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Environmental Toxicology and Chemistry

Environmental Toxicology

PARTITIONING OF NANOPARTICLE-ORIGINATED DISSOLVED SILVER IN NATURAL AND ARTIFICIAL SEDIMENTS

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PARTITIONING OF NANOPARTICLE-ORIGINATED DISSOLVED SILVER IN NATURAL AND ARTIFICIAL SEDIMENTS

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Abstract: Sediments are believed to be a major sink for the silver nanoparticles (AgNP) in the aquatic environment, but there is a lack of knowledge about the environmental effects and behaviour of AgNP in sediments. The release of highly toxic Ag^+ through dissolution of AgNP is one mechanism leading to toxic effects in sediments. We applied an ultrasound-assisted sequential extraction method to evaluate the dissolution of AgNP and to study the partitioning of dissolved Ag in sediments. Ag was spiked into artificial (AS) and 2 natural sediments (HS, KS) as silver nitrate ($AgNO_3$), uncoated AgNP (uc-AgNP), or polyvinylpyrrolidone-coated AgNP (PVP-AgNP). In addition, the total body burdens (TBB) of Ag in the sediment dwelling oligochaete *Lumbriculus variegatus* were assessed over a 28-day exposure period. The dissolution rate was found to be similar between the uc-AgNP and PVP-AgNP. In all sediments dissolved Ag was mainly bound to the residual fraction of the sediment, followed by iron and manganese oxides or natural organic matter. In KS, dissolved Ag that originated from PVP-AgNP was relatively more bioaccessible resulting also in higher TBB in *L. variegatus*, than that from uc-AgNP or $AgNO_3$. In AS and HS, $AgNO_3$ was significantly more bioaccessible than AgNP. Our results highlight the importance of sediment properties and AgNP surface chemistry when evaluating the environmental exposure of AgNP. This article is protected by copyright. All rights reserved

Keywords: Sediment chemistry, Nanoparticles, Sequential extraction, Nanoecotoxicology, Silver

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INTRODUCTION

Silver nanoparticles (AgNP) have the highest degree of commercialization of all the nanomaterials and are applied in a wide variety of products ranging from anti-odor textiles to paints and medical products [1]. The expanding use results in the release of AgNP into the surface waters, mainly through untreated wastewaters and effluents from wastewater treatment plants [2].

Residence time of AgNP in the surface waters is typically short due to aggregation and further precipitation from the water column to sediments, which are believed to be the final sink for the particles in the aquatic environment [2,3]. Aggregation is induced by high ionic strength, when the electrolytes in the water suppress the repulsive diffuse double layer of the particles [3]. To prevent the aggregation, AgNP are typically coated to increase the repulsive force between the particles. Steric stabilization by polyvinylpyrrolidone (PVP) is a common way to increase the stability of AgNP [3]. Under environmental conditions where electrolytes and suspended solids are present, aggregation and sedimentation of AgNP are likely to happen despite the coating agents [4,5]. Effects and behavior of AgNP in surface waters have been intensively studied, but only a few articles concerning the environmental effects in sediment have been published.

The lack of sediment studies derives from the limited methodology to quantify and characterize the nanoparticles in complex environmental media. The tendency of AgNP to dissolve makes the quantification challenging, as it is difficult to separate dissolved Ag ions (Ag^+) from AgNP in complex media. Dissolution rate is dependent on the particle properties such as size, surface area and capping agent [6-8]. Environmental factors such as pH, ionic strength, thermodynamics, and the amount of stabilizing agents (e.g. natural organic matter, NOM) also affect the dissolution rate [8-11]. It has been suggested that the toxicity of AgNP can

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be explained solely by dissolved Ag^+ [12]. Recent studies indicate that AgNP are toxic also in the nanoparticle form and the toxicity of AgNP can be higher than Ag^+ alone [13-15].

