# Uptake of Silver from Polyvinylpyrrolidine Coated Silver Nanoparticles in a Terrestrial System

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***Abstract—*** *The widespread use of silver nanoparticles (Ag NPs) has facilitated their uninterrupted entry into various ecosystems. Nanoparticles are stabilized using a variety of approaches for various applications. The present study has investigated the uptake of polyvinylpyrrolidine (PVP) coated Ag NPs in a terrestrial system. Two insect (Acheta domesticus and Tenebrio molitor) and two plant species (Sorghum vulgare and Helianthus annuus) were used in the study. The effect of concentration and size of PVP-coated Ag NPs was investigated. The test species were maintained in soil spiked with 0, 1, 5, 25, 125, and 625 mg/kg PVP-coated 30-50 nm Ag NPs to test the effect of concentration of Ag NPs on uptake. Similarly, the test species were maintained in soil spiked with 25 mg/kg of 20, 30-50, and 50-80 nm PVP-coated Ag NPs to study the effect of size of nanoparticles on uptake. The PVP-coated Ag NPs were characterized using transmission electron microscopy, dynamic light scattering and powder X-ray diffraction techniques. The levels of silver in test samples were measured using inductively coupled plasma-optical emission spectroscopy. A concentration dependent increase in the levels of Ag in both the insect species was observed as a function of increasing concentrations of coated Ag NPs in soil. An increase in the levels of Ag as a function of increasing size of coated Ag NPs was observed with Acheta domesticus. No apparent trend was observed with Tenebrio molitor species. A concentration dependent increase in the levels of Ag in the roots of both the plants was observed as a function of increasing concentrations of coated Ag NPs in soil. Additionally, the translocation of Ag to other plant tissues was observed in Helianthus annuus, a dicot plant.*

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

## I. INTRODUCTION

The widespread use of silver nanoparticles (Ag NPs) for a variety of consumer, industrial and medical applications has resulted in an increase in the anthropogenic release of silver in to the environment [1,2]. Silver from many Ag NP containing products is predicted to enter into the wastewater streams and eventually wastewater treatment plants [3]. Much of the silver at the treatment plants is partitioned in to sewage sludge [2,4,5]. Ag NPs eventually find their way in to the terrestrial ecosystems through the application of sewage sludge to agricultural lands [4].

The colloidal stability of nanoparticles significantly affects their mobility, uptake/bioavailability, and toxicity in a given ecosystem [6]. Environmental conditions present in an ecosystem such as pH, ionic strength, background electrolyte composition, etc. affect the colloidal stability of nanoparticles. Additionally, the presence of capping agents/coatings on nanoparticles also influences their colloidal stability [7,8]. Ag NPs are highly reactive due to their high surface area-to-volume ratio. This leads to phenomenon such as particle aggregation and settling in Ag NPs, unless stabilized/protected by suitable coatings [9,10]. Coatings prevent the aggregation of nanoparticles through electrostatic repulsions, steric repulsions, and a combination of both [9,11]. Coatings that are commonly used during the synthesis of Ag NPs include chemicals such as citrate, sodium borohydride ($NaBH_4$), and polyvinylpyrrolidine (PVP) [9,11].

PVP coatings are known to sterically stabilize Ag NPs. The mechanism of steric stabilization of nanoparticles involves the adsorption of uncharged polymer on the surface of nanoparticles [6,12,13]. Another mechanism involves the formation of weak coordinative bonds between PVP and $Ag^+$ ions [14,15]. The effect of surface coatings on the stability, transformation, uptake, and toxicity of nanoparticles has been discussed in literature [13, 16-21]. The present study investigates the uptake of PVP-coated Ag NPs (henceforth referred to as coated Ag NPs) in a terrestrial system by two insect (*Acheta domesticus* and *Tenebrio molitor*) and plant species (*Sorghum vulgare* and *Helianthus annuus*). PVP-coated Ag NPs were chosen as they demonstrated excellent shape and size stability characteristics in an ecotoxicological medium compared to other charge stabilized (citrate coated) Ag NPs [13]. The insect and plant species selected for the study were found to be native to the region where the soil was collected. Therefore, investigating the uptake of Ag NPs by these species would help us understand

the role of these species in the transport of metal contaminants along the food chain of native insectivorous and granivorous species.

## II. MATERIAL AND METHOD

### 2.1 Soil collection and preparation

All soil used during the insect and plant exposure experiments were collected in Mitchell County, Texas at an elevation of 684 m above sea level. The soil was collected from the top 10 cm of soil and is shoveled into clean plastic containers. Once transported to The Institute of Environmental and Human Health (TIEHH) at Texas Tech University (TTU) in Lubbock, TX, the soil was processed for homogeneity. Large clumps of soil were crushed. Large rocks, roots, living organisms, and other organic matter were removed. This was followed by sieving of the soil through a 2 mm wire screen into another clean plastic storage container. Once processed, the soil was covered and stored indoors until ready for use.

### 2.2 Soil analysis

The analysis of various characteristics of soil samples was performed at the Midwest Laboratories Inc. (Omaha, NE). 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 Nanoparticle Characterization

Silver nanoparticles coated with 0.2% PVP (20, 30-50, and 50-80 nm) were purchased from US Research Nanomaterials, Inc. (www.us-nano.com, Houston, TX). The US Research Nanomaterial, Inc reported that all Ag NPs consisted of $\geq$ 99.99% Ag.

