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Phytoavailability of silver at predicted environmental concentrations: does the initial ionic or nanoparticulate form matter?†

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Silver nanomaterials (Ag NMs) have been known for centuries. Silver nanomaterials (Ag NMs) have been a part of our daily lives in the past few years due to their antimicrobial properties. Their success in applications including human health, surface coatings, food packaging, and textiles is due to the effective silver concentrations required for antimicrobial properties that are lower at the nanoscale than bulk materials and in some cases ionic Ag. Based on the life-cycle of Ag NM-enabled products, the main release pattern of Ag NMs into the environment is through wastewater treatment plants and sludge spreading in agricultural soils as fertilizer. Other unquantified sources of Ag NMs are pesticides where they are used against plant pathogens and insect pests, and deposits from incineration fumes. The predicted environmental concentrations (PEC) of Ag NMs in the soil range from 6 to 21 ng kg\(^{-1}\) in agricultural soils and 50–530 ng kg\(^{-1}\) in sludge-treated soils in Denmark.

Electronic supplementary information (ESI) available: Silver K-edge XANES spectra, isoelectric points, principal component analysis of the dataset, and soil characteristics. See DOI: 10.1039/c8en00644j
Ag NMs in the EU to range from 30 to 80 ng kg$^{-1}$ in natural and urban soils and from 1290 to 1390 ng kg$^{-1}$ in sludge-treated soils by 2020. In soils, porous waters, sludge, etc., Ag NMs are known to be physically and chemically unstable. They quickly undergo physical–chemical transformations (oxidation, sulfidation, dissolution, and adsorption/complexation with natural colloids) thereby altering their properties, mobility, fate and interactions with living organisms.\(^8\)

In realistic release scenarios, Ag NMs are expected to reach plant crops. Several studies have already assessed the phytotoxicity and phytoavailability of Ag NMs. However, most of them were hydroponic studies. Ag NMs were found to alter seed germination, plant biomass, and plant physiology, and to generate oxidative stress. The non-exhaustive list of factors governing Ag NM phytotoxicity included the size, shape, and coating of the NMs, the plant genotype, and the experimental methods.\(^9\) Plant roots were considered as the main route of Ag NM entry into plants with subsequent translocation to the shoots and leaves.\(^10–12\) Stegemeier \textit{et al.} \textbf{(2015)}\(^13\) studied the uptake and distribution of silver in alfalfa \textit{(Medicago sativa)} exposed to pristine Ag NMs, sulfidized Ag$_2$S NMs andionic Ag at 3 mg L$^{-1}$ in hydroponics. Despite marked differences in the release of Ag$^+$ ions from the nanoparticles, Ag NMs, Ag$_2$S NMs, and Ag showed similar quantitative patterns of association with plant roots and similarly limited (<1%) translocation of Ag to shoots. However, the local distribution of silver in plant roots was Ag speciation-dependent. Exposing wheat plants to Ag NMs, Ag$_2$S NMs and ionic Ag (3 mg L$^{-1}$) in hydroponics, Pradas del Real \textit{et al.} \textbf{(2017)}\(^14\) showed that various chemical transformations occurred in plant roots, leading to the dynamic exposure of the plant to multiple forms of silver.

The interactions between NMs and mineral phases, natural organic matter, and microbiota\(^15\) have to be taken into account when studying Ag phytoavailability and phytotoxicity in the soil.\(^16,17\) Dimkpa \textit{et al.} \textbf{(2013)}\(^18\) exposed wheat to Ag NMs in a sand matrix (0.5–2.5 mg kg$^{-1}$) and observed a dose-dependent reduction in growth. Pradas del Real \textit{et al.} \textbf{(2016)}\(^19\) spread Ag NM-amended sludge (18 and 400 mg kg$^{-1}$) on soil and detected Ag in the rhizosphere of wheat and rape, as nanosized Ag$_2$S, Ag–S species and mixed metallic sulfides with Zn and Cu. Following such exposure, Ag was undetectable in the soil pore water and poorly accumulated in plant shoots. Doolette \textit{et al.} \textbf{(2015)}\(^20\) studied the bioavailability of Ag in lettuce using a sandy soil amended with biosolids containing Ag$_2$S NMs and reported limited accumulation in shoots. Ag$_2$S NMs applied directly to a sandy soil at 1 mg kg$^{-1}$ were less bioavailable than Ag$_2$S NM-containing biosolids. In an outdoor lysimeter study, Schlich \textit{et al.} \textbf{(2017)}\(^21\) exposed canola and wheat to Ag NMs (1.7 and 8.0 mg kg$^{-1}$) via sewage sludge in a loamy-sand soil. Despite low remobilization of Ag from the soil to the percolating water, Ag uptake by roots showed that the chemical conditions in the rhizosphere induced Ag remobilization from the incorporated sewage sludge.