From the ecotoxicological point of view, bioaccessible Ag can be considered a more relevant parameter than total concentration of the Ag in sediment. Bioaccessibility refers to the amount of chemical that can be taken up by the organism if the organism has access to the chemical [16]. The bioaccessibility of metals in environmental media can be investigated by sequential extraction methods. These methods have also been applied for use with AgNP in soil [17,18]. The partitioning of Ag spiked as uncoated AgNP (uc-AgNP) was found to be strikingly different compared to Ag spiked as silver nitrate ($AgNO_3$) or citrate-coated AgNP. Both AgNP were quickly immobilized, but the bioaccessible fraction of Ag spiked as uc-AgNP was slowly increasing over time resulting in values multiple times higher than those in soil spiked with $AgNO_3$. The differences among Ag compounds was more pronounced in the soil containing more organic carbon, and both the surface chemistry of the AgNP and the matrix properties were important to the partitioning of Ag [18]. Similar studies have not been conducted in the sediment before the present work. As sediments are a major sink for AgNP, it is crucial to study the fractionation of AgNP-derived Ag to better evaluate possible environmental effects.

The aim of our work was to study the partitioning of dissolved Ag in different sediments spiked with 2 types of AgNP, and to compare the results to $AgNO_3$-spiked sediments. Our hypotheses based on the soil study conducted by Coutris el al.[18] were that aging of AgNP can lead to increasing bioaccessible concentrations of Ag in spiked sediments, and that the sediment and AgNP properties have an effect on the partitioning of Ag. To test these hypotheses, ultrasound-assisted sequential extractions of Ag were performed [19] over a 28-day period in Ag-spiked artificial sediment and 2 natural sediments collected from lakes with different sediment

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characteristics. The effect of the polyvinylpyrrolidone (PVP) as a capping agent of AgNP was assessed by spiking the sediment with uncoated AgNP (uc-AgNP) and PVP-AgNP of roughly similar size. $AgNO_3$ was used as a reference material for the partitioning of dissolved Ag. In addition, the dissolution of AgNP was estimated using the sequential extraction data and the bioaccessible fraction determined by the sequential extraction was compared to the total body burdens (TBB) of Ag in the benthic oligochaeta *Lumbriculus variegatus*.

MATERIAL AND METHODS

Silver nanoparticles

Polyvinylpyrrolidone-coated AgNP (PVP-AgNP, purity 99.9 %, diameter 30-50 nm, polyvinylpyrrolidone content = 0.2 wt %) and uncoated AgNP (uc-AgNP, purity 99.5 %, nominal size 35 nm), were obtained as dry powder from Nanostructured and Amorphous materials Inc. (Nanoamor, Houston, USA). Analytical grade silver nitrate ($AgNO_3$, supplied by J.T. Baker, Deventer, Netherlands) was used as a Ag reference. The size distribution of AgNP was estimated by measuring the diameter of 344 uc-AgNP and 205 PVP-AgNP from 3 grids using the 25 000 x magnification of a transmission electron microscope (JOEL JEM-1400). The amount of dissolved Ag in readily suspended nanoparticles was estimated by inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin-Elmer model Optima 8300) after ultracentrifugation. Details of the characterization are given in the supporting information (SI).

Sediments

Natural sediments collected from Lake Höytiäinen (HS) and Lake Kuorinka (KS) and artificial sediment (AS) prepared in the laboratory were used in the experiments. HS and KS have been used as clean reference sediments in toxicity tests and contain minimal amounts of organic contaminants [20]. AS was prepared following OECD guideline 225 [21] and consisted

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of 75 % combusted quartz sand (grain size 100 - 200 µm), 5 % finely ground (< 500 µm) sphagnum peat (Kekkilä, Finland) and 20 % kaolin. Powder made out of the leaves of *Urctica dioica* (collected from Jyväskylä, Finland) was used as a food source and added simultaneously with the Ag compounds 24 h before the start of the experiment. The pH of the AS sediment was adjusted to 7.0 with calcium carbonate. Sediments were characterized for dry weight percent (dw%), loss of ignition (LOI), organic carbon content (OC), background Cd, Cr, Cu, Ni, Pb and Zn concentrations, total Ag concentrations, easily reducible Fe and Mn oxides (Fe_{ox}, Mn_{ox}), acid volatile sulfides (AVS) and simultaneously extracted metals (SEM). The details of the characterization and reagents used are given in the SI.

