# The Influence of Vermiculite 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 Vermiculite, typical 2:1 clay. Two insect species, Acheta domesticus and Tenebrio molitor, and two plant species, Helianthus annuus and Sorghum vulgare, were exposed to silver nanoparticles in the presence of increasing concentrations of Vermiculite in soil. Silver nanoparticles were characterized using techniques including transmission electron microscopy, dynamic light scattering, and powder X-ray diffraction. The levels of silver in test species exposed to silver nanoparticles were measured using an inductively coupled plasma-optical emission spectrometer. An increase in the cation exchange capacity of soil was observed with the increase in the concentration of vermiculite in soil. The results suggested a decrease in the uptake of silver from silver nanoparticles in soil by Acheta domesticus as a function of increasing concentrations of Vermiculite in soil. No apparent trend was observed in the remaining species. Both plant species were found to accumulate silver in their roots. The translocation of silver to stems and leaves was observed in the case of Helianthus annuus. Results from this study suggest that the presence of Vermiculite in soil could possibly decrease the uptake of silver from silver nanoparticles.*

***Keywords— Silver nanoparticles, Acheta domesticus, Tenebrio molitor, Helianthus annus, Sorghum vulgare, inductively coupled plasma-optical emission spectrometer, Cation exchange capacity.***

## I. INTRODUCTION

The widespread use of silver nanoparticles (Ag NPs) for a variety of applications has resulted in an increase in the concentrations of Ag NPs in terrestrial ecosystems. Typically, Ag NPs find their way into terrestrial ecosystems through the application of sewage sludge as fertilizer to land [1-3]. The environmental behavior, fate, and ecotoxicity of metal-based nanoparticles in a terrestrial ecosystem are known to be influenced by the physicochemical characteristics of both the nanoparticles themselves and the soil. Physical properties include size and shape of nanoparticles while the chemical characteristics include acid-base character, aqueous solubility and surface coatings, if any [4]. The physicochemical properties of soil can influence the mobility, bioavailability and toxicity of pollutants in a terrestrial ecosystem. These properties include pH, soil texture, organic matter, and cation exchange capacity (CEC), etc [5,6].

The texture of a soil is comprised of sand, silt, and clay. The particle size of sand, silt, and clay are > 50 μm, 2-50 μm, and < 2 μm respectively [7,8]. Clay particles belong to a group of minerals described as hydrous silicates [9]. Clay particles typically have a negative charge [10]. Additionally, clay particles considerably contribute to the CEC of soil. CEC of soil is the quantity of positively charged ions that could be held by the negatively charged surface of clay minerals [9]. The CEC of a soil provides electrostatic binding sites for cations like silver ions ($Ag^+$) thus rendering them electrostatically immobilized [11]. The negative charge of clay minerals, the high surface area of clay due to small particle size [12] and the contribution of clay minerals to the CEC of soil [9] play a key role in determining the fate of metal contaminants in soil. The four major groups of clay minerals include: 1) the kaolinite group; 2) the mont-morillonite/smectite group; 3) the illite group and 4) the chlorite group [9]. The mont-morillonite/smectite group of clay minerals are known to possess high surface area and CEC compared to the remaining three groups of clay minerals. The general formula of mont-morillonite/smectite group of clay minerals is $(Ca,Na,H)(Al,Mg,Fe,Zn)_2(Si,Al)_4O_{10}(OH)_2\text{-}XH_2O$ [9].

A member of the mont-morillonite/smectite group of clay minerals, Vermiculite, is used in the present study. The effect of increasing concentrations of Vermiculite in soil on the uptake of silver from Ag NPs in a terrestrial system was investigated. Terrestrial isopods have been used as model organisms to understand the uptake, kinetics and transformation of metal nanoparticles [13-15]. The uptake of nanoparticles in terrestrial isopods occurs exclusively through the oral route. Negligible

surface uptake of nanoparticles is observed in terrestrial isopods facilitating the uptake and transformation studies of metal nanoparticles [16]. However, it is equally important to investigate the uptake of metals from metal nanoparticles by other components of terrestrial ecosystems that serve a crucial link in the metal transport chains between trophic levels in the food web.

