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Effect of Coating Agents of Silver Nanoparticles on Their Accumulation in and Growth of Radish (*Raphanus sativus* L.)

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ABSTRACT

The widespread use of silver Nanoparticles (AgNPs) in various industries has raised concerns about the fate of these materials. Therefore, the present study aimed to assess the effects of coating agents of Ag particles in soil and how they interact with plants as the first step in the human food chain. Radish (*Raphanus sativus*) was exposed to silver Nitrate ($AgNO_3$) as well as AgNPs with different coatings: Citrate (AgNPs-Cit), Polyethyleneimine (AgNPs-PEI), and Polyvinylpyrrolidone (AgNPs-PVP) at different concentrations. The effect of concentration (5, 25, 125 mg kg^{-1} soil) and the type of coatings on the dry weight of radish were compared with the control. The results revealed that the type of treatments affected dry weight of radish and, among all treatments, $AgNO_3$ had the highest weight loss, in which shoot dry weight decreased by 51%. Total silver measurement in radish root, tuber, and shoot indicated that the accumulation of AgNPs was influenced by the type and concentration of the coating. The AgNPs with positive charge coating (PEI) had a higher transfer ratio than other treatments. The findings indicated that radish had the ability to store silver in its root, tuber, and shoots in large amounts, thus having the potential to act as a source of silver contamination for humans.

Keywords: AgNPs, Citrate coating, Polyethyleneimine, Polyvinylpyrrolidone.

INTRODUCTION

Release of metallic nanoparticles into the environment has attracted international attention (Rajput *et al*., 2018).The important issues in this case are the unknown effects of large amounts of these particles entering the soil and various parts of the environment (Belal and El-ramady, 2017). One of the most important nanoparticles that can be considered potentially toxic and dangerous for the environment is AgNPs. The unique properties of this element and its nanoparticulate form have led to its widespread use in various industries (Noori *et al*., 2019). In this regard , it is important to study behavior of AgNPs in the environment (Tkalec *et al*., 2019). There are many sources of entry for AgNPs into soil, for example, the use of wastewater sludge as a soil amendment, the use of nano fertilizers, aerosols, or waste disposal in landfills (El Hadri *et al*., 2018). Release of AgNPs into the soil can lead to their leaching into groundwater, or they may enter the human body directly by breathing or swallowing (Inshakova and Inshakov, 2017). After accumulating in the soil, they can be absorbed by edible plants and vegetables and enter the human food chain and potentially induce adverse effects (Inshakova and Inshakov, 2017).

Aggregation behavior, size, and surface properties of Engineered Nanomaterials

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(ENMs) are the most important factors in controlling their mobility and transport in aquatic and terrestrial systems (Badawy *et al*., 2010). Those factors determine the dependence and interaction with sorptive surfaces, such as biofilms, algae, plants, fungi, humic substances, and soil sediments (Navarro *et al*., 2008). One of the most important issues in this case is the surface coatings of the nanomaterials. ENMs are usually coated in order to tune their surface properties (Reinhart *et al*., 2010). These coatings are usually small ligands (e.g., citrate), polymers (e.g., polyvinylpyrrolidone) or fibers (He *et al*., 2019), which can render the surface charge of nanoparticles neutral, positive, or negative . These kinds of charges can create widespread changes in behavior of these nanoparticles (Abbaszadegan *et al*., 2015), which has caused concerns in the environmental field.