Transmission electron microscopy (TEM) was used to confirm the size range and shape of the coated and uncoated Ag NPs. The preparation of samples involved the dispersion of Ag NP powder in ethanol (EtOH). Samples were sonicated for 10 minutes before being drop cast onto a carbon coated copper grid. Samples were air dried prior to analysis and TEM (Hitachi H-8100 TEM) images were taken at 200 kV using a tungsten filament side-mounted camera.

The size of Ag NPs was also confirmed using Dynamic light scattering (DLS). A mixture of 10 mg of Ag NP powder in 10 ml of reagent grade acetone (Fisher Scientific, MA, USA) 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 Powder x-ray diffraction (PXRD). A Rigaku Ultima III X-Ray Diffractometer was used. Ag NPs were analyzed using a Cu Kα radiation as x-ray source. The following instrument parameters were used to analyze the Ag NPs: 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.

### 2.4 Exposure of insects to coated Ag NPs

Two 37.8 L terrariums were prepared for each insect treatment group. Exactly 2.5 kg of prepared soil was weighed out into each clean terrarium. The insect species were maintained in soil spiked with a range of concentrations of 30-50 nm coated Ag NPs (0, 1, 5, 25, 125, and 625 mg/kg) to understand the effect of concentration of Ag NPs on uptake. Additionally, the effect of size of nanoparticles (20, 30-50, and 50-80 nm) on their uptake by the test species was investigated by spiking the soil with 25 mg/kg coated Ag NPs. Once the terrariums were prepared, insects were purchased from Reptilefood (reptilefoods.com, Ohio, USA). Either 300 small crickets or 400 large mealworms were placed in each of the terratiums and were provided with fresh food and water as needed for the duration of the 28 day exposure period. After the 28 day exposure had run to completion, 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. Freeze drying of the insects (FreeZone 2.5 Liter Freeze Dry System, Labconco, Corp. Kansas City, MO) for at least 48 hours was performed to ensure the removal of all moisture. Finally, the freeze dried insects were then crushed into a fine powder and stored in a freezer until further use.

### 2.5 Exposure of plants to coated Ag NPs

This set of experiments was performed in commercially available 7.6 L plastic nursery containers. The containers were filled with approximately two inches of commercial pond pebbles to aid in proper drainage. As detailed in the insect sample

section above, the plant species were maintained in soil spiked with a range of concentrations of 30-50 nm coated Ag NPs (0, 1, 5, 25, 125, and 625 mg/kg) to understand the effect of concentration of Ag NPs on uptake. Additionally, the effect of size of nanoparticles (20, 30-50, and 50-80 nm) on their uptake by the test species was investigated by spiking the soil with 25 mg/kg coated Ag NPs.

Seeds for the two plant species were planted into their own completed nursery containers and were transported to the TTU greenhouse. The plants were maintained in the TTU greenhouse until maturity, approximately three months for *H. annuus* and six months for *S. vulgare*. The plants received shaded sunlight and were maintained at 60°F or above in the TTU greenhouse. The entire plant was harvested after it reached maturity. The roots of the plants were separated rinsed using tap water for one 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 use.

### 2.6 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, if possible. Dry weights were used in the case of insects and wet weights were used in the case of plants. 10 ml of 70% nitric acid ($HNO_3$, reagent grade) was added to 1 gm of insect or plant samples in a 100 ml beaker. 10 ml of 30% hydrogen peroxide ($H_2O_2$, reagent grade) was added to this mixture. The beakers were covered with a Telfon watch glass and the samples were heated in increments of 50 ˚C until the solutions begin to reflux gently. To aid in the effective digestion of all samples, the beakers were periodically swirled. The digestions were stopped when the volume of sample in each of the beakers reduced to approximately 5 ml. This was followed by placing the beakers in an ice bath to cool. The samples were then filtered into 50 ml centrifuge tubes (Corning CentriStar $^{TM}$, Corning, NY) using ashless filter paper (Whatman No. 41, Fisher Scientific, PA). Filtering at this step ensures all lipids and other solids were removed from the samples. The original sample beakers were additionally rinsed twice with 10 ml of 5% $HNO_3$ and the rinse contents were added to the 50 ml centrifuge tube. The extracts were stored at room temperature until further analysis. A solution containing 10 ml $HNO_3$ and 10 ml $H_2O_2$ was used as the reagent blank during the analysis of samples.

### 2.7 ICP-OES analysis

The samples were analyzed using a Teledyne Instruments (Hudson, New Hampshire) Prodigy High Dispersion Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). The analysis of samples was performed at three wavelengths: 224.643, 328.068, and 338.289 nm. A 10 ppm silver standard solution (SPEX CetriPrep) was used to align all the three wavelengths of interest. Ultimately, the data from 338.289 nm was chosen for analysis.

### 2.8 Statistical analysis

A duplicate analysis of samples was performed in the present study (n=2). A normality check was performed and the data was normalized using Johnson transformation approach (p=0.05). ANOVA was performed on Ag concentrations more than 0.005 µg/g. Samples that contained silver concentrations below instrument detection limits (<0.005 µg/g) were treated as zeroes. All statistical analysis was performed with 95% confidence interval. The ANOVA test was followed by a multiple comparison test (Tukey) to identify the significant difference among the treatment groups. Statistical analyses were performed using MINITAB 17 [Minitab 17 Statistical Software (2010). Computer software, State College, PA: Minitab, Inc. www.minitab.com].