Two species of insects, *Acheta domesticus* and *Tenebrio molitor*, and two species of plants, *Helianthus annuus* (a dicot plant) and *Sorghum vulgare* (a monocot plant) were used. The insect and plant species were exposed to Ag NPs in soil containing a range of concentrations of Vermiculite. Insects serve an important role in the metal-transport chains between trophic levels in food webs [17]. For instance, during the breeding season, insects and larvae constitute an important food source for insectivorous birds [18]. Therefore, it is important to determine if insects are able to bioaccumulate Ag from Ag NPs in soil. As seeds are another important food source for granivorous birds [19], the possibility of translocation of Ag to plant tissues was investigated. Results from this study would help understand the role of plants in bioaccumulation of metal nanoparticles.

## II. MATERIAL AND METHOD

### 2.1 Soil collection and preparation

Soil used in the present study 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 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. The soil was processed for homogeneity at TIEHH. 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

The characterization of soil samples was performed at Midwest Laboratories Inc. (Omaha, NE). Soil samples were characterized by evaluating parameters such as 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 ($K^+$, $Mg^{2+}$, $Ca^{2+}$, $H^+$), CEC, and sulfur (S) content.

***Nanoparticle Characterization***

Uncoated silver nanoparticles (30-50 nm) were purchased from US Research Nanomaterials, Inc. (Houston, TX). All silver nanoparticles were reported by US Research Nanomaterial, Inc (www.us-nano.com) to consist of $\geq$ 99.99% Ag.

In order to confirm the size range and shape of the nanoparticles, transmission electron microscopy (TEM) was used. Samples were prepared by dispersing the Ag NP 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 size Ag NPs. The sample preparation process for DLS involved placing approximately 10 mg of Ag NP powder in 10 mL of reagent grade acetone (Fisher Scientific, MA, USA). Samples were 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).

Powder x-ray diffraction (PXRD) was used to confirm the composition of Ag NPs. A Rigaku Ultima III X-Ray Diffractometer was used for this purpose. 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.

### 2.3 Insect treatment groups

Two 37.8 L terrariums were prepared for each insect treatment group, including a control group. The clay content of soil was adjusted before the terrariums were spiked with Ag NPs. The required amount of clay (Sta-Green® vermiculite) was weighed

out to constitute 1, 5, 10, 15, and 20% of clay (by weight) in 2.5 kg soil for each treatment group. Each terrarium was spiked with 62.5 mg of uncoated 30-50 nm Ag NPs so that a final soil concentration of 25 µg/g Ag NPs was obtained.

Once the terrariums were prepared, insects were purchased from Reptilefood (reptilefoods.com, Ohio, USA). Each terrarium received either 300 small crickets or 400 large mealworms. Insects were provided with fresh food and water as needed for the duration of the 28-day exposure period. After 28 days, 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. 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 further analysis.

### 2.4 Plant treatment groups

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. The clay content in the soil was adjusted as was done with the insect treatment groups. This soil was then spiked to have a final soil concentration of 25 µg/g Ag NPs in soil. The spiked soil was transferred into the plastic nursery containers.

Seeds of each plant species were planted into the prepared nursery containers and were transported to the TTU greenhouse. The plants remained in the greenhouse until 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. To prevent the soil from drying out, plants were misted for three minutes every eight hours in a day. 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.

### 2.5 Sample digestions

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. For each sample, either plant or insect, approximately 1 grams were weighed into a 100 mL beaker. Wet weights (ww) were taken into account in the case of plants samples. 10 ml of 70% nitric acid ($HNO_3$, reagent grade) was added to each sample. This was followed by an addition of 10 ml of 30% hydrogen peroxide ($H_2O_2$, reagent grade). A solution containing 10 ml $HNO_3$ and 10 ml $H_2O_2$ was used as the reagent blank. All beakers were covered with a Teflon watch glass and placed on hot plates overnight for sample digestions. All samples were slowly heated in increments of 50 ˚C until the solutions began to reflux gently. Care was taken to ensure that none of the solutions boiled over resulting in a loss of sample. All samples were swirled periodically during the reflux process to aid in the digestion of the samples. The digestions were considered complete when the volume of samples in the beaker was reduced to approximately 5 ml. Once complete, beakers were removed from the hot plate and placed in an ice bath to cool. Cooling was followed by filtering the samples into 50 ml centrifuge tubes (Corning CentriStar $^{TM}$, Corning, NY) using ashless filter paper (Whatman No. 41, Fisher Scientific, PA). This was to ensure all remaining solids and/or digested lipids were removed from the samples. The original sample beakers were then rinsed twice with 10 ml of 5% $HNO_3$ and the rinse contents were added to the centrifuge tubes. The centrifuge tubes with sample extracts were stored at room temperature until analysis by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES).