Test organisms

Lumbriculus variegatus oligochaetes were cultured at the University of Jyväskylä in large aquaria filled with artificial fresh water (AFW, hardness 1.0 mM L^{-1} as Ca + Mg) at a constant temperature of 20 °C and 16:8 light:dark regimes. Cellulose paper tissues were used as a substrate and worms were fed with ground tetra fish food once a week (Tetrawerke, Melle, Germany). *L. variegatus* were synchronized by cutting them in half 14 days before the experiment and only the posterior part were used. Synchronization was performed to ensure similar reproductive states of the test organisms.

Spiking of the sediment

Dry AgNP powder or $AgNO_3$ stock solution (25 g L^{-1}) were mixed to the sediments in glass jars with a rotating metal blade, and the jars were mixed at 12 rpm in an overhead rotating mixer for 24 h. The nominal Ag concentration for AgNP was 300 mg kg^{-1} (dw) and the concentration of $AgNO_3$ was 20 mg kg^{-1} (dw). The used concentration of AgNP was higher than estimated to be found in the environment [22]. The concentration was selected according to

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previous studies done in soil, where dissolution of AgNP and association of dissolved Ag with soil fractions was low [17,18]. To ensure the analytical precision, higher concentration of AgNP than $AgNO_3$ was used in the present study. Despite the elevated AgNP concentration, the processes studied in the present paper are environmentally relevant.

Experiment set-up and samples

The experiment was conducted in 3 replicates in 50 mL glass beakers. The spiked sediment (4.8 g of HS, 6.8 g of KS and 15 g of AS as dw) was transferred to the bottom of each test unit and the beakers filled with AFW (hardness 1.0 mM L^{-1}). Test units were let to equilibrate for 24 h before 3 synchronized *L. variegatus* were added to each beaker. The amount of sediment was calculated roughly to match a 1:50 ratio (w:w) as dw of test organisms to the amount of NOM in the sediment [21]. Temperature in the test room was kept constant at 20 °C and 16:8 light:dark regimes were used. The test units were constantly aerated, and pH was measured from the overlying water once a week.

The kinetic association of Ag with different sediment components and the total body burdens (TBB) of Ag in the test organisms were measured after 2, 5, 9, 14 and 28 days of exposure. After the exposure period, the worms were sieved out from the sediment and transferred into clean AFW for 6 h to empty their gut, dried at 105°C overnight, weighed and stored at -20°C for further metal extractions. The TBB of Ag in worm tissue was normalized for the total dry biomass (µg g^{-1} of Ag in dry tissue). When the TBB were linearly increasing as a function of time, the sediment uptake coefficients (k_s) were calculated from the slope (µg g^{-1} d^{-1} of Ag in dry tissue). For the sequential extractions, a sample of wet sediment (corresponding to 250 mg of dry sediment) was taken from each experimental unit, and processed immediately. The total metal concentrations were analyzed from the aqua regia -digested dry sediment

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samples (200 mg). In addition, a 5 mL water sample (acidified with 0.5 mL of 65 % HNO_3) was collected from each unit for the analysis of Ag in the overlying water (Discussed in SI). All the metal concentrations were analyzed by ICP-OES. Details of the sample treatment and analysis can be found in the SI.

Ultrasound-assisted sequential extraction

The ultrasound-assisted sequential extraction method was used to evaluate the association of Ag with 6 different sediment fractions [23]. Free Ag concentrations in pore water, bound to the exchangeable fraction, bound to carbonates, bound to Fe_{ox} and Mn_{ox}, and bound to NOM and sulfides were determined from the extraction solutions by ICP-OES. The residual Ag was calculated by subtracting the sum of other fractions from the total concentration of Ag in the sediment. The sum of free Ag in pore water and Ag in the exchangeable fraction is referred to as the bioaccessible fraction. Results are given as percentage of Ag based on the estimated dissolved Ag concentration in the treatment. Full experimental details can be found in the SI.