Therefore, many studies have been done to determine the behavior of nanoparticles and especially AgNPs in the environment. Most of the studies deal with biological and chemical effects on specific plants, such as *Arabidopsis thaliana*. Some studies have reported that AgNPs accumulated in the plant when grown in various environments such as soil (Geisler-Lee *et al*., 2013), agar (Kaveh *et al*., 2013) and Hoagland (Nair and Chung, 2014). The various effects of accumulation in this specie have been reported, including mortality (Kaveh *et al*., 2013), reduction in root/shoot weight (Qian *et al*., 2013) and reduction of chlorophyll content (Sosan *et al*., 2016). Due to the negative effects observed for plants, numerous studies have been conducted on aquatic media (Jiang *et al*., 2017) and edible plants (Torrent *et al*., 2020). Recently Torrent *et al*. (2020) reported accumulation of silver in various tissues of lettuce and indicated the effect of toxicity on this plant. Meanwhile, Tripathi *et al.* (2017) reported that AgNPs reduced growth parameters, photosynthesis, and ascorbic and glutathione pigments content in pea (*Pisium sativum*). In another study, Quah *et al.* (2015) investigated the toxicity, accumulation, and transport of AgNPs with different properties in two different agricultural products *Glycine max* and *Triticum aestivum*. They reported that the toxicity and fate of AgNPs were different, depending on their size and type. In a study to investigate the effect of AgNPs, TiO_2, fluorine and carbon nanotubes on the suppression of mosaic virus, concentrations of 100, 200 and 500 mg L^{-1} of these nanomaterials were used (Adeel *et al*., 2021). The results showed that fluorine and carbon nanotubes had the most suppressive effect. Cvjetko *et al* (2017a) considered the toxicity of silver nitrate and three types of AgNPs in onion (*Allium cepa*) and reported that the toxicity of these particles was directly dependent on their shape, surface properties, and type of surface coating agents.

Edible plants such as radish, cabbage, lettuce, play an essential role in the nutritional transmission of pollutants from soil to human bodies. They are considered very important in human health assessment. Radish (*Raphanus sativus L.*) is a popular vegetable with high global consumption and can mature in three to four weeks under favorable growth conditions. Radish is also an underground vegetable, with its edible tissues directly exposed to AgNPs in soil. Therefore, radish may accumulate high concentrations of nanoparticle in their edible tissues (Zhang *et al*., 2017). A previous study demonstrated that the radish tubers grown in a loamy sand soil with 250 and 500 mg kg^{-1} of CeO_2 NPs accumulated high concentrations of Ce, posing potential risks for human exposure (Corral-Diaz *et al*., 2014). However, detailed distribution of Ce in the tubes and the role of soil properties were not reported in that study. On the other hand, in previous studies less attention was paid to radish, which is capable of storing pollutants in its tubers and even its leaves(Zhang *et al*., 2017). Gui *et al.* (2017) studied the phytotoxicity of cerium oxide nanoparticles on radish plant and reported that nanoparticles can enter the human food

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chain through soil and consumption of edible parts.

Indeed, detailed information on the behavior of AgNPs with different coatings in soil and plant, in particular for radish, is still missing. Therefore, the aims of this study were: (1) Investigating the effect of AgNPs with different coatings and concentrations on its uptake and distribution in different parts of radish, and (2) Evaluation of toxic effects of silver nanoparticle on shoot, root, and tuber dry weight of radish.

MATERIALS AND METHODS

Reagents and Apparatus

AgNPs with citrate, polyethyleneimine and polyvinylpyrrolidone coating agents as well as silver nitrate were purchased from ASEPE Nano Tech (Tabriz, Iran). For the digestion procedures of radish tissues, nitric acid (HNO_3 69%) hydrochloric acid (conc. HCl, 37% w/w), concentrated perchloric acid, and hydrogen peroxide (H_2O_2 30%) were purchased from Sigma Aldrich (Tehran, Iran). Distilled de-ionized water was used in all the experiments. Ag concentrations were determined using AA990 Atomic Absorption (Petrović *et al*., 2001).

Table 1. The main characteristics of the test soils.

