Human Health (TIEHH) at Texas Tech University (TTU) in Lubbock, TX. The soil was then processed for homogeneity. Large rocks, roots, living organisms and other organic matter was removed, and large clumps of soil was crushed. This was followed by sifting the soil through a 2 mm wire screen into another clean plastic storage container. All processed soil was covered and stored indoors until further analysis.

2.2 Analysis of soil properties

The analysis of soil properties was performed at the Midwest Laboratories Inc. (Omaha, NE). The soil was characterized by evaluating the 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 S content. The effect of increasing concentrations of humic acid on some important properties of soil was also investigated.

2.3 Characterization of Ag NPs

Ag NPs (30-50 nm) containing $\geq$ 99.99% Ag were purchased from the US Research Nanomaterials, Inc. (Houston, TX). Additionally, the Ag NPs were characterized by studying their size, shape and composition.

The size and shape of Ag NPs was confirmed using transmission electron microscopy (TEM) was used. Sample preparation included dispersing the Ag NP powder in ethanol (EtOH). The mixture was sonicated for 10 minutes before being drop cast onto a carbon coated copper grid. Samples were air dried and analyzed on a Hitachi H-8100 TEM operated at 200 kV using a tungsten filament side-mounted camera.

The size of Ag NPs was also confirmed using the technique of Dynamic light scattering (DLS). 10 ml of reagent grade acetone (Fisher Chemical) was added to 10 mg of Ag NP powder and the mixture was sonicated until Ag NPs remained suspended in solution. Samples were analyzed using a Nanotrac NPA252 Combination (Microtrac Inc. Montgomery, PA) and Mictrotrac Flex Software (Version: 10.3.14).

The composition of Ag NPs was confirmed using the technique of Powder x-ray diffraction (PXRD). A Rigaku Ultima III X-Ray Diffractometer was used and samples were analyzed using a Cu Kα radiation as x-ray source. Parallel-beam geometry with a step width of 0.03° and a count time of one second was used. The divergence, scattering, and receiving slits were set at one. The diffraction patterns were compared and matched to the phases in the International Center for Diffraction Data (ICDD) powder diffraction file (PDF) database.

2.4 Exposure of insects to increasing concentrations of Humus in soil

37.8 L terrariums were used to conduct the exposure studies of *T. molitor* and *A. domesticus* to increasing concentrations of Humus in soil. The treatment groups include concentrations of 0 (control), 1, 5, 10, 15 and 20% of Hapi-Gro Peat Humus (by weight) in 2.5 kg of soil. The soil was then spiked with 62.5 mg of Ag NPs to obtain a final concentration of 25 ppm Ag NPs in soil. Either 300 crickets or 400 large mealworms were added to each treated soil groups to conduct the exposure studies. The exposure study was carried out over a period of 28 days and all insects were provided with fresh food and water for the entire duration of study. After 28 days, insects were removed from individual terratiums in to glass jars. The jars were placed in a -80°C freezer to ensure all insects are deceased. The insects were then freeze dried (FreeZone 2.5 Liter Freeze Dry System, Labconco, and 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 further analysis.

2.5 Exposure of plants to increasing concentrations of Humus in soil

The plant exposure studies were performed in commercially available plastic nursery containers filled with approximately two inches of pond pebbles to aid in adequate drainage. The preparation of soil with increasing concentrations of Humus and the subsequent spiking of soil was performed in a similar fashion to that of the insect exposure studies. Seeds for two plant species, *H. annuus* and *S. vulgare* were planted into the prepared nursery containers and were 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 *S. vulgare*. While in the greenhouse, plants received shaded sunlight and were maintained at 60°F or above. 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 samples were stored in a freezer until further analysis.

2.6 Sample Digestions

A modified version of EPA Method 3050B was used to perform all sample digestions. No hydrochloric acid was used during the process of sample digestions in order to prevent the formation of silver chloride.