Several studies have deciphered the interactions between soil properties and Ag NMs\(^22,23\) but only a few studies have addressed the silver phytoavailability at PEC. The aim of the present study was to evaluate the impact of soil properties on Ag phytoavailability in tall fescue \textit{(Festuca arundinacea)}, using a set of soils with contrasting properties (clay, organic and carbonate contents, cationic exchange capacity, pH, etc.). We also studied the role of the form of silver using nanoparticulate Ag with inorganic (SiO$_2$) and organic (polyvinylpyrrolidone, PVP) coatings, and dissolved Ag (AgNO$_3$) as silver sources. To study Ag soil–plant transfers, we used a RHIZOTest device,\(^24\) which is based on a root mat placed in close contact with the soil. This technique allows easy and rapid harvesting of the whole plant and the soil. Recently, the RHIZOTest was shown to be a useful tool to analyze the phytoavailability of CeO$_2$ NMs at concentrations close to PEC in fescue and tomato.\(^16\) Here, we selected two concentrations lower than the ones already published in studies dealing with the phytoavailability of Ag NMs in soils i.e. close to Ag PEC in soils (0.0015 mg kg$^{-1}$) and 100 times higher than PEC (0.15 mg kg$^{-1}$).

### Materials and methods

**Ag nanoparticles and dissolved Ag**

Two Ag nanoparticles of similar size (TEM core diameter $\sim$50 nm) and shape (spherical) but with different coatings (PVP and SiO$_2$) with the trade names ‘50 nm PVP Nanoxact™ Silver’ and ‘50 nm silica coated Nanoxact™ Silver’ were obtained from nanoComposix (San Diego, CA). Dissolved silver was obtained from AgNO$_3$ (Sigma-Aldrich, Saint Louis, MI) dissolved in Milli-Q water. In the commercial stock suspensions, SiO$_2$-AgNPs were suspended in isopropyl alcohol at 1000 mg Ag L$^{-1}$ and PVP-AgNPs in Milli-Q water at 20 mg Ag L$^{-1}$. These stock suspensions were diluted in Milli-Q water (4.5 mg Ag L$^{-1}$ and 45 mg Ag L$^{-1}$) before soil contamination and characterized by transmission electron microscopy (JEOL JEM 2011), X-ray absorption spectroscopy (at the Ag K-edge on the BM30b FAME beamline, ESRF, Grenoble, France), dynamic light scattering (Nano ZS, Malvern, UK), and zeta potential measurements (Zetasizer, Malvern, UK).

After dilution in Milli-Q water, the SiO$_2$- and PVP-AgNPs contained crystallites of metallic silver (see ESI† Fig. S1, XANES Ag K-edge), with a bimodal TEM size distribution with two populations centered at $\sim$50 nm and $\sim$5 nm (Fig. 1). The population at 50 nm matched the size provided by the supplier. According to the TEM observation, the population at $\sim$5 nm was likely attributed to the dissolution/re-precipitation of silver due to changes in chemical equilibrium as a result of dilution (Fig. 1). Respectively, 2% and 6.5% of the total silver is in the ionic form in the diluted PVP-AgNP and SiO$_2$-AgNP suspensions as measured by ICP-MS following ultra-filtration (Amicon tubes, 3 kDa). The TEM pictures also revealed that the thickness of the SiO$_2$ coating around the Ag core was heterogeneous (varying between 10 and 30 nm). The zeta potentials of the PVP-AgNPs were negative ($–11 \pm 2$ mV in Milli-Q water) between pH 2 and 6 due to the negatively-charged PVP coating\(^25\) (see ESI† Fig. S2). However, for the SiO$_2$-AgNPs, a positive/negative inversion of the zeta potential occurred around pH 4, attributed to the SiO$_2$ coating\(^26\) (see ESI† Fig. S2).
Soil characteristics and treatments

Four soils with different pH values, organic matter and carbonate contents, cation exchange capacities (CEC) and particle size distributions were selected (Fig. 2 and ESI† Table S1). Clay and sandy loam were purchased from LUFA Speyer and were classified (WRB) as a vertisol coming from Hanhofen (Germany) and as a gleysol from Siebeldingen (Germany). The loam with high OM content was a luvisol sampled in a field under permanent pasture (Cote Saint-André, France) and the loam with high carbonate content was a rendosol sampled in a field that had not been cultivated for more than 10 years (Collias, France).