Parameter	Amount
pH	7.97
EC (dS m^{-1})	0.45
CEC (cmolc kg^{-1})	8.5
OM	0.58
$CaCO_3$ (%)	17
Sand (%)	63
Silt (%)	13.3
Clay (%)	23.7
Texture class	Sandy clay loam

Soil Treatment and Plant Culture

Soil samples used in this study were obtained from 0-15 cm field in Khorasan Razavi Agricultural Research Education and Extension Organization (AREEO). The main characteristics of the test soils were measured, and are listed in Table 1. This experiment was conducted using a completely randomized design with factorial arrangement including 12 treatments with three replications (Figure 1). $AgNO_3$ and AgNPs with three types of coating agents, namely, Citrate (Cit), Polyethyleneimine (PEI), and Polyvinylpyrrolidone (PVP), were weighed. Then, AgNPs and silver nitrate were mixed with 2 kg soil, using an electrical mixer, in three concentration levels of 5, 25,

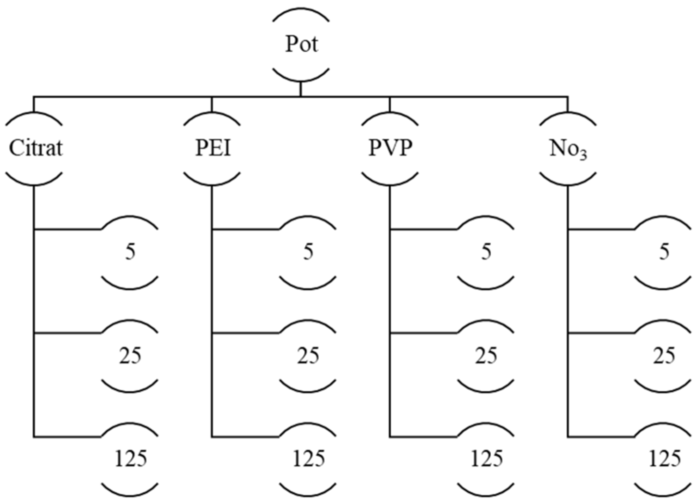

Figure 1. General schematic of the experimental design.

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and 125 mg kg^{-1} and were transferred to plastic pots, as a simulation of adding sludge amendment or fertilizers directly to the soil. Previous studies reported that up to 5 mg kg^{-1}, no serious toxicity symptoms were seen in different plants (Tripathi *et al*., 2017). In order to investigate the effect of increasing concentration in this study, treatments were selected from 5 mg kg^{-1} to 125. For better drainage, a 4 cm layer of uncontaminated sand was added at the bottom of the pots following previously reported methods (Vittori Antisari *et al*., 2015). Thirty-six pots treated with AgNPs and silver nitrate were randomly placed in the greenhouse of Ferdowsi University of Mashhad, where temperature and light were controlled. The 35-day length of the growth period to the reproductive stage was set to 16 hours of light and 8 hours of darkness at 20±5°C. Before planting, radish seeds were placed in tap water overnight, then, they were planted in the pots. After 24 hours, the first sprouts were observed in the pot. After five days, three plants per pot (and 3 pots for each treatment) were maintained and the rest were removed (Figure 2). Irrigation was done daily and, in order to avoid the release of AgNPs and $AgNO_3$ from the pots, it was done in such a way that no water drained out of the pots.

Sampling and Analyses

At the end of the growth period (35 days), each radish plant was removed from the pots and leaves, tubers, and fine roots were separated, washed with deionized water and weighed (wet-weight). They were then dried at 60°C until constant weight and weighted again (dry weight) prior to grinding using an electrical mill (Naniwa 100w/N95/"F" class). After that, the samples were stored in the freezer in vacuum plastic containers until digestion.

Digestion on a heating plate was used to determine the amount of silver in different parts of plant. Half a g of the milled samples were transferred to a beaker, then 8 mL of nitric acid (69%) and 2 mL of H_2O_2 (30%) were added to each sample (v/v= 4:1). After covering the dishes with watch glass, the samples were heated at 250°C for 1 hour. After cooling, the samples were transferred to a 25 mL volumetric flask, which was filled up to the mark with deionized water. The samples were finally filtered using Whatman filter paper and stored in a

Figure 2. The plants situation in the greenhouse.