2.7 Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) Analysis

All samples were analyzed using a Teledyne Instruments (Hudson, New Hampshire) Prodigy High Dispersion ICP-OES. The samples were analyzed for silver at three different wavelengths: 224.643, 328.068, and 338.289 nm. Ultimately, the data from 338.289 was chosen for statistical analysis.

2.8 Statistical Analysis

All statistical analyses was performed using MINITAB 17 software [20]. All samples were analyzed in relicates (n=2). A two-way ANOVA was performed to analyze the data for effect of insect species and percent humus content on the uptake of AgNPs. A multi- way ANOVA was performed to examine the effect of plant type, percent humus, and plant tissues on the uptake of Ag NPs. The post analysis comparison was performed with Tukey test. Any statistical significance was established at an alpha level of 5% (p=0.05).

III. RESULTS AND DISCUSSION

3.1 Soil Characterization

The control soil was found to be sandy loam in nature, with 54% sand, 36% silt, and 10% clay. Additional analyses revealed that the soil contains 0.01% humic matter, 1.7% organic matters, and 9 ppm S. The pH of the control soil was slightly basic (8.1 pH units) and the CEC of the soil was calculated to be 18.0 meq/100g.

The effect of increasing concentrations of humus in soil on the various properties of soil is summarized in TABLE 1. As is evident from TABLE 1, increasing concentrations of humus in soil has resulted in an increase in the humic matter (%), sulfur content (ppm), CEC (meq/100g), and the organic matter (%) of soil.

TABLE 1
EFFECT OF INCREASING CONCENTRATIONS OF HUMUS ON PROPERTIES OF SOIL.

Treatment Group	Humic Matter (%)	Sulfur Content (ppm)	CEC (meq/100 g)	Organic Matter (%)
0% Humic Content	0.01	9	19.8	1.7
1% Humic Content	0.01	17	18.2	1.6
5% Humic Content	0.03	36	19.0	1.9
10% Humic Content	0.07	82	21.4	2.5
15% Humic Content	0.07	101	20.7	2.6
20% Humic Content	0.11	127	23.1	2.9

3.2 Transmission electron microscopy analysis

The 30-50 nm uncoated silver nanoparticles were found to be heavily aggregated after being dispersed in EtOH. However, the TEM was able to confirm the spherical shape of the nanoparticles (Figure 1). The average particle size of Ag NPs was found to be 30-50 nm, with outliers on either side of the range.

3.3 Dynamic light scattering analysis

The size of Ag NPs used in the study is confirmed by DLS. It was observed that approximately 95% of the 30-50 nm Ag NPs had a size between 30.70 to 52.90 nm (Figure 2). The average size of Ag NPs used in the present study was found to be 41.80 nm.

3.4 Powder X-Ray Diffraction analysis

The composition of Ag NPs used in the present study is confirmed using the technique of Powder X-ray diffraction (Figure 3). The diffraction patterns of Ag NPs obtained matched both those in the ICDD and those provided by the manufacturer.