## 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. 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/100g. Other data from soil analysis is summarized in Table 1.

### 3.2 Transmission electron microscopy

A representative TEM image of coated 20 nm Ag NPs is presented in Fig. 1. As expected with the presence of PVP coatings, the phenomenon of aggregation is observed to be minimal. The presence of bulk organic materials like PVP on the surface of Ag NPs prevents the phenomenon of aggregation of nanoparticles through steric repulsions [6,12,13]. A reduction in aggregation of coated Ag NPs may likely facilitate their uptake in the test species. Additionally, the TEM image confirmed the spherical shape of coated Ag NPs.

### 3.3 Dynamic light scattering

The DLS analysis of coated Ag NPs revealed the average size to be much higher than expected. The size of coated 20 nm Ag NPs ranged from 39.20-154.3 nm, with an average particle size of 48.80 nm. A representative size distribution for the coated 20 nm Ag NPs is presented in Fig. 2. The size of coated 30-50 nm Ag NPs ranged from 40.50-121.5 nm with an average size of 62.40 nm. And size of coated 50-80 nm samples ranged from 78.90- 171.5 nm with an average size of 101.7 nm. The presence of an organic layer (PVP) may have possibly resulted in the observed increase in size of coated Ag NPs. Larger particles are thought to be less likely to aggregate, but also have lower dissolution rates. Large particles and large aggregates are thought to be less mobile in porous materials, including soils [22-24].

**TABLE 1**
**CHARACTERISTICS OF SOIL**

| Analysis | Results |
|---|---|
| Organic Matter | 1.7% |
| Exchangeable Potassium | 263 ppm |
| Exchangeable Magnesium | 114 ppm |
| Exchangeable Calcium | 3273 ppm |
| pH | 8.1 |
| Cation Exchange Capacity | 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% |
| Slit Content | 36% |
| Clay Content | 10% |