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polypropylene centrifuge tube at 4°C prior to AAS analysis.

Statistical Analyses

The statistical design was completely randomized with the factorial arrangement and data were considered as three replicates. The data were analyzed using JUMP software and Tukey's test.

RESULTS AND DIS CUSSION

Fine Root Dry Weight

As shown in Figure 3, the dry weight of the fine roots decreased with increasing concentration of silver, with significant differences (P< 0.05) between most treatments, including the lowest concentration, compared to the control. This reduction in the dry weight of the fine root can reduce growth of the plant and other organs of the plant and thus decrease the dry weight of the tubers and shoots. The most significant reduction in fine root dry weight was seen in the $AgNO_3$ treatments. According to the results in the $AgNO_3$ treatment at concentrations of 25 and 125 mg kg^{-1}, fine roots dry weight decreased 32 and 76%, respectively. This sharp decrease in dry weight could be attributed to the release of silver ions causing toxic effects on the plant, decreasing growth, and/or due to Reactive Oxygen Species (ROS) mediating damages to the biomolecules linked with the photosynthetic apparatus (Tripathi *et al*., 2017b). In contrast, roots dry weight in $AgNO_3$ 5 mg kg^{-1} treatment increased significantly about 4%. Such an increase in dry weight of fine roots, tubers, and shoots at 5 mg silver nitrate per kg soil was, probably, because the concentration of silver was still not high enough to cause toxicity to the plant and, on the other hand, the nitrate fraction of this treatment could act as a growth stimulant to improve growth conditions.

Tuber Dry Weight

In the case of the tubers, there was also a decreasing trend, except for $AgNO_3$-5 that that increased (Figure 3). The decrease can be due to the negative or, perhaps, toxic effects of silver on the roots, which prevented root development and reduced the growth of the plants. The problem with tubers is that they are in direct contact with soil and silver can also have negative effects on the shape of the tubers. Also Vishwakarma *et al.* (2017) reported that AgNPs and $AgNO_3$ treatments induced morphological modifications not only on the

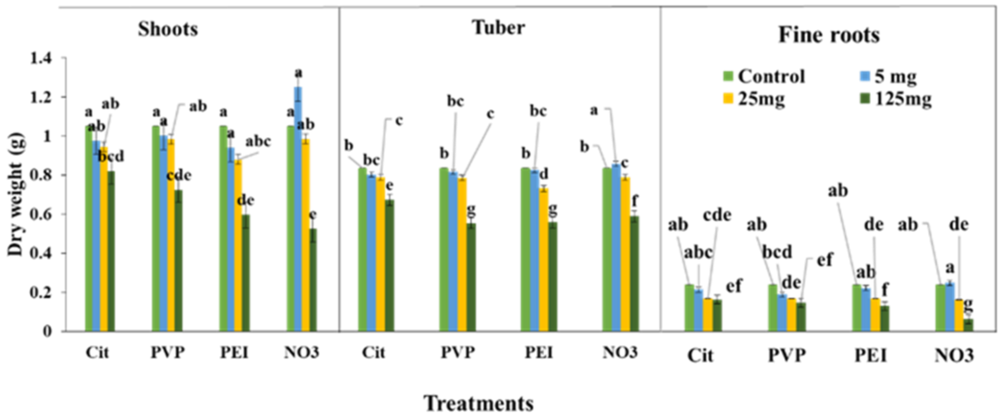

Figure 3. Effect of $AgNO_3$ and AgNPs with 3 different coating types on dry weight of radish seedlings. [Data are means±standard error of three independent experiments with three replicates (n= 9). Bars followed by different letter(s) show significant differences (P< 0.05) according to the Tukey's test.

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contact parts of the roots but also in the tubers, stem, and leaves. There was a significant difference between AgNPs-Cit-125, AgNPs-PEI-25, $AgNO_3$-5 and AgNPs-Cit-5 ($P< 0.05$). Other treatments at different concentrations and with different coatings reduced dry weight compared to the control.