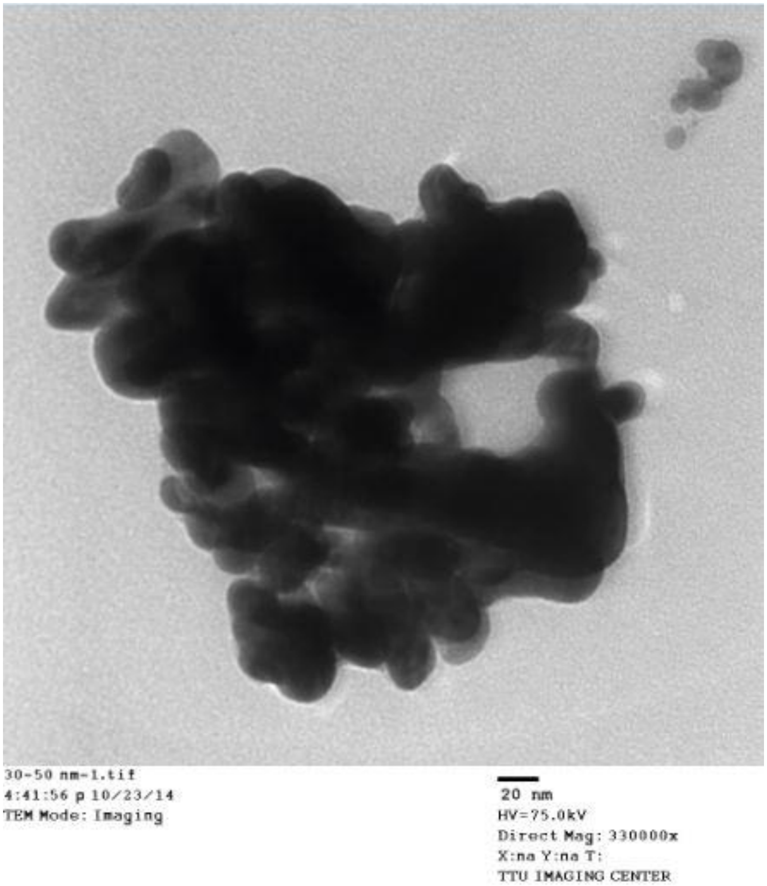


FIGURE 1: REPRESENTATIVE TRANSMISSION ELECTRON MICROSCOPY IMAGE OF 30-50 nm Ag NPs USED IN THE STUDY.