Dissolution of AgNP

Total extracted concentration of Ag in each sampling point ($\boldsymbol{exC}_{\mathbf{Agx(t)}}$) was calculated

$$\boldsymbol{exC}_{\mathbf{Agx(t)}} = \boldsymbol{F1}_{\mathbf{Agx}} + \boldsymbol{F2}_{\mathbf{Agx}} + \boldsymbol{F3}_{\mathbf{Agx}} + \boldsymbol{F4}_{\mathbf{Agx}} + \boldsymbol{F5}_{\mathbf{Agx}} \quad (1)$$

where, $\boldsymbol{F1}_{\mathbf{Agx}}$, $\boldsymbol{F2}_{\mathbf{Agx}}$, $\boldsymbol{F3}_{\mathbf{Agx}}$, $\boldsymbol{F4}_{\mathbf{Agx}}$ and $\boldsymbol{F5}_{\mathbf{Agx}}$ represent the concentration of Ag (mg kg^{-1} dw) in the extracted fractions of Ag spiked test sediment. The $\boldsymbol{exC}_{\mathbf{Agx(t)}}$ was used to estimate the dissolution rate, $\boldsymbol{d\%}_{\mathbf{AgNP(t)}}$, of AgNP in each test sediment at every exposure time

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$$d\%_{AgNP(t)} = exC_{AgNP(t)} \times (exC_{AgNO3(t)} / totC_{AgNO3}) / totC_{AgNP} \times 100 \quad (2)$$

where, $totC_{AgNO3}$ is total $AgNO_3$ concentration in the sediment and $totC_{AgNP}$ is total AgNP concentration in the sediment. The Equation 2 assumes that the partitioning of Ag^+ spiked as AgNP is similar with the partitioning of Ag^+ released from the AgNP.

Data treatment and statistical tests

The Ag measurements that fell under the method level of detection of ICP-OES (1.9 µg L^{-1}) were considered as zero. The non-parametric pairwise Wilcoxon rank sum test was used for the comparisons of the dissolution rates of the AgNP among the sediments, and exact Wilcoxon rank sum test for the comparison of the average bioaccessible Ag (9,14 and 28 days) among the treatments normalized to the total dissolved Ag. The significance threshold was set at 0.05 after Holm-Bonferroni step-down correction [24]. One-way ANOVA with Tukeys HSD post-hoc test ($p < 0.05$) was used to compare the absolute bioaccessible concentration of Ag among the different exposure times and different Ag treatments. Normality of the data was tested with the Shapiro-Wilk normality test and homogeneity of variance with Levene's test. Occasionally the assumption of normality was violated but concerning the robustness of the test, this does not preclude the use of parametric tests [25]. Analyses were performed using R version 3.0.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS AND DISCUSSION

Characterization of AgNP

The diameter of uc-AgNP measured from the pictures taken by transmission electron microscope varied from 11.9 to 48.8 nm resulting in a mean (± SD) diameter of 39.4 nm (± 12.6 nm), and from 7.5 to 74.8 nm for PVP-AgNP, resulting in a mean (± SD) diameter of 28.6 nm (±

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5.8 nm) (Supplemental data, Figure S1). Both AgNP formed large agglomerates when suspended in water (Supplemental data, Figure S1). Further characterization of AgNP in water suspension was not considered relevant, as AgNP were spiked into the sediment as dry powder. The amount of readily dissolved Ag in the freshly prepared AgNP suspension was 0.075 % (± 0.006) for PVP-AgNP and 0.30 % (± 0.01) for uc-AgNP.