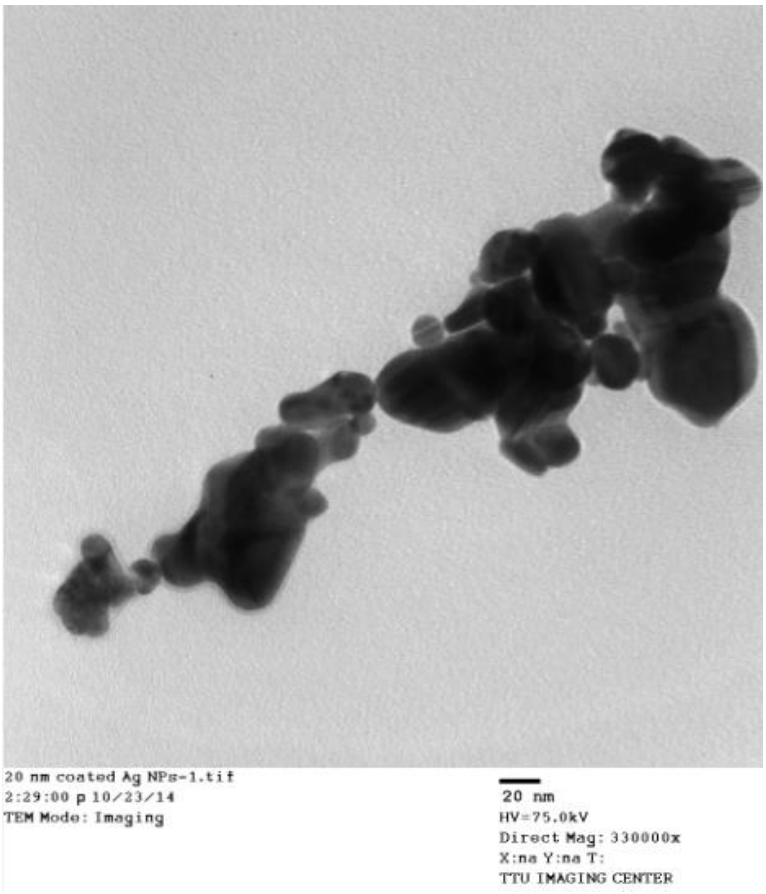


**FIG. 1: TRANSMISSION ELECTRON MICROSCOPY IMAGE OF 20 nm COATED Ag NPs.**

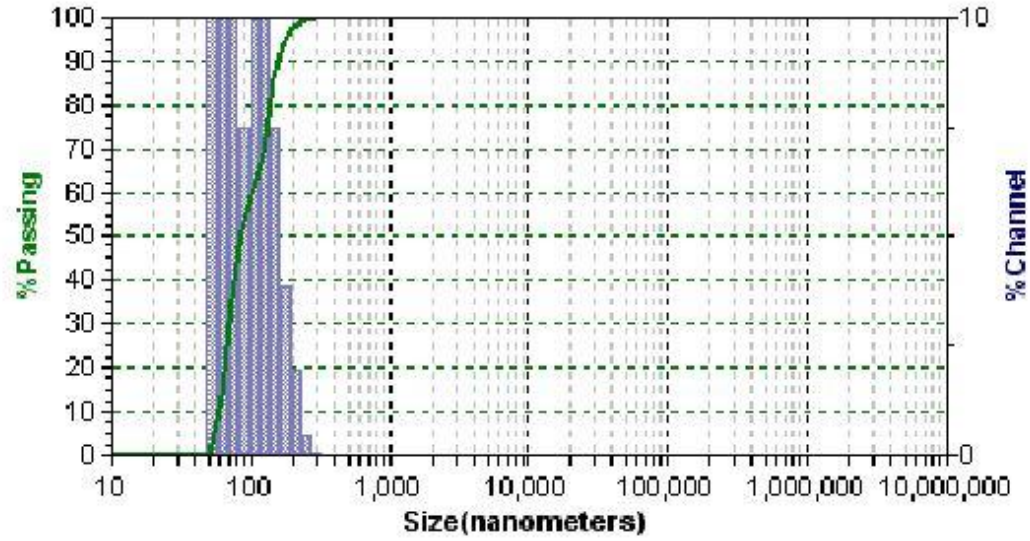


**FIG. 2: SIZE DISTRIBUTION OF COATED 20 nm Ag NPs AS DETERMINED BY DYNAMIC LIGHT SCATTERING.**

### 3.4 Powder X-ray diffraction

The PXRD analysis of the silver nanoparticles confirmed their composition. The measured crystalline structure was slightly larger most likely due to the 0.2% PVP coating. The diffraction patterns still matched both those in the ICDD and those provided by the manufacturer. A typical diffraction pattern can be seen in Fig. 3.

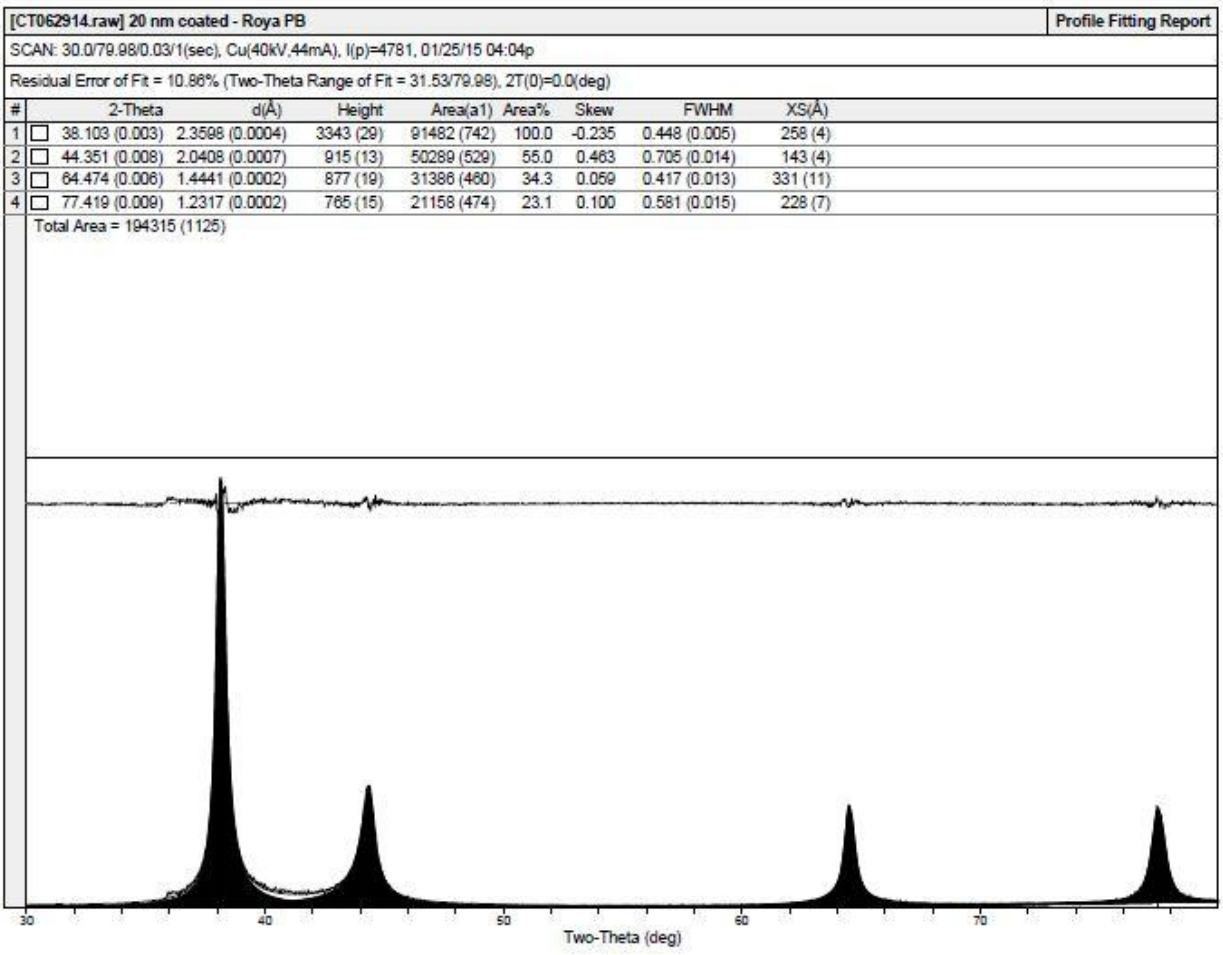


**FIG. 3: DIFFRACTION PATTERN FOR 20 nm COATED Ag NPs AS DETERMIEND BY POWDER X-RAY DIFFRACTION.**

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

No detectable amount of silver was observed in the control soil used for the insect experiments. However, increased levels of Ag were observed in *A. domesticus* and *T. molitor* samples that were maintained in soil spiked with 1 mg/kg coated Ag NPs. An error in any of the sample processing steps would have accounted for the observed increase in the levels of Ag in the insects maintained in soil spiked with 1 ppm coated Ag NPs.