The soils were treated with PVP-AgNP and SiO₂-AgNP suspensions and dissolved silver solution with 0.0015 and 0.15 mg of Ag per kg of soil (dry mass). The lowest concentration was selected based on the range of PEC estimated.7

RHIZOtest experiment

The experimental procedure is detailed elsewhere.16,24 The RHIZOtest is a two-step procedure. The plant seedlings were first grown (preculture period) for 14 days in hydroponics in a cylinder closed at the bottom with a 30 μm polyamide mesh to promote the development of a dense, planar root mat. In the second step (culture period), the plants were pressed down firmly onto a 6 mm-thick layer of soil for a period of eight days. Immediately preceding the contact between the plant and soil, 300 μL of silver (in either the nanoparticulate or dissolved form) were added to the soil surface. Five replicates were performed for each condition (silver treatment × silver concentration × soil type) and control (no Ag added), representing a total of 140 plant pots. The whole RHIZOtest experiment was conducted in a growth chamber under controlled climatic conditions (16 h day, 200–400 μmol of photons m⁻² s⁻¹, 75% relative humidity and a temperature of 25 °C; 8 h night, 70% relative humidity and a temperature of 20 °C).

At the end of the culture period, shoots have been separated from roots by cutting and both parts were dried at 50 °C to measure their biomass. The plant samples were then burned for two hours in a platinum cup at 500 °C. The ashes were mixed with a few drops of pure water and 2 mL of nitric acid (6 mol L⁻¹) and the solutions were filtered using ashless filter paper in a volumetric flask. The filters containing the residues were burned for 30 min at 500 °C, moistened with a few drops of ultra-pure water, mixed with 2 mL of concentrated hydrofluoric acid and boiled on a hot plate until evaporation. The residues were mixed with a few drops of pure water and 2 mL of nitric acid (6 mol L⁻¹), and the solutions were transferred to the same volumetric flasks, which were topped up to the gauge level with ultra-pure water. A set of control plants was harvested at the end of the preculture period in hydroponics to determine the pool of silver in the plants before exposure to soil.

Ag concentrations and fluxes in plants

The total silver concentrations were determined with an inductively coupled plasma-mass spectrometer (Thermo Scientific iCAP Q ICP-MS using the kinetic energy discrimination mode and He as the collision gas). An internal solution containing Be, Sc, Ge, Ir and Rh was added on-line to the samples to correct signal drifts. A calibration curve including 4 points (0, 1, 5 and 10 ppb) was analyzed every 20–30 samples. For quality control, in-house reference samples and
Principal component analysis (PCA) was used to detect patterns in the dataset and to describe linear relations between the response variables (Ag flux, plant mass, pH, DOC and the concentration of Ag in the soil solution). The three first components (PC1, PC2, and PC3) accounted for 77.7% of the variability. The score plots showed clustering based on the soil type on the first component (see ESI† Fig. S3). The correlation loading plots (ESI† Fig. S3) indicated no significant correlation between the Ag flux and the other variables (pH and DOC in soil solution and biomass).

To test the simultaneous responses of the Ag flux variable to the environmental variables (soil characteristics, Ag treatments and concentrations), a permutation-based method (PERMANOVA) was used which requires the independence of the variables but not their normal distribution. According to the pseudo-Fisher ratio ($F$) and the $p$-values, the PERMANOVA revealed a highly significant effect of the Ag exposure concentration ($F = 90.2$, $p$-value < 0.001), the soil type ($F = 15.0$, $p$-value < 0.001) and the treatment ($F = 7.1$, $p$-value < 0.001) on the Ag flux.