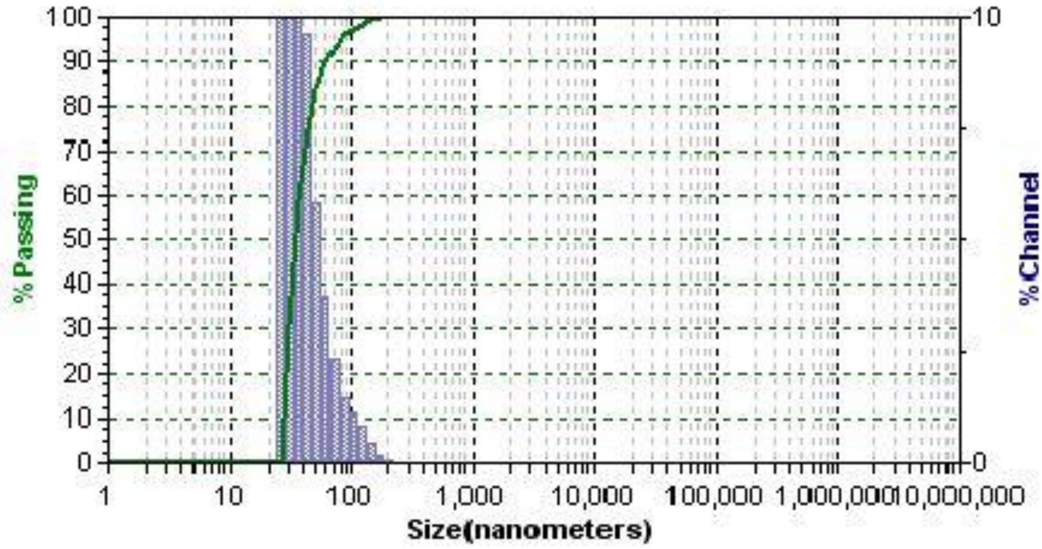
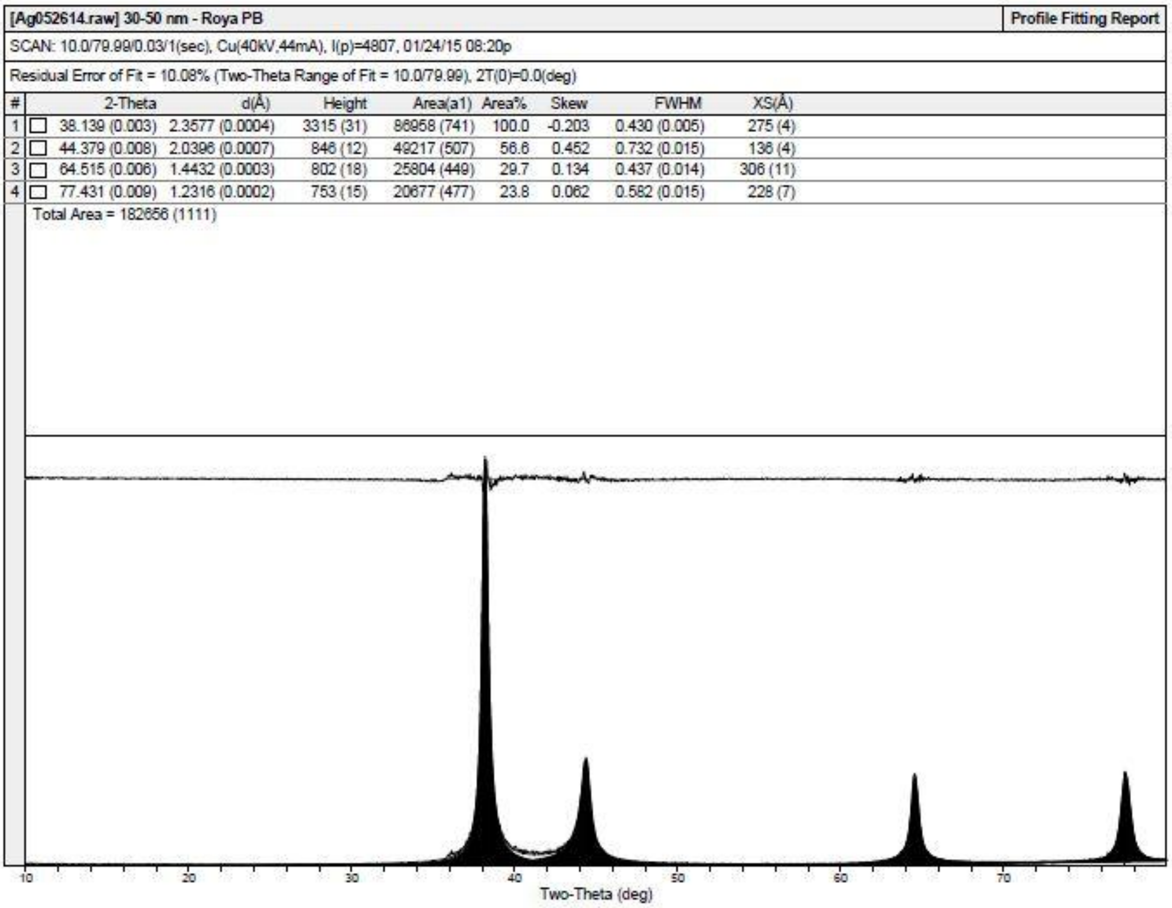


FIGURE 2: REPRESENTATIVE SIZE DISTRIBUTION OF 30-50 nm Ag NPs USED IN THE PRESENT STUDY OBTAINED USING DYNAMIC LIGHT SCATTERING.



#	2-Theta	d(Å)	Height	Area(a1)	Area%	Skew	FWHM	XS(Å)
1	38.139 (0.003)	2.3577 (0.0004)	3315 (31)	86958 (741)	100.0	-0.203	0.430 (0.005)	275 (4)
2	44.379 (0.008)	2.0396 (0.0007)	846 (12)	49217 (507)	56.6	0.452	0.732 (0.015)	136 (4)
3	64.515 (0.006)	1.4432 (0.0003)	802 (18)	25804 (449)	29.7	0.134	0.437 (0.014)	306 (11)
4	77.431 (0.009)	1.2316 (0.0002)	753 (15)	20677 (477)	23.8	0.062	0.582 (0.015)	228 (7)

FIGURE 3: REPRESENTATIVE DIFFRACTION PATTERN OF 30-50 nm Ag NPs USED IN THE PRESENT STUDY AS DETERMINED BY POWDER X-RAY DIFFRACTION.