With the experiments involving *A. domesticus*, no silver was observed in insects maintained in soil spiked with 1 ppm coated Ag NPs. Trace amounts of Ag (>0.02 mg/kg) were observed in insects maintained in soil spiked with 25 mg/kg coated Ag NPs. Quantifiable amounts of Ag (>0.1 mg/kg) was observed in *A. domesticus* maintained in soil spiked with 125 and 625 mg/kg coated Ag NPs. In general, an increase in the levels of Ag in *A. domesticus* was observed as a function of increasing concentrations of coated Ag NPs in soil (Fig. 4). Similar results were obtained in the case of *T. molitor* samples (Fig. 4). Additionally, the levels of Ag in both the insect species maintained in soil spiked with 625 mg/kg coated Ag NPs was found to be significantly higher than the levels of Ag in insect species maintained in soils spiked with 1, 5, and 25 mg/kg coated Ag NPs ($p<0.05$).

The uptake of Ag from coated Ag NPs by both *A. domesticus* and *T. molitor* in soil was first observed in soil spiked with 25 mg/kg coated Ag NPs. Hence, spiking with 25 mg/kg coated Ag NPs was considered to investigate the effect of size of nanoparticles on uptake of Ag from coated Ag NPs in soil (Fig. 5). An increase in the levels of Ag as a function of increasing size of coated Ag NPs was observed with *A. domesticus*. No apparent trend was observed with *T. molitor* species. Nevertheless, *T.molitor* was found to have a significantly higher level of Ag compared to *A. domesticus* ($P<0.05$). This suggests that *T.molitor* is accumulating higher levels of Ag from coated Ag NPs in soil compared to *A. domesticus*.

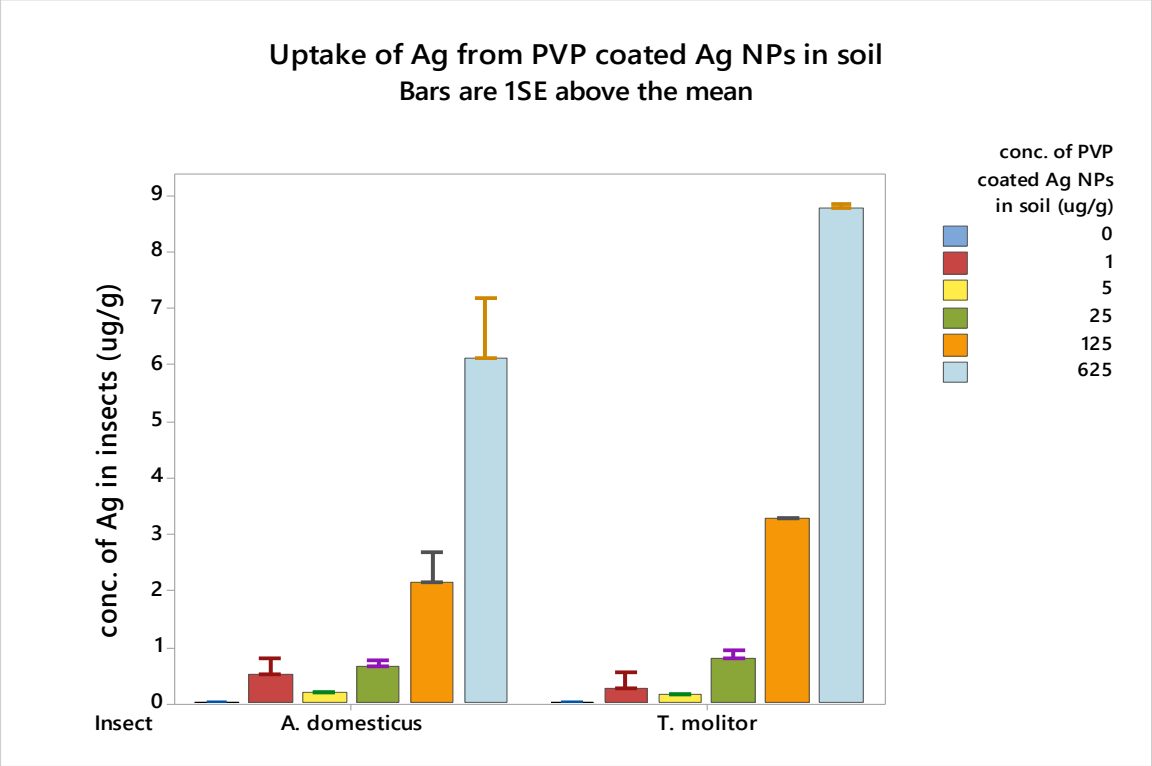


**FIG. 4: LEVELS OF Ag IN *A. domesticus* AND *T. molitor* SAMPLES MAINTAINED IN SOIL SPIKED WITH DIFFERENCE CONCENTRAITONS OF COATED Ag NPs.**

The difference in the uptake of Ag from coated Ag NPs in soil by *A. domesticus* and *T. molitor* could be inherent. The mechanism of uptake by either of these two species is not yet clearly established. However, the level of uptake of metals by soil-dwelling organism is found to be dependent on their habitat, diet, and physiological responses [25]. Another explanation could be the phenomenon of avoidance. Certain species like earthworms (*Eisena fetida*) are better able to sense the presence of nanoparticles in soil and avoid. This way, they tend to avoid their dwelling in such soils and the uptake of contaminants [26]. The phenomenon of avoidance by one species over another in the present study cannot be discounted.