Shoot Dry Weight

As shown in Figure 3, the amount of shoot-dry-weight decreased with increasing concentration of all treatments, except silver nitrate treatment at a concentration of 5 mg kg^{-1}. Meanwhile, the highest weight loss was related to the Ag-NO_3-125, in which shoot dry weight decreased by 51%. This effect might be due to the toxic effects of silver ions on the plant reducing its yield (Vishwakarma *et al*. 2017). However, this also could be attributed to the increased uptake of silver by plants (Harris and Bali, 2008). In the $AgNO_3$-5 treatment, dry weight increased by about 19% compared to the control. Changes in the shoot dry weight indicate a negative effect of experimental treatments on the growth rate and the consequent dry weight of radish. As can be seen in the Figure 3, there is a statistical difference at some of treatments (AgNPs-Cit-125, AgNPs-PVP-125, AgNPs-PEI-125, AgNPs-PEI-25, $AgNO_3$-125 AgNPs-Cit-125, AgNPs-PEI-125) ($P< 0.05$). Accordingly, the difference in shoot-dry-weight at high concentrations of treatments is significant. AgNPs-PEI treatment, after silver nitrate, had the highest dry weight reduction, possibly due to the positive charge on the surface of these nanoparticles, which may affect its behavior and transformations in the soil as well as toxicity of this particle to plants (Tkalec *et al*., 2019). Also it can increase amount of uptake of these particles by negative charges on the root surface of the plant (Yang *et al*., 2013). Cvjetko *et al.* (2017) reported that the highest impact of AgNPs with different coating charges was recorded for positively charged AgNP-CTAB, which induced changes significantly different from the control and other AgNPs in the majority of tested parameters in *Allium cepa* roots. Among the treatments, the lowest weight loss was observed in citrate-coated nanoparticles. This could be due to their surface's negative charge which causes the adsorption of these nano particles by ligands and multivalent cations such as Ca2+ and Mg2 + in the soil, preventing their uptake and impact on the plant. (Beer *et al*., 2012). Moreover, due to the aggregation as well as the negative charge, uptake of AgNP-citrate by root was probably difficult. Another reason could be the decrease in the AgNPs available surface for interaction with organic molecules and the AgNP-citrate toxic effects (Šinko *et al*., 2014).

Overall, the results show that dry weights were more affected by the concentration of treatments than the type of coatings or speciation. In other words, the effective factor in reducing the dry weight of radish plant was the silver element, and increasing its concentration caused toxicity to the plant and, as a result, reduced plant growth. Nonetheless, the $AgNO_3$ treatments had slightly higher impacts on the dry weight, suggesting that most of the toxic effect results from the silver ions and that nanoparticles dissolved fast in the system, hence, producing an effect independent from their initial state. This is in agreement with previous results from Yasur and Rani (2013) who described that AgNPs expressed lower toxicity than $AgNO_3$, as a result of their coating that can stabilize AgNPs towards dissolution and reduce their toxicity. Likewise, the toxicity of treatments was more pronounced in roots compared to the shoots, because roots are the main sites of interaction while the plant's self-defense mechanism involve translocation of the AgNPs from roots to shoots and thus restrict its accumulation in tubers completely or partially (Vannini *et al*., 2014). Previous studies have investigated the effect of AgNPs on different plants such as wheat (Jiang *et al*., 2012; Dimkpa *et al*., 2013) , cucumber (Shams *et al*., 2013), *B. juncea* (Vishwakarma *et al*., 2017), and watermelon and corn (Almutairi and Alharbi, 2015). All these researchers reported that the AgNPs decreased the root and shoot dry weight. Galazzi and Arruda (2018) also investigated