3.5 Effect of increasing concentrations of humus on the uptake of Ag from Ag NPs in soil by insect and plant species

Figure 4 summarizes the effect of increasing concentrations of humus in soil on the uptake of Ag from Ag NPs in soil by *A. domesticus* and *T. molitor*.

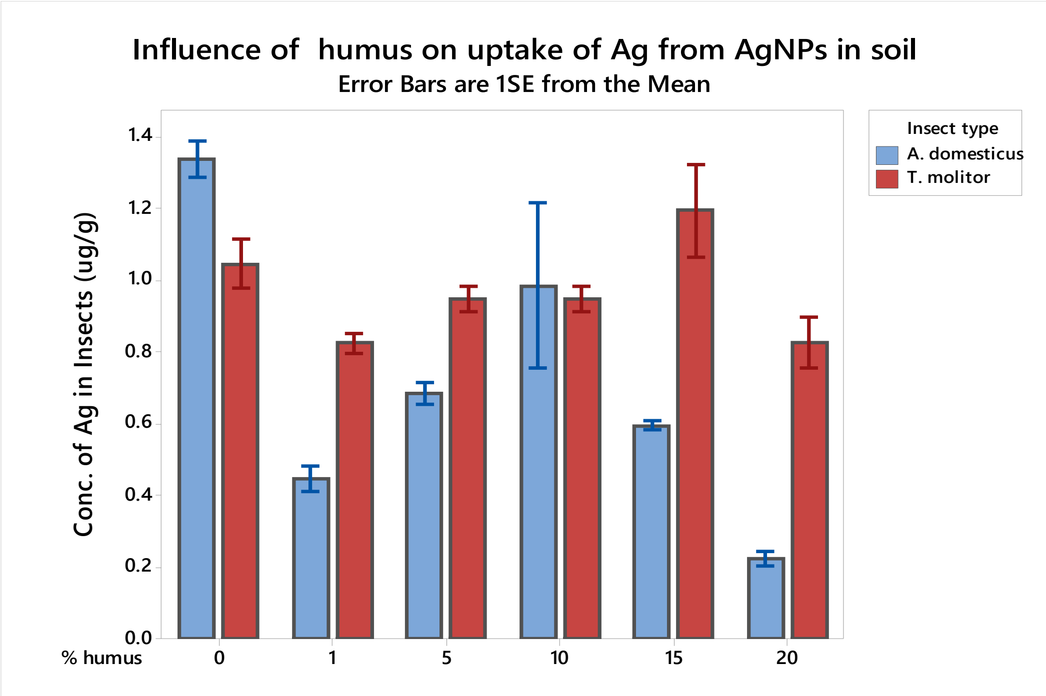


FIGURE 4: INFLUENCE OF INCREASING CONCENTRATIONS OF HUMUS ON THE UPTAKE OF Ag FROM Ag NPs IN SOIL BY INSECT SPECIES (n=2).

The control samples from the insect species were found to contain no detectable concentration of silver. No apparent trend was observed to decipher the effect of increasing concentrations of humus in soil on the uptake of Ag from Ag NPs in soil by the insect species. However, a decrease in the uptake of Ag from Ag NPs in soil was observed in the case of *A. domesticus*, especially at high concentrations of humus in soil. The uptake of Ag from Ag NPs in soil by *A. domesticus* was observed to be significantly lower when the soil had 20% humus compared to the remaining soil treatment groups. However, the overall uptake of Ag from Ag NPs in soil was found to be higher in the case of *T. molitor* when compared to *A. domesticus*.

Figures 5 and 6 summarize the effect of increasing concentrations of humus in soil on the uptake of Ag from Ag NPs in soil by H. annuus and S. vulgare, respectively.