Sediment characterization

In the HS sediment the pH stayed relatively constant (5.8 - 6.2) throughout the experiment, decreased in the KS sediment from 6.2 to 4.7 and increased from 7.0 to 8.3 in AS during the 28-day test period (Supplemental data, Figure S3). The AVS of the sediments was measured from the storage containers before the experiment (Table 1). The test sediments were then treated under oxic conditions and thus the amount of AVS at the start of the exposure is considered negligible in all sediments. The KS sediment was the only sediment containing a measurable amount of AVS, which suggests that AVS can be reformed in the anoxic layer of the sediment during the 28-day exposure period [26]. The concentration of Fe_{ox}, Mn_{ox}, Cd, Cr, Cu, Ni, Pb, Zn and OC was highest in the HS sediment, followed by KS and AS (Table 1). The variation of the total Ag concentrations in the spiked sediments was highest with the uc-AgNP, indicating possible heterogeneous distribution of uc-AgNP in the sediments (Table 1).

Dissolution rate of AgNP

Aquatic toxicity of AgNP is typically found to be several times lower than toxicity of Ag^+ [27]. Toxic effects often derive from Ag^+ released from the AgNP by dissolution. It is difficult to measure the dissolution rate of AgNP in sediment, as the dissolved Ag is rapidly bound by the sediment components [17,18]. In the present study, the dissolution rate was evaluated by assuming the partitioning of Ag^+ spiked as $AgNO_3$ and dissolved Ag released from the AgNP to

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be similar. A sequential extraction method accompanied with ICP-OES analysis was used to assess the concentration of Ag in different sediment fractions. These methods do not take into account the form of the Ag in the extracts of the final samples, but our assumption is that the amount of AgNP is minimal and Ag is mostly present as dissolved compounds (See discussion in the SI). Using Equations 1 and 2, the dissolution rate was estimated individually at each sampling point (Table 2). The association of Ag released from AgNP may however be slower than Ag spiked as $AgNO_3$, as AgNP need to first dissolve and $AgNO_3$ was spiked readily dissolved. Thus we considered the average dissolution among the sampling points to be the best estimate of the dissolution rate of AgNP in the test sediments. In HS the extractable concentration of Ag spiked as $AgNO_3$ at 14 days was unexpectedly low (Figure 1), leading to relatively high estimates of dissolution (Table 2). This estimate was excluded from the average, as the estimates of dissolution rates among the other sampling points were close to each other and similar decrease was not observed in the AgNP spiked sediments at 14 days. In KS and AS, the dissolution rate is given as an average of all sampling points (Table 2).

In the present experiment, the PVP-coating did not have an effect on the average dissolution of AgNP, as no significant difference was observed in the dissolution rates between the uc-AgNP and PVP-AgNP in the HS, KS or AS sediment ($p = 1.000$) (Table 2). Surface chemistry is reported to be one of the major factors influencing the dissolution rate of AgNP in addition to size and morphology of the particles [7,28]. Sterically stabilized particles, such as PVP-AgNP, have typically reduced dissolution rates compared to uncoated or electrostatically stabilized particles due to the physical protection provided by the coating [29]. However, the PVP-coating does not totally eliminate the dissolution of AgNP [30]. The similar dissolution rate between PVP-AgNP and uc-AgNP in our study can be explained by the interaction of AgNP with

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NOM in the sediments. NOM is known to increase the stability of AgNP by coating the particles both sterically and electrostatically protecting initially uncoated particles [31-33]. Also PVP-AgNP are influenced and coated by the NOM by displacing the PVP-coating or associating with the coating, which adds an additional electrostatic barrier [34]. NOM rich in sulfur and nitrogen complex has a great affinity to Ag and high potential to decrease the dissolution rate of AgNP by blocking the active sites on the surface [35]. In conclusion, we believe that the NOM-AgNP interactions protect particles from oxidation and further dissolution in a somewhat similar manner, leading to similar dissolution rates for uc-AgNP and PVP-AgNP.