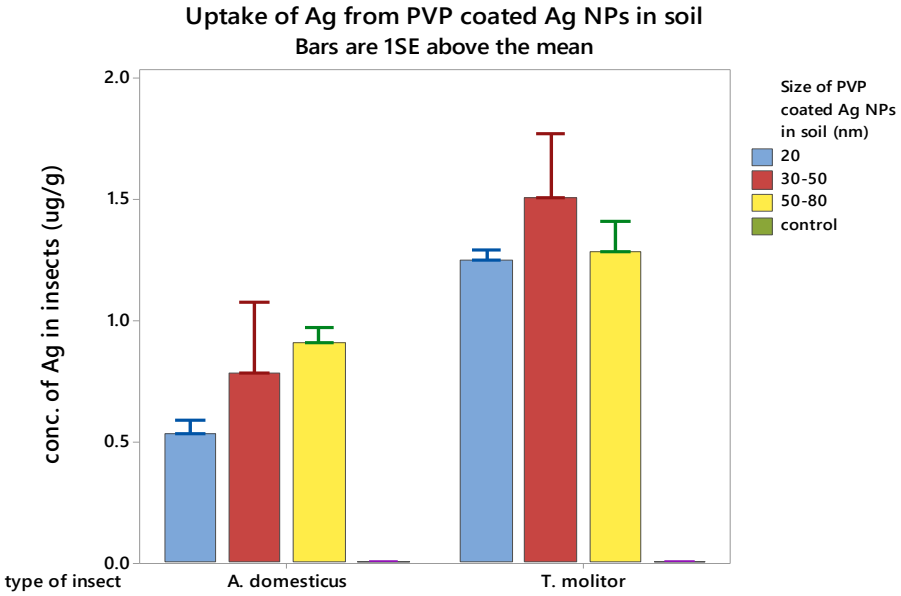


**FIG. 5: LEVELS OF Ag IN *A. domesticus* AND *T. molitor* SAMPLES MAINTAINED IN SOIL SPIKED WITH DIFFERENT SIZED COATED Ag NPs.**

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

The present study considered a monocot (*S. vulgare*) and a dicot (*H. annuus*) plant species to investigate the difference in the accumulation of Ag from coated Ag NPs in soil. In both plants, the roots have accumulated significantly higher levels ($p<0.05$) of Ag from coated Ag NPs in soil compared to other plant tissues (stem, leaves, seeds, etc.). In the roots of both the plants, a concentration dependent increase in the levels of Ag is observed as a function of increasing concentrations of coated Ag NPs in soil. Additionally, the levels of Ag in roots of plants maintained in soil spiked with 625 mg/kg coated Ag NPs is found to be significantly higher than the levels of Ag in roots of plants maintained in soil spiked with 1, 5, 25, and 125 mg/kg coated Ag NPs. Finally, the dicot plant (*H. annuus*) has accumulated more levels of Ag in its system compared to the monocot plant (*S. vulgare*). The results are summarized in Fig. 6 and 7.