In order to study the relationships between the response variables and the environmental variables, the principal component analysis (PCA) was completed by redundancy canonical analysis (RDA) using the ade4 (ref. 29) and adegraphics packages.²⁰

The PCA was carried out in the statistical environment R²⁸ using the ade4 (ref. 29) and adegraphics packages.²⁰

Differences among groups with multiple response variables were assessed using permutation-based multivariate analysis of variance (PERMANOVA, 1000 permutations) in Vegan.³¹

As the Ag flux fits a non-normal distribution, we used the non-parametric Kruskal–Wallis test followed by Dunn’s post hoc test in the R environment²⁸ to compare the Ag flux among the treatment groups. The $p$-values were corrected for false discovery rates due to multiple post hoc comparisons using the Benjamini–Hochberg (BH) method.³²

Simple regression was implemented in StatGraphics Centurion XVI.II and XLSTAT to describe the relationships between the outcome variable Ag flux and the predictor variables Ag concentration applied or in soil solution among the treatment groups. The lack-of-fit test was used to assess whether the selected model adequately described the observed data. The Durbin–Watson statistic tested the residuals to determine any significant autocorrelation based on the order in which they occur in the data file.

Results and discussion

Soil type-dependent Ag phytoavailability at PEC and 100× PEC

Principal component analysis (PCA) was used to detect patterns in the dataset and to describe linear relations between the response variables (Ag flux, plant mass, pH, DOC and the concentration of Ag in the soil solution). The three first components (PC1, PC2, and PC3) accounted for 77.7% of the variability. The score plots showed clustering based on the soil type on the first component (see ESI† Fig. S3). The correlation loading plots (ESI† Fig. S3) indicated no significant correlation between the Ag flux and the other variables (pH and DOC in soil solution and biomass).

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< 0.001) on the Ag flux. When the values of the control (no Ag added) were removed from the data set, the treatment no longer had an impact on the Ag flux variable (p-value > 0.05), showing that the phytoavailability of silver was basically the same under AgNP (PVP and SiO2-coated) and AgNO3 treatments.

As none of the response variables were correlated, redundancy analysis (RDA) was used to describe the response variables (Ag flux, plant mass, pH, DOC and Ag concentration in soil solution) with the set of environmental variables (soil characteristics (see ESI† Table S1), Ag treatments and concentration), used as explanatory variables. The results of the RDA are shown in Fig. 3. The angle between the response variables (black arrows) or between the environmental variables (red arrows) indicates the degree of linear correlation between them. The right-angled projection of centroid points (blue circles), indicating non-quantitative variables, onto a response variable (black arrows) approximates the relationship between these variables. The constrained part of the RDA explained 57.8% of the total variability. The first (F1) and second (F2) components accounted for 55.3% and 29.2% of the constrained variability, respectively. The pH and DOC in soil solution were the main contributors to F1 and both were negatively correlated (R = −0.92, R² = 0.85). The Ag flux and Ag in soil solution were the main contributors to F2. The contribution of the plant mass was low on both axes.

A permutation test revealed a highly significant linear relationship between the response variables and the explanatory environmental variables (p-value < 0.0001). The Ag flux was positively and strongly correlated with the exposure concentration of Ag (R = 0.97, R² = 0.95), negatively and strongly correlated with soil clay content (R = −0.84, R² = 0.88) and CEC (R = −0.91, R² = 0.84), moderately correlated with the DOC (R = −0.49, R² = 0.24) and carbonate concentration (R = 0.43, R² = 0.20), and poorly correlated with the soil organic matter content (R² = 0.05) and pH (R² = 0.01). The concentration of silver in the soil solution was best correlated with the clay content (R = 0.91, R² = 0.84) and the exposure concentration of Ag (R = 0.86, R² = 0.74) and poorly correlated with the other environmental variables (R² ≤ 0.35). The right-angled projections of the centroids for the Ag treatment variable set up a gradient along the Ag flux variable, with a negative contribution for controls (no Ag added), whereas in the other treatments, AgNO3, SiO2- and PVP-AgNPs, the contributions were positive. This highlights the fact that the trends in the Ag flux were similar under the SiO2-AgNP, PVP-AgNP, and AgNO3 treatments. The right-angled projections of the centroids of the soil variable on the Ag flux made some soils stand out: the clay soil that contributed negatively to the Ag flux and the loam with high carbonate content that contributed positively. The same analysis of the concentration of silver in soil solution variable emphasized the positive contribution of loam with high OM content, and the clay and loam with high carbonate content with similar but negative contributions.