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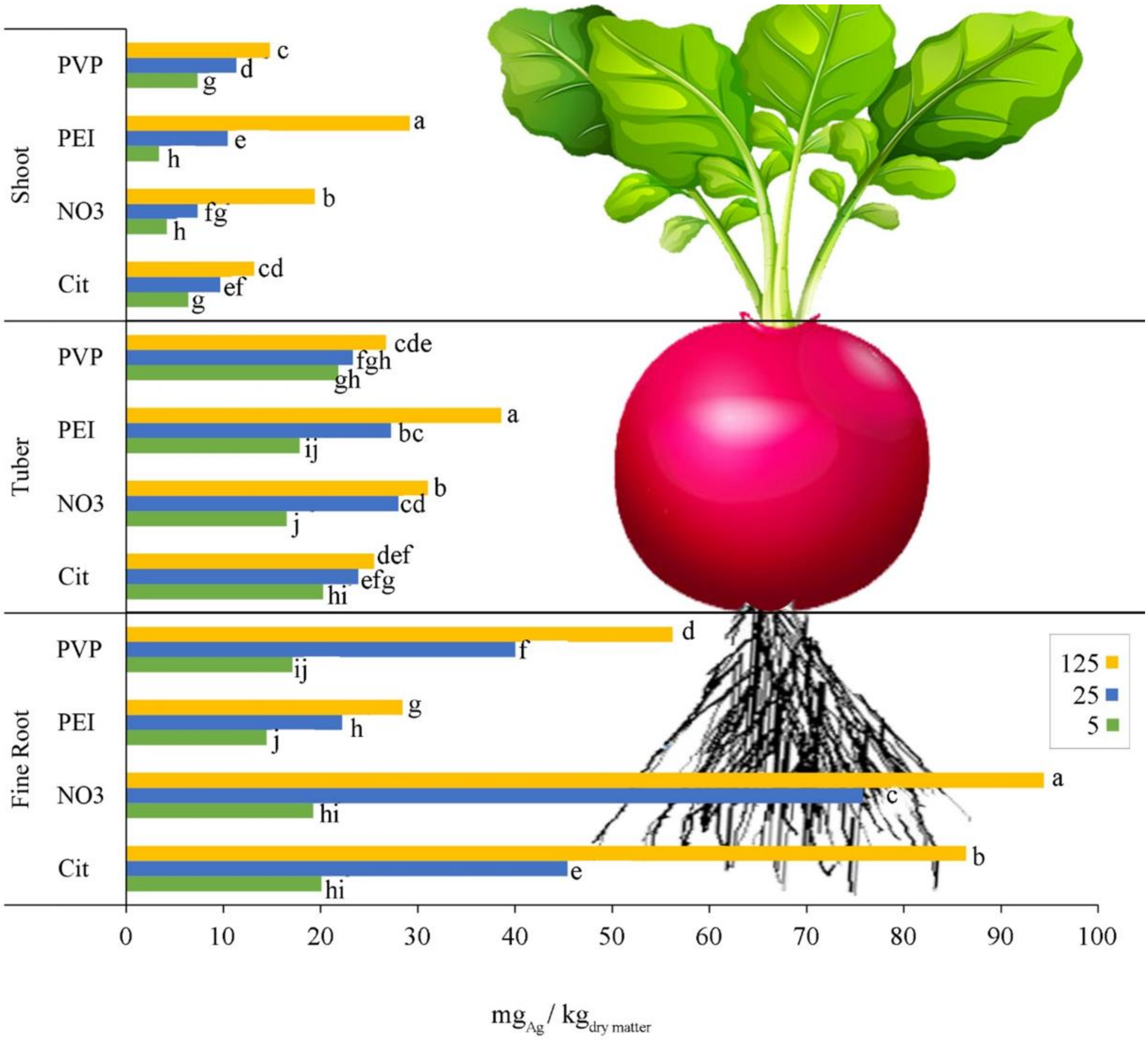

Figure 4. The effect of different treatments ($AgNO_3$ and AgNP-Citrate, AgNPs-PVP and AgNPs-PEI) on silver concentration in radish plant. Bars followed by different letter(s) show significant differences ($P< 0.05$) according to the Tukey's test. Control samples were < limit of detection (LOD).

the effects of 50 mg kg^{-1} AgNPs and $AgNO_3$ on soybean and they reported 19 to 25% total weight reduction compared to the control.