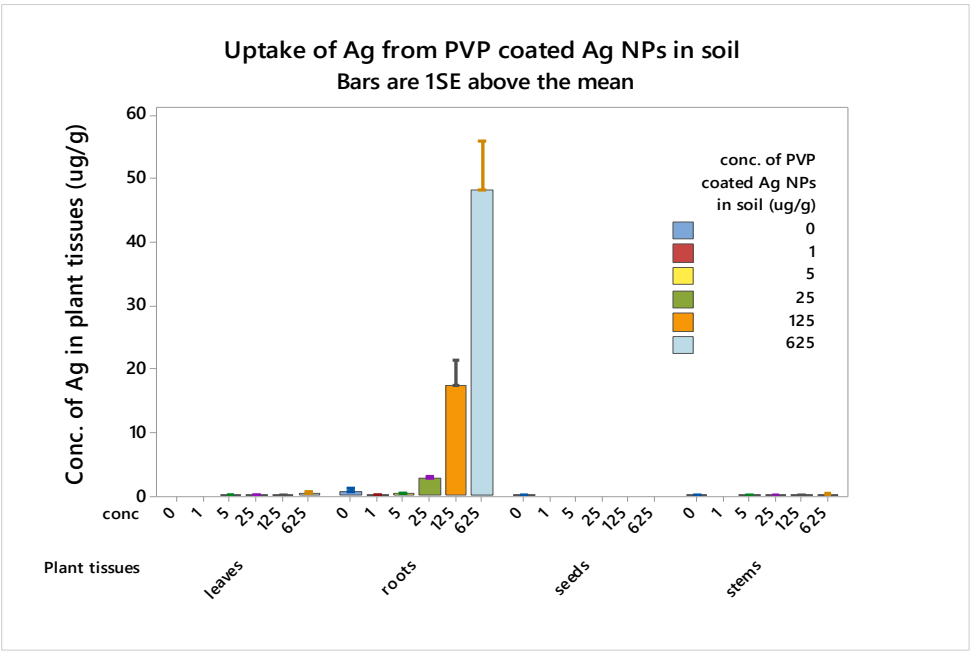


**FIG. 6: LEVELS OF Ag IN *S. vulgare* MAINTAINED IN SOIL SPIKED WITH DIFFERENCE CONCENTRATIONS OF COATED Ag NPs.**

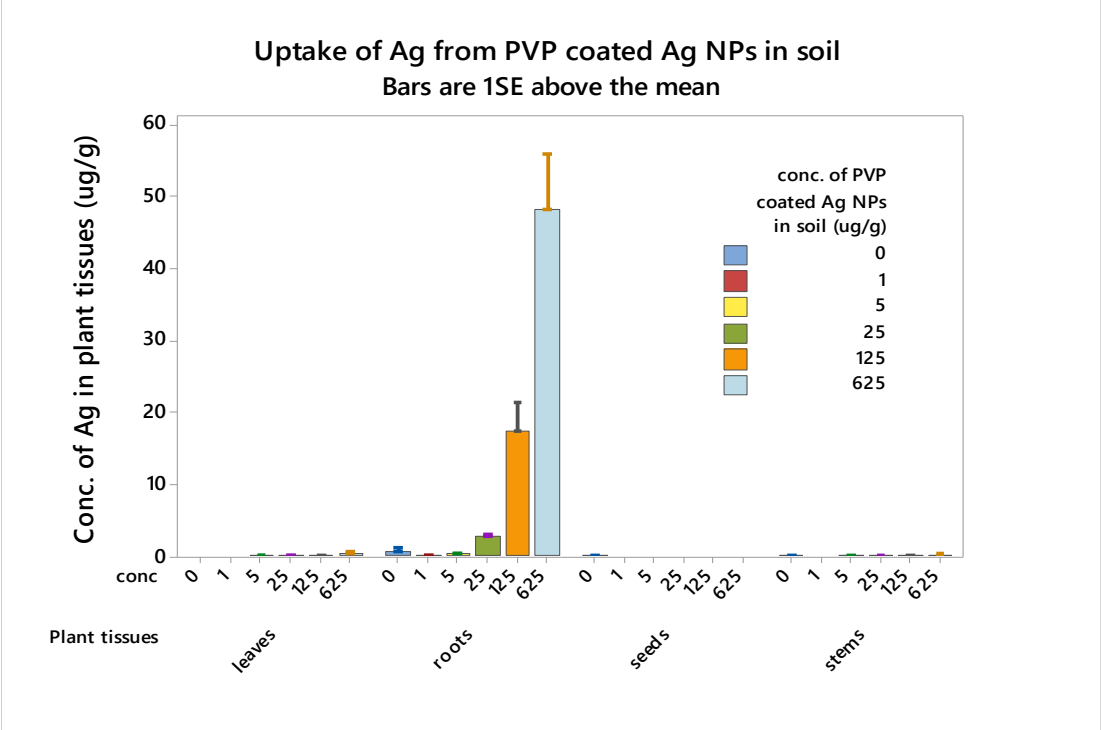


**FIG. 7: LEVELS OF Ag IN *H. annuus* MAINTAINED IN SOIL SPIKED WITH DIFFERENCE CONCENTRTATIONS OF COATED Ag NPs.**

Fig. 8 and 9 represent the levels of Ag in tissues of *S. vulgare* and *H. annuus*, respectively, when maintained in soil spiked with different sized coated Ag NPs. Both the plants accumulated significantly higher concentrations of Ag in their roots ($p<0.05$) compared to the remaining plant tissues (stems, seeds, and leaves). Additionally, the translocation of Ag from roots to other plant tissues is observed in the case of *H. annuus*, a dicot plant (Fig. 9). No translocation phenomenon is observed in the case of *S. vulgare*, a monocot plant (Fig. 8).