### 2.6 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 wavelengths: 224.643, 328.068, and 338.289 nm. The three wavelengths were aligned using a 10 ppm silver standard solution (SPEX CetriPrep). The instrument was calibrated using a range of silver concentrations from 0-20 ppm.

### 2.7 Statistical Analysis

All data was compared using ANOVA with Rstudio software [20]. The Shapiro test was used to test the normality of data [21]. All statistical analysis was compared with 95 % confidence interval. Data was analyzed using a one-way ANOVA, followed by a multiple comparison test (Tukey HSD) to identify significant differences among the treatment groups ($\alpha<0.05$).

## III. RESULTS AND DISCUSSION

### 3.1 Soil characterization

The control soil was characterized as sandy loam soil as it contained 54% sand, 36% silt, and 10% clay. The soil was also found to contain 0.01% humic matter, 1.7% organic matter, and 9 ppm S. The pH of the soil was slightly basic, 8.1. The CEC of the soil was calculated to be 18.0 meq/100g. However, the CEC of soil was affected by the presence of increasing concentrations of Vermiculite in soil and is summarized in Table 1.

**TABLE 1**
**EFFECT OF VERMICULITE ON THE CEC OF SOIL**

| Treatment Group | CEC (meq/100 g) |
|---|---|
| Control | 18.0 |
| 1% Vermiculite | 20.7 |
| 5% Vermiculite | 22.4 |
| 10% Vermiculite | 25.4 |
| 15% Vermiculite | 25.1 |
| 20% Vermiculite | 26.3 |

As is evident from Table 1, an increase in CEC of soil was observed as a function of increasing concentration of Vermiculite in soil.

### 3.2 Transmission electron microscopy

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 (Fig. 1). And most of the particles were found to be within the in 30-50 nm range, although there were outliers on either side of the range.