Plant Uptake and Accumulation of Silver

Figure 4 shows the amount of silver uptake by radish plants' parts in a soil polluted with different treatments of silver over 35 days. Accordingly, silver was not only absorbed by the plant but also transferred to the tubers and leaves after absorption. As can be seen in all treatments, concentration of silver in the roots was higher than tubers and shoots, suggesting that the main pathway for the Ag-NPs is through the root, e.g. through water uptake. There is a significant difference between all treatments in terms of accumulation of silver in shoots ($P< 0.05$). This might be due to the surface characteristics of the treatments or the ability of radish. As Torrent *et al*. (2020) indicated, the uptake and accumulation of AgNPs in different plants as well as their subsequent toxicological effects depend on various factors such as nanoparticle

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physicochemical properties, metal concentration, and type of plants. Also, the AgNPs-PEI treatment (positively charged) had the highest accumulation rate in both tuber and the shoot with even higher concentration than in the roots, contrasting with the other coatings. This result is in agreement with Wang *et al.* (2014) who reported the uptake and accumulation of nanoparticles with positive charge coatings such as PEI were higher than those with negative charge in poplar trees (*Populus deltoides nigra*). Meanwhile, Zhu *et al* (2012) reported that positively charged AuNPs accumulated in radish to a greater extent than those with neutral and negative charge.

Based on the results, the lowest absorption and accumulation in tubers and shoots were observed in AgNPs-PVP and AgNPs-Citrat treatments. The low accumulation rate of AgNPs-Citrate in the tuber and shoots may be attributed to the fact that the electrostatic repulsion between the root surface and the nanoparticles with negative surface charge reduces the rate of absorption (Anjum *et al*., 2013). These results are in agreement with Cvjetko *et al.* (2017) who reported that the amount of uptake and accumulation of PVP and citrate treatments in the root of *Allium cepa* was similar and the lowest among the treatments. Seemingly, AgNPs (PEI-AgNPs) and $AgNO_3$ with positively charged surface were more easily transported from tuber to shoots compared to negatively charged AgNPs (citrate-AgNPs).

Based on the results of this study, it was found that accumulation of AgNPs by the plant was also affected by the type of coating of nanoparticles, while Torrent *et al.* (2019) reported that accumulation of AgNPs was influenced by size and concentration, but not by nanoparticle coating. Also, silver nitrate treatment had less absorption and accumulation than AgNPs-PEI treatment. This result is incompatible with published studies with other edible plants (Cvjetko *et al*., 2017b; Soria *et al*., 2017; Torrent *et al*., 2020; Vinković *et al*., 2017; Vishwakarma *et al*., 2017), as they indicated that the absorption and accumulation of silver nitrate treatment in plants were higher than AgNPs. Figure 3 clearly reveals that the amount of Ag accumulated at the fine-roots differs from the tuber and shoots. Accordingly, the highest accumulation was with $AgNO_3$ application, which was significantly different from the other treatments ($P< 0.05$). The amount of silver present in radish's fine root in $AgNO_3$-125 treatment was almost similar to the soil, indicating a high accumulation in the fine-roots. The high accumulation of AgNPs-citrate in the roots, which is the highest accumulation after $AgNO_3$, is also notable. However, due to the negative charge of these particles and root surface, the absorption rate of this type of nanoparticles is expected to be lower than the others. Meanwhile, the lowest accumulation was observed in AgNPs-PEI treatment, which was significantly different from other treatments ($P< 0.05$). Therefore, due to the high concentration in the tubers and shoots, it is clear that Ag was rapidly translocated from the roots, and this could be a serious threat to human health. Overall, as indicated by Gupta *et al*. (2019), it seems that the source and form of metals as well as the concentration of available metal in soil are the factors affecting AgNPs accumulation**.** As previously noted, plant properties also contribute to the uptake and accumulation of nanoparticles, and this may be related to the radish uptake properties. To the best of our knowledge, the mechanism of nanoparticle interaction with plants is not entirely understood, however, it is accepted that, depending on their properties, some nanoparticles may form complexes with membrane transporter proteins or root exudates resulting in their translocation into the plant system (Yadav *et al*., 2014). It is conceivable that such mechanisms are selective towards nanoparticles having a certain surface composition. One the other hand, one cannot exclude the possibility that nanoparticles completely dissolve during the exposure duration. The observed selectivity would then be related to the formation of