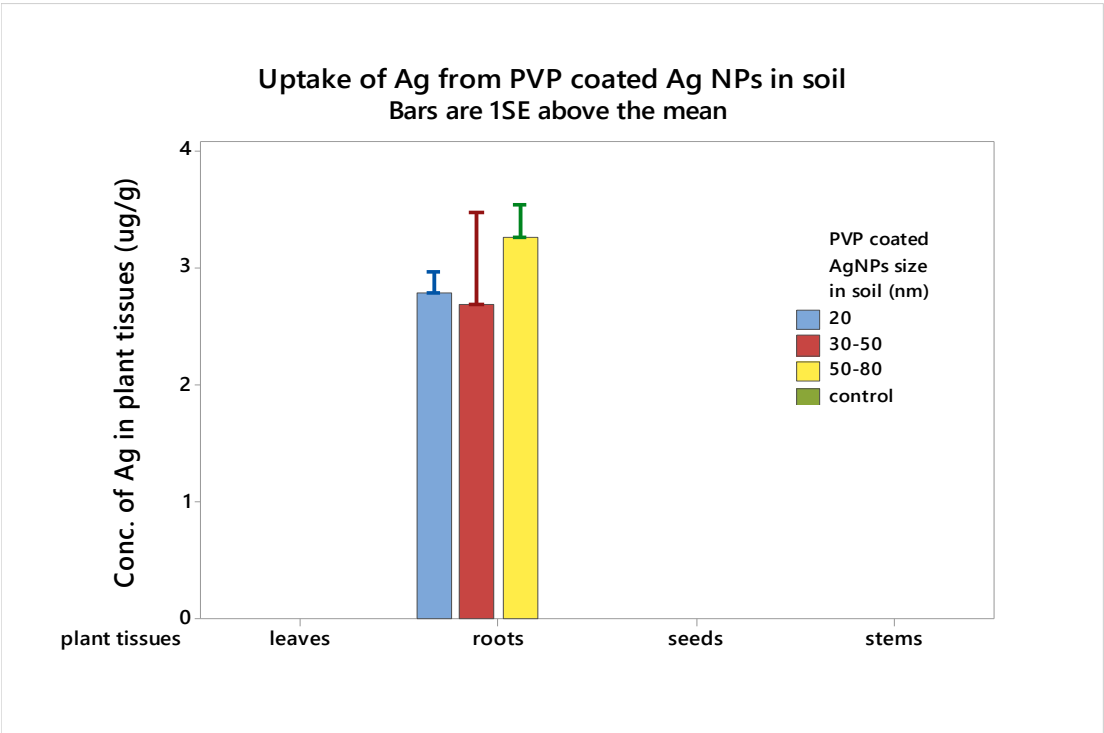


**FIG. 8: LEVELS OF Ag IN *S. vulgare* MAINTAINED IN SOIL SPIKED WITH DIFFERENCE SIZED COATED Ag NPs.**

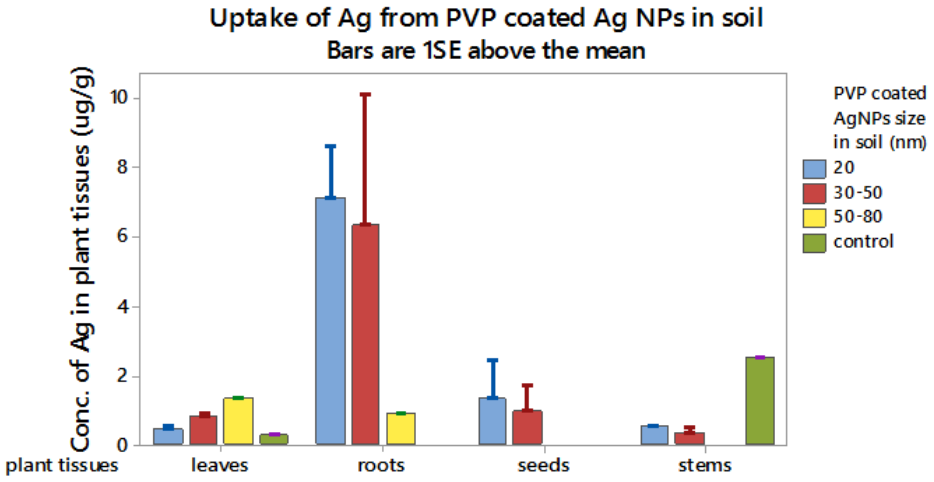


**FIG. 9: LEVELS OF Ag IN *H. annuus* MAINTAINED IN SOIL SPIKED WITH DIFFERENCE SIZED COATED Ag NPs.**

Considerable accumulation of nanoparticles in the roots was observed in the case of both monocot and dicot plant species used in the study. This observation is consistent with results from other studies [27-30]. The uptake of Ag from coated Ag NPs in soil by monocots (*S. vulgare*) could be attributed to their root morphology. The presence of thin and numerous roots in monocot plants provide a very high surface area for the penetration and accumulation of nanoparticles [31]. No translocation of Ag into other plant tissues was observed in *S. vulgare*. Studies have suggested that monocot plants usually are very resistant to metal oxide and nanoparticle uptake [32-34]. On the other hand, translocation of coated Ag NPs was observed in the case of *H. annuus*, a dicot plant. The mechanism of translocation of nanoparticles to tissues such as leaves involves the accumulation of nanoparticles in plant vascular system, i.e., xylem [21]. Similarly, phloem transport explains the presence of Ag NPs in the seeds of *H. annuus* [35]. Yan et al. 2009 [36] have reported that PVP coating confers hydrophilicity to Ag NPs [36]. The hydrophilicity of coated Ag NPs could enable their translocation into various plant tissues through xylem and phloem transport mechanisms.

The differences in uptake, accumulation, and translocation of coated Ag NPs between the two plant species used in the study could also be attributed to the inherent physicochemical variations such as difference in hydraulic conductivity, pore size of the cell walls, etc [37].

Finally, PVP coating is known to prevent the oxidation of PVP-coated Ag NPs thereby affecting the chemical state of Ag NPs [6,17]. Three different Ag species, $Ag^0$ (Ag NPs), Ag-PVP complexes, and free $Ag^+$ could be present in a given suspension of PVP-stabilized Ag NPs [38]. ICP-OES measures total Ag in a sample. Hence, the effect of PVP coating on dissolution of Ag ions could not be validated in the present study. It is difficult to know for certain whether the samples contained silver ions or silver nanoparticles.

## IV. CONCLUSION

The uptake of PVP-coated Ag NPs in a terrestrial system by insect and plant species was investigated. The phenomenon of aggregation of Ag NPs in the presence of a PVP coating was found to be minimal. Also, the size of PVP-coated Ag NPs was found to be larger than pristine Ag NPs. The composition of PVP-coated Ag NPs was confirmed by powder X-ray diffraction. A concentration dependent increase in the uptake of Ag from PVP-coated Ag NPs in soil was observed. No effect of size of PVP-coated Ag NPs on their uptake by the test species was observed. Considering the increasing use of Ag NPs for a variety of applications, the results from this study would be helpful in elucidating the role of coatings on Ag NPs in determining their uptake by plant and insect species. Eventually, the results from this study would also help understand the role of coatings in affecting the bioaccumulation and biomagnification of Ag NPs along the food webs.

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