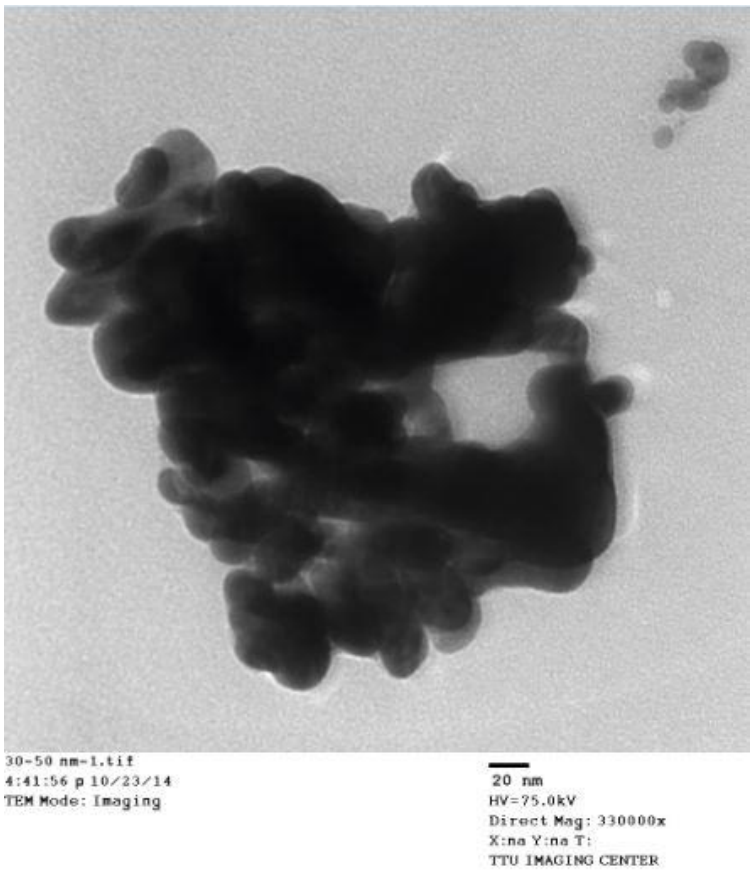


**FIG. 1: TRANSMISSION ELECTRON MICROSCOPY IMAGE FOR 30-50 nm UNCOATED SILVER NANOPARTICLES.**

### 3.3 Dynamic light scattering

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

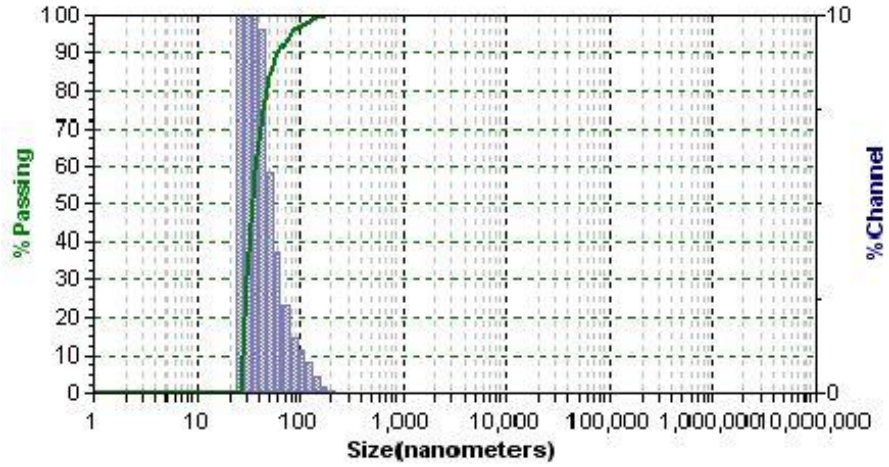


**FIG. 2: SIZE DISTRIBUTION OF 30-50 nm UNCOATED SILVER NANOPARTICLES DETERMINED BY DYNAMIC LIGHT SCATTERING**

### 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 can be seen below (Fig. 3).

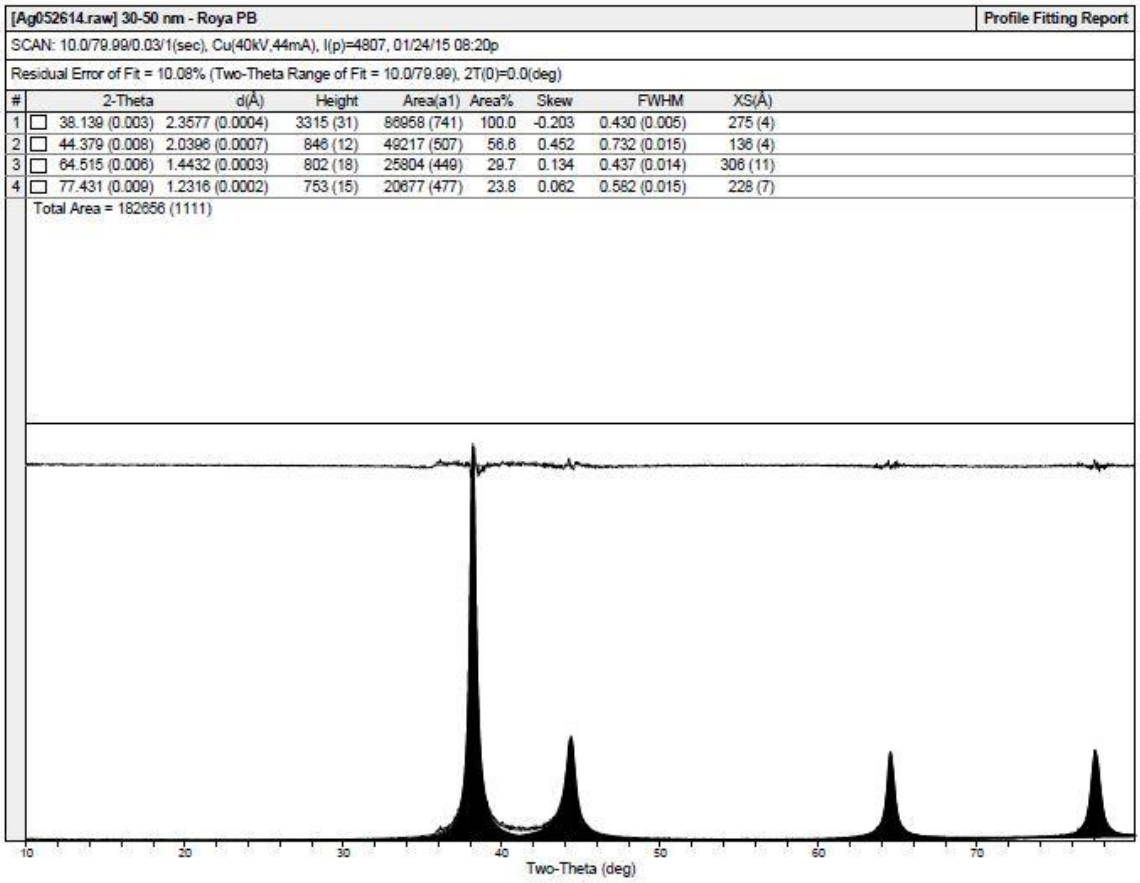


**FIG.3: DIFFRACTION PATTERN OF 30-50 nm UNCOATED SILVER NANOPARTICLES DETERMINED BY POWDER X-RAY DIFFRACTION**

### 3.5 Uptake of Ag from Ag NPs in soil by insects

Fig. 4 summarizes the results of the uptake of Ag from Ag NPs in soil by insect species in the presence of increasing concentrations of Vermiculite.

In the case of *A. domesticus*, a decrease in the uptake of Ag from Ag NPs in soil was observed as a function of increasing concentrations of Vermiculite in the soil. No uptake of Ag from Ag NPs in soil was observed at the highest concentrations of Vermiculite employed in this study, i.e. 20%. More importantly, the concentration of silver in the control group (0% Vermiculite in soil) is found to be significantly higher than the concentration of Ag in the insects from the treated group that had various concentrations of Vermiculite. No specific trend in the uptake of Ag from Ag NPs as a function of increasing