The general trend showed the clay soil to have the lowest Ag concentration in the soil solution and the lowest Ag flux, the loam with the high carbonate content to have the lowest concentration of Ag in soil solution but the highest Ag flux, and the loam with high OM content to have a high concentration of Ag in soil solution but a low Ag flux. The sandy soil contributed poorly to these two variables. These relationships are discussed in more detail in the following sections.

Ag phytoavailability from AgNPs and AgNO3 at PEC is indistinguishable from that of geogenic silver.

In the literature, the lowest Ag concentration used in soil-plant transfer was 0.5 mg kg⁻¹ in a sand matrix.33 Here, we used the RHIZOtest as a bioassay to detect differences in Ag phytoavailability between treatments within the range of AgNP PEC in agricultural soils.7 Fig. 4 shows the relationship between the silver flux as a function of soil type and modalities. A modality is defined here as a silver treatment at a given exposure concentration. For the lowest concentration tested (0.0015 mg kg⁻¹), the Ag flux induced by AgNPs (SiO2- or PVP-coated) or AgNO3 did not significantly differ (p-value > 0.05) from the controls (no Ag added).

While the RHIZOtest was unable to detect differences with controls in Ag phytoavailability at 0.0015 mg kg⁻¹, it was sufficiently sensitive to detect a soil-dependent Ag flux at 0.15 mg kg⁻¹. Fig. 4 shows that for 7 out of 12 of the modalities, the Ag fluxes in fescue exposed to 0.15 mg kg⁻¹ of silver, as AgNO3 or AgNPs (SiO2- or PVP-coated), were significantly higher than those in the control groups. The Ag fluxes in fescue exposed to the clay and the loam soils with high carbonate content differed from those in the controls, regardless of the Ag treatment. In contrast, the Ag flux in fescue exposed to the loam soil with high OM content or the sandy soils did not significantly differ from those in the controls, except for the AgNO3 treatment in the loam soil with high OM content.

This result highlights the sensitivity of the RHIZOtest as a bioassay to detect differences in phytoavailability in soils.
w.png}

with contrasting properties, even at a Ag exposure concentration lower than the concentrations already published (0.15 mg kg⁻¹ in this study versus 0.5 mg kg⁻¹ (ref. 33)). The sensitivity of the RHIZOtest has already been highlighted with CeO₂ NMs.¹⁶

**Antagonist clay and carbonate effects on Ag phytoavailability at 100× PEC**

Fig. 4 shows that for a given modality, Ag fluxes always increased in the order clay < loam with high OM content < sandy loam < loam with high carbonate content (Ag fluxes being significantly lower in the clay soil than in the loam soil with high carbonate content). Fig. 5 shows the Ag concentration in soil solution plotted as a function of the exposure concentration and also highlights a repetitive pattern for a given modality. Significantly lower Ag concentrations in soil solutions were measured in the clay soil and loam soil with high carbonate content (pH 7.97), they could locally favor Ag immobilization in the vicinity of the root apex due to the plant exudation and the decrease in pH. This suggests that even if clays do not interact with Ag at the pH of the clay soil (pH 7.45) and loam soil with high carbonate content (pH 7.97), they could locally favor Ag immobilization in the vicinity of the root apex due to the plant exudation and the decrease in pH.

The clay and loam soils with high carbonate content mainly differed in their clay content, CEC, and carbonate content (Fig. 2). It is noteworthy that CEC and clay content are related since CEC arises from various negative charges on soil particle surfaces, especially those of clay minerals and soil organic matter. Together with the RDA (Fig. 3), the results presented in Fig. 4 and 5 show that (i) interactions between Ag and clay minerals hindered the Ag flux, and (ii) suggest a potential role for carbonates in Ag phytoavailability in the soil. Several studies already highlighted the major role of the physical–chemical properties of the soil in the mobility and transformation of Ag NPs in natural soils. Using three soils with property gradients (pH 4.6 to 8, clay content 12% to 27%, CEC 8.5 to 15 cmol kg⁻¹, and organic matter content 0.66 to 2.5%), Wang et al. (2015)¹⁴ correlated the mobility of PVP-AgNPs positively with pH, CEC, and organic matter content. Cornelis et al. (2012)¹⁵ used a larger set of 16 natural soils with a broader range of chemical and physical properties (pH 4 to 7.58, clay content 1 to 59%, CEC 0.2 to 65.7 cmol kg⁻¹, and carbon content 0.9 to 7%). These authors correlated the partitioning of Ag with the soil clay content and suggested the preferential adsorption of the negatively charged citrate-coated AgNPs on the positively-charged surface sites of clay-sized minerals. Recently, Zhou et al. (2017)¹⁶ demonstrated that negatively-charged AgNPs do not alter the stability of montmorillonite clay at pH 8, but interact with the edges of clays and coagulate at pH 4. At the apex of the plant roots, exudation of protons and organic acids usually locally decreases the pH to close to 4.5 to enable the uptake of nutrients. This suggests that even if clays do not interact with Ag at the pH of the clay soil (pH 7.45) and loam soil with high carbonate content (pH 7.97), they could locally favor Ag immobilization in the vicinity of the root apex due to the plant exudation and the decrease in pH.