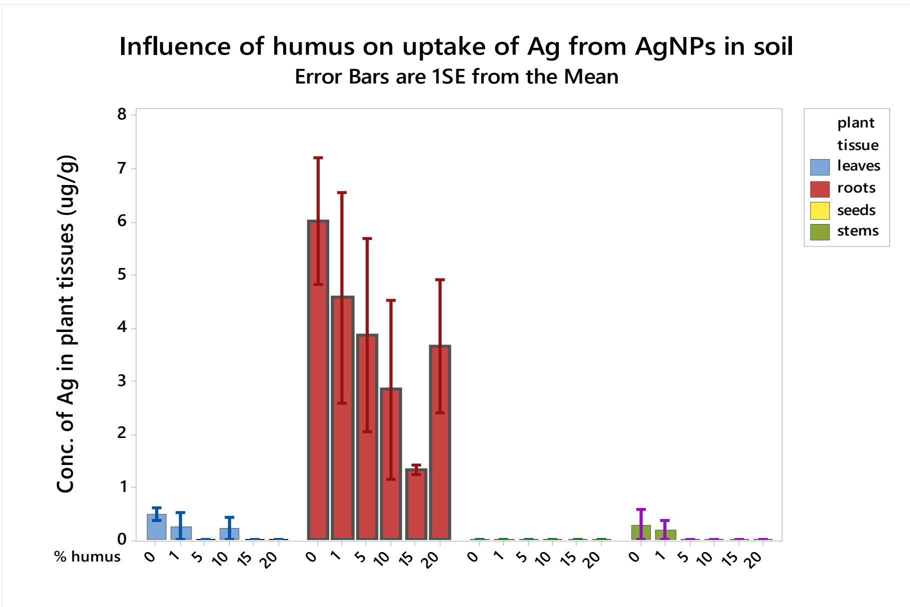


FIGURE 5: INFLUENCE OF INCREASING CONCENTRATIONS OF HUMUS ON THE UPTAKE OF Ag FROM Ag NPs IN SOIL BY *H. annuus* (n=2).