concentrations of Vermiculite in soil was observed in the case of *T. molitor*. However, a decrease in the uptake of Ag from Ag NPs in soil was observed in the presence of Vermiculite. The decrease in the uptake of Ag by insects in the presence of Vermiculite could be attributed to the increase in CEC of soil due to Vermiculite [22,23].

Despite the levels of Ag found in both insect species, it is important to consider the type of soil used in the present study is Sandy loam. Some of the important characteristics of sandy loam soil include low organic matter content, low organic carbon content, low clay content and low CEC. All these properties of sandy loam soil usually facilitate the uptake of metals [6]. The discrepancies in the levels of Ag uptake observed in both the species may be attributed to the inherent differences in the ability of soil-dwelling invertebrates to uptake metals in contaminated soils. Due to the difference in habitats, diet and physiological responses, some invertebrates are known to accumulate metals preferentially [24].

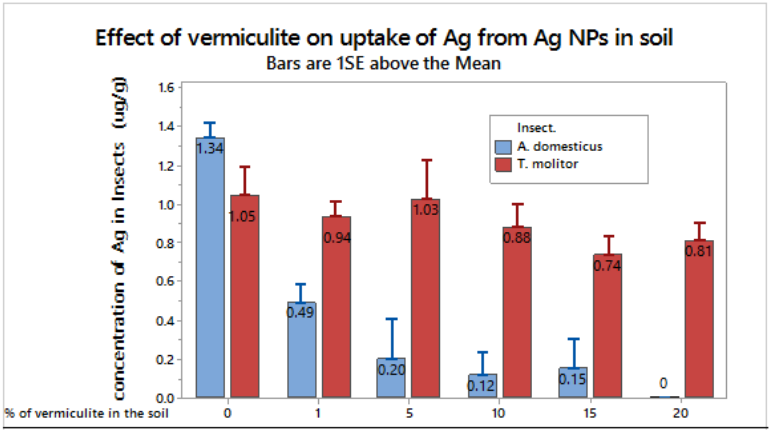


**FIG. 4: EFFECT OF VERMICULITE ON THE UPTAKE OF Ag FROM Ag NPS IN SOIL (n=2).**

### 3.6 Uptake of Ag from Ag NPs in soil by plants

Fig. 5 and 6 summarize the uptake of Ag from Ag NPs in soil by the plant species *S. vulgare* and *H. annuus*, respectively) used in the study as a function of increasing concentrations of Vermiculite.