In the loam soil with high carbonate content (with low clay content), the formation of Ag₂CO₃ could account for the low Ag concentration in the soil solution observed at 0.15 mg kg⁻¹ of AgNO₃, PVP- and SiO₂-AgNPs. Sekine et al. (2014)¹⁷ studied carbonate–Ag interactions in neutral (pH 6.9) and alkaline soils (pH 7.8). Ag₂CO₃ was only detected in the soils freshly spiked with AgNO₃ followed by its disappearance after three months of incubation. In the loam soil with high carbonate content, Ag₂CO₃ species are likely to precipitate (Ksp =
Fig. 6 Silver concentration in fescue leaves and roots (mg kg⁻¹) on the four soils (n = 5, ±SD). * represents significant differences (Kruskal-Wallis test) compared to the control.

8.5 × 10⁻¹²) as already observed by XAS.⁵⁷ Several studies have highlighted the ability of plants to change the pH of their rhizosphere. For instance, the roots of tomato and rape were shown to cause systematic acidification of calcareous soil.⁵⁸ Therefore, a decrease in pH in the vicinity of the root apex in the loam soil with high carbonate content could favor the dissolution of Ag₂CO₃ over time and hence influence Ag phytoavailability. This root-induced change in pH could explain the high fluxes observed in fescue in the loam soil with high carbonate content despite the low concentration of Ag in the soil solution.

Ag from AgNO₃ and Ag NPs is similarly phytoavailable and translocated at 100× PEC

Fig. 6 reports the silver concentrations in the roots and shoots of fescue exposed to AgNPs and AgNO₃ at PEC and 100× PEC, in the different soils. PERMANOVA revealed a significant impact of the Ag exposure concentration (F = 13.1, p-value < 0.0001), soil type (F = 4.1, p-value < 0.01), and treatment (F = 3.3, p-value < 0.01) on the Ag concentration in the shoots. Interestingly, no linear correlation was found between the silver concentration in the shoots and in the roots (R = 0.32, R² = 0.09).

The silver concentrations in the roots mainly mirrored the trend observed in Ag flux discussed in the previous sections. At PEC, the Ag concentration in the fescue shoots and roots following exposure to AgNPs (SiO₂- or PVP-coated) or AgNO₃, did not significantly differ (p-value > 0.05) from the controls (no Ag added). However, at 100× PEC, in 4 out of 12 modalities, the concentrations of Ag in shoots were significantly higher than those of the respective controls, as in the loam soil with high carbonate content for both Ag NPs (PVP- and SiO₂-AgNPs) and AgNO₃ and in the clay soil for SiO₂-AgNPs. This confirms the previous conclusion that soil parameters prevail over the form of silver (nanoparticulate or dissolved), in Ag phytoavailability and translocation to plant shoots.

At 100× PEC, neither the nanoparticulate (irrespective of the organic or inorganic coating) nor the ionic form modified the flux of Ag in fescue (Fig. 4). The behavior of Ag NPs in soil pore water (dissolution, complexation and aggregation) was investigated³⁹ and the coating (citrate vs. PVP) was shown to strongly affect the physical–chemical behavior of Ag NPs in terms of the aggregation state and partitioning to pore water. Here, we found that regardless of the coating (organic vs. inorganic) and the form (nanoparticulate vs. ionic) at 100× PEC, the Ag flux was not impacted. The RHIZOTest outputs should be however considered as an evaluation of the phytoavailability measured under standardized conditions, which consequently do not per se take into account some important processes in comparison with field experiments (e.g. chronic exposure to contaminants, plant life cycle, etc.).

Conflicts of interest

The authors have no conflicts to declare.

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