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Ag-complexes with the free ligands from the coating and with soil organic matter.

CONCLUSIONS

In this study, it became clear that different forms of silver, including bulk silver nitrate and silver nanoparticles with different coatings, had negative effects on dry weight of root, tuber, and shoot of radish. These effects increased significantly with increasing concentration of treatments, due to the increase in silver content in root media. These different coatings of silver nanoparticle also had different levels of accumulation in the root, tuber, and shoot. The silver nitrate form probably accumulated more in the roots due to its higher solubility and lower interaction with the soil components. Positively charged particles behave differently in terms of uptake than negatively charged particles or ions. Due to the positive charges on the surface of the nanoparticles, PEI treatment was rapidly absorbed by the negative charges of the root surfaces and was highly transmitted to the tuber and shoots, which can be a threat to human health. Based on the results, oral consumption of radish plant cultivated in contaminated soil, which is capable of storing large amounts of nanoparticles and silver nitrate, may have detrimental effects on human health. These findings should be completed by looking at the speciation of Ag in the different part of the plant and in the soil. In addition, we could also look for other application scenarios. For example, from aerial deposition on the leaf or from sludge amendment using contaminated sludge.

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اثرات عوامل پوشش سطح نانوذرات نقره بر تجمع آنها و رشد گیاه تربچه

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چکیده

استفاده گسترده از نانوذرات نقره در صنایع مختلف باعث بروز نگرانی های بسیاری در مورد سرنوشت این مواد شده است. از این رو مطالعه حاضر با هدف بررسی اثرات پوشش های سطح نانوذرات نقره در خاک و چگونگی برهمکنش آنها با گیاهان به عنوان اولین مرحله از زنجیره غذایی انسان انجام شد. گیاه

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تربچه(*Raphanus sativus*) در معرض نیترات نقره و نانوذرات نقره با سه نوع پوشش سیترات، پلی اتیلن ایمین و پلی وینیل پیرولیدون در غلظت های مختلف قرار گرفت. اثر غلظت (۵، ۲۵ و ۱۲۵ میلی گرم بر کیلوگرم خاک) و نوع پوشش سطح بر وزن خشک با مقدار کنترل مقایسه شد. نتایج نشان داد که نوع تیمار بر وزن خشک اثر معنی داری نشان داده و در بین تیمار ها نیترات نقره با ۵۱ درصد کاهش بیشترین کاهش وزن خشک برگ را نشان داد. مقدار کل نقره در ریشه، غده و برگ تربچه نشان داد که تجمع نانوذرات نقره تحت تاثیر پوشش و غلظت است. نانوذرات با بار مثبت (پلی اتیلن ایمین) بیشترین میزان انتقال را نسبت به تیمارهای دیگر نشان داد. در نهایت یافته های مطالعه حاضر نشان داد که تربچه توانایی ذخیره نقره در ریشه، غده و برگ ها در مقدار بالا را دارد بنابراین این گیاه پتانسیل دارد تا به عنوان یک منبع آلودگی نقره برای انسان تلقی شود.

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