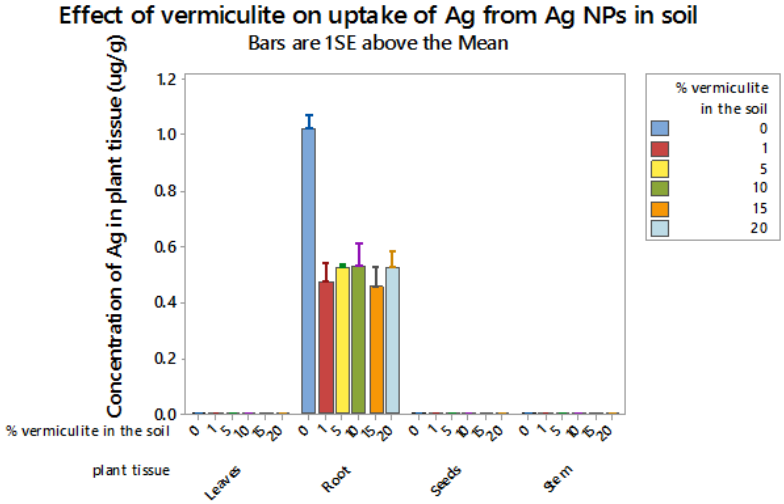


**FIG. 5: EFFECT OF VERMICULITE ON THE UPTAKE OF Ag FROM Ag NPS IN SOIL BY *S. vulgare* (n=2).**

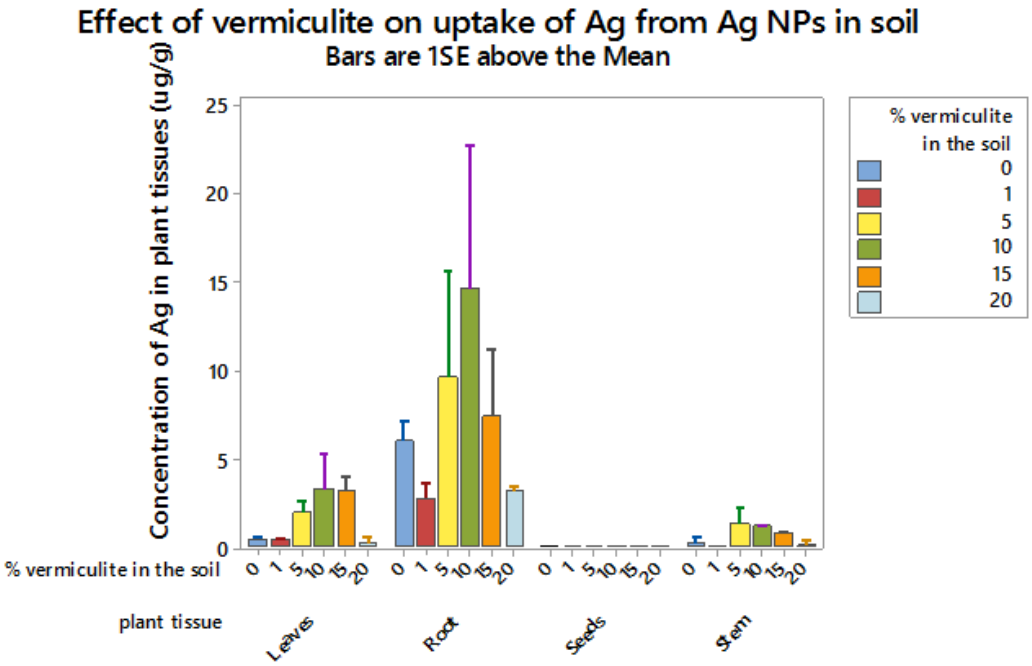


**FIG. 6: EFFECT OF VERMICULITE ON THE UPTAKE OF Ag FROM Ag NPs IN SOIL BY *H. annuus* (n=2).**

Fig. 5 suggests an accumulation of Ag in the roots of *S. vulgare*. Although no translocation of Ag to other parts of the plant tissues was observed, a decrease in the uptake of Ag from Ag NPs in soil by roots was observed in the presence of Vermiculite. This could be attributed to the increased CEC of soil in the presence of Vermiculite. The increase in CEC of soil due to the presence of Vermiculite would facilitate electrostatic binding of Ag to the soil [22,23] thereby inhibiting its uptake by the roots of *S. vulgare*.

The observed uptake of metals by monocot plants like *S. vulgare* is predominantly attributed to their root morphology. The presence of numerous thin roots in monocot plants presents a very high surface area for the penetration and accumulation of nanoparticles [25]. However, the lack of translocation of heavy metals has been observed in other monocot plants like maize, rice, and wheat [26,27,28]. This retention of metals at the roots could be partially explained by the insolubilization of metals at the root surface and in the root apoplast [26,27].

Fig. 6 suggests an accumulation of Ag in the roots of *H. annuus* and subsequent translocation of Ag to other parts of the plant tissues such as leaves and stems. The concentration of Ag in the roots were found to be significantly higher than the levels of Ag in other tissues ($p<0.05$). The translocation of heavy metals like Ag to other plant tissues occurs by the phenomenon of pholem transport. Phloem-mobile metals are known to translocate to other plant tissues like leaves, stem, seeds, etc [29].

It is also evident from Figure 6 that the roots of *H. annuus* have accumulated higher concentrations of Ag than those of *S. vulgare*. Dicot plants have been known to accumulate metals better than monocots [30]. The root exudates of dicot plants contain organic acids such as citric acid, maleic acid, ascorbic acid, and oxalic acid [31,32]. These organic acids lower the pH near the vicinity of roots thereby solubilizing metals. This results in an increased uptake of metals by dicot plants compared to monocots [33,34,35]. Additionally, the decrease in pH of the soil also decreases the CEC of soil. The decrease in CEC of soil also facilitates an increase in the uptake of Ag from Ag NPs in soil [36].