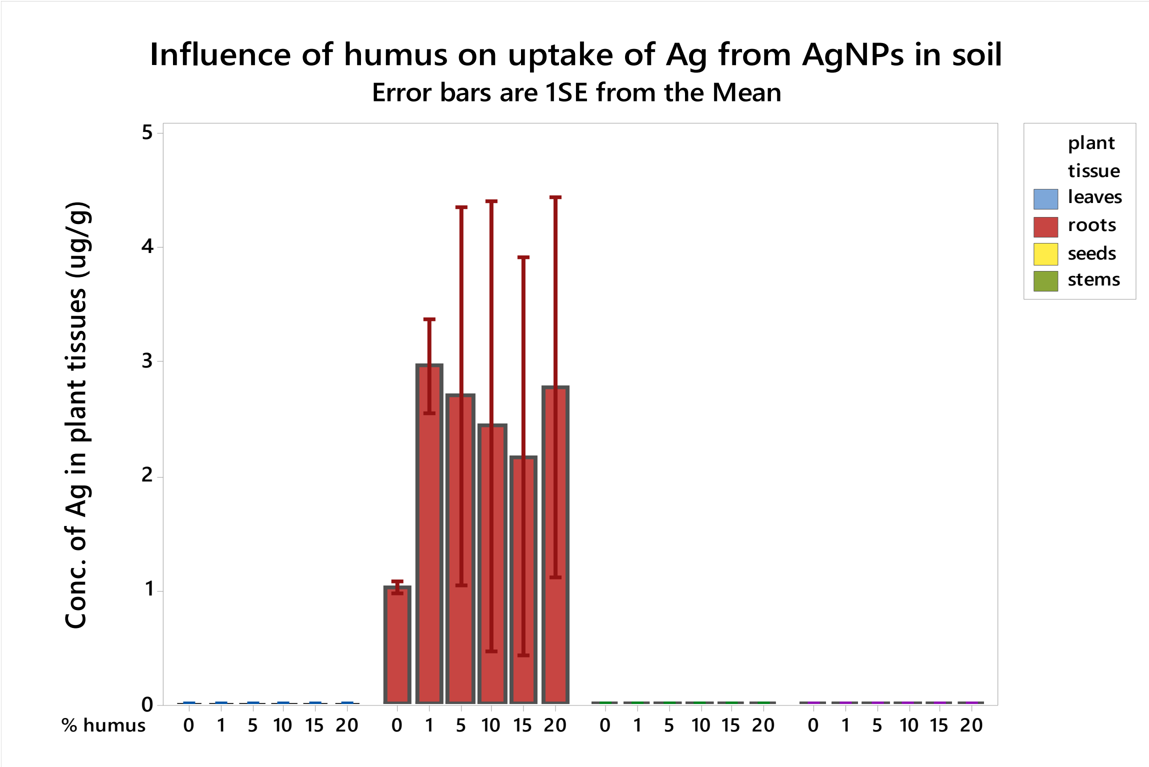


FIGURE 6: INFLUENCE OF INCREASING CONCENTRATIONS OF HUMUS ON THE UPTAKE OF Ag FROM Ag NPs IN SOIL BY *S. vulgare* (n=2).

The blank samples from both the plant species used in the study were found to have no detectable levels of silver. In the case of both the plants, the concentrations of Ag in the roots at all treatment levels was found to be significantly higher than the concentrations of Ag in the remaining plant tissues ($p<0.05$). The translocation of Ag from the roots to other plant tissues like leaves was observed in the case of *H. annuus*. Nevertheless, a general decrease in the levels of Ag in roots of both plants was observed as a function of increasing concentrations of Humus in soil.

Unintentional contamination of glassware during the process of sample digestions has compromised the use of all three replicates for statistical analyses. Hence, only two replicates per sample were used in the present study. This explains the lack of any definitive trend in the concentration of Ag in insects and plants as a function of increasing concentrations of Humus in soil. Regardless, a general decrease in the concentrations of Ag in both the plants and *A. domesticus* was observed as a function of increasing concentrations of Humus in soil. The decrease in the uptake of Ag in the presence of increasing concentrations of Humus in soil could be explained using TABLE 1. As is evident from TABLE 1, increasing the concentrations of Humus in soil has resulted in an increase in the CEC and Sulfur content of soil. CEC of soil is defined as the quantity of positively charged ions that could be help by the soil [21].It provides electrostatic binding sites for cations like silver (Ag+) thus inhibiting their availability for uptake in a terrestrial ecosystem [22]. Hence, an increase in the CEC of soil due to increasing concentrations of Humus (TABLE 1) in soil does result in a decrease in the availability of Ag for uptake by plants and insect species in a terrestrial system.

Additionally, it is also evident from TABLE 1 that increasing concentrations of Humus in soil has resulted in an increase in Sulfur content of soil. The increase in Sulfur content could be of significance especially in the case of Ag NPs. Silver has a very high affinity for S and will form Ag_2S which is highly insoluble (K_{sp}= 8.0 x 10^{-51}) . To be available to uptake by plants and insect species in a terrestrial system, Ag ions ought to be in soil solution. However, Ag_2S being highly soluble in water renders Ag unavailable for uptake [23-29]. Finally, the presence of negatively charged functional groups in SOM (Foth 1978, cited in [12]) may also decrease the availability of Ag ions in a terrestrial system

IV. CONCLUSIONS

The effect of increasing concentrations of soil organic matter (Humus) on the uptake of Ag from Ag NPs in soil by two insect and plant species was investigated. Ag NPs were thoroughly characterized using the techniques of transmission electron microscopy, dynamic light scattering and powder X-ray diffraction analyses. In general, it was observed that the presence of Humus decreases the uptake of Ag from Ag NPs in soil by insect and plant species. Hence, it can be inferred that the presence of increasing concentrations of Humus in soil decreases the bioavailability of Ag from Ag NPs in a terrestrial system.

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