No apparent trend in the uptake of Ag form Ag NPs in soil was observed as a function of increasing concentrations of Vermiculite in soil. This could be attributed to the limited sample size (n=2) used for the study. Despite starting with three replicates for each insect and plant species used in the study, the data from only two of the replicates could be used for data analysis. Contamination from the glassware and during the process of sample digestions limited our ability to consider all three replicates for analysis.

Nevertheless, it could be inferred that the uptake of Ag from Ag NPs in soil will decrease as a function of increasing concentrations of Vermiculite in soil. Clay minerals such as Vermiculite possess permanent negative surface charges. These permanent negative charges are an inherent property due to non-stoichiometric/isomorphous substitution of cations within the structure of Vermiculite. These substitutions include $Al^{3+}$ instead of $Si^{4+}$ in the tetrahedral sheet, etc [37,38,39]. As the concentrations of Vermiculite in the soil increases, the presence of these negative charges become independent of the pH of

soils. Additionally, the phenomenon of cation adsorption in expandable clay minerals such as Vermiculite occurs predominantly in inter-layer spaces, not on the relatively inactive planar surfaces [38].

## IV. CONCLUSION

The influence of a clay mineral, Vermiculite, on the uptake of Ag from Ag NPs in soil by insect and plant species was investigated. The presence of Vermiculite resulted in an increase in the cation exchange capacity of soil. The presence of Vermiculite has also potentially resulted in a decrease in the uptake of Ag from Ag NPs in soil by insect and plant species. The increase in CEC of soil due to the presence of clay minerals, the increased surface area of clay minerals due to their small particle size, and the negative charge of clay minerals may cumulatively result in a decrease in the uptake of Ag from Ag NPs in soil. Consequently, clay minerals may be used as sorbents for heavy metals in soil thereby decreasing the possibility of their entrance into food webs.

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