The quality of NOM was not assessed in the present study, but may be one factor explaining the differences in the dissolution rates among the sediments, as the dissolution rate of AgNP was significantly higher for PVP-AgNP in HS than KS or AS ($p < 0.001$) but similar between KS and AS (Table 2). Significant difference was also found for uc-AgNP between HS and AS ($p < 0.001$) but not between KS and AS ($p = 0.747$). The high OC content was not observed to be a significant dissolution reducing factor, as dissolution increased with the sediment OC content (HS > KS > AS). Low pH has also been found to promote the dissolution of AgNP in water but in soils the dissolution was independent of pH possibly due to the increased coagulation of AgNP [8,10]. The dissolution in the soil suspension was however reported to be fast, and unmodified AgNP were concluded not to be persistent in the soil suspensions [10]. Our results indicate that the role of pH on the dissolution among different sediments is small but, in contrast to the soil suspensions, AgNP seemed to be persistent in the sediment, probably due to the fast interaction with NOM in the sediments protecting AgNP from dissolution.

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The partitioning of dissolved Ag in the sediments

The concentrations of Ag recovered from each fraction in AgNP-spiked sediments were normalized with the estimated total dissolved Ag, calculated using the average dissolution rates of AgNP. This was performed to add comparability between $AgNO_3$ and AgNP treatments. The association of AgNP with different sediment fractions was not assessed in the present study, as they were removed from the samples by the centrifugation.

The residual fraction. Ag in the residual fraction is considered to be highly stable and not expected to be released under conditions encountered in nature [19]. It consists of components such as resistant Ag-sulfides, Ag trapped into cavities of amorphous clays or minerals and metallic Ag reduced from Ag^+ by humic substances [19,36,37]. In all treatments, the residual fraction was the most dominant fraction binding dissolved Ag (Figure 1). Results compare well with the soil studies, where the majority of Ag is also reported to lie in the residual fraction [38,39]. In AS, the concentration of residual Ag was increasing during the experiment with all Ag compounds, indicating slower association of Ag in the AS sediment compared to natural HS and KS (Figure 1). The coarse grain size of AS can explain this observation. The AS sediment consisted mainly of 100 - 200 µm quartz sand while natural sediments HS and KS are reported to consist mainly of material under 65 µm; 79.0 % in HS and 77.9 % in KS [40].

The NOM and sulfides and the Fe_{ox}/Mn_{ox} fractions. Affinity of Ag towards NOM, sulfides, Fe_{ox} and Mn_{ox} is high [41]. The role of sulfides in our experiment can be considered negligible, as the test sediments were treated under oxidized conditions where sulfides are depleted**.** Thus, the NOM and sulfides fraction is considered to consist only of Ag bound by NOM.

The Fe_{ox}/Mn_{ox} fraction bound relatively more Ag (7.7-17 % of $AgNO_3$, 13-16 % of uc-AgNP and 10-13% of PVP-AgNP) than NOM (0.0-5.6 % of $AgNO_3$, 2.9-13 % of uc-AgNP and

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7.3-9.5 % of PVP-AgNP) with all Ag compounds in HS (Figure 1ABC). The high concentration of Fe_{ox} and especially Mn_{ox} in HS may explain the relatively higher association (Table 1).The NOM fraction seemed to be more important in binding Ag spiked as uc-AgNP (5.3-18 %) and PVP-AgNP (10-15 %) also in KS, as it was dominant over the Fe_{ox}/Mn_{ox} fraction (4.6-7.7 % with uc-AgNP and 4.4-6.0 % with PVP-AgNP) (Figure 1DEF). In contrast, the Fe_{ox}/Mn_{ox} fraction bound more Ag (7.0-18 %) than the NOM fraction (4.8-13%), when spiked as $AgNO_3$ in KS (Figure 1DEF).

As discussed earlier, NOM is likely to coat initially uncoated uc-AgNP and partially replace the PVP-coating on the particles [31-34]. Interactions between AgNP and NOM can increase the total surface area of NOM, as the molecules get well arranged on the surface of AgNP, opening more specific binding sites for Ag. The coating process is reported to be fast, and therefore expected to take place simultaneously with dissolution of AgNP [34]. The association can thus be intensified by the close distance of freshly released Ag^+ and NOM. Alternatively, formed NOM-coatings might be prone to oxidation by the H_2O_2 extraction, making also the surface of AgNP susceptible. This can lead to overestimation of the NOM-bound dissolved Ag in the samples.

In AS, NOM-associated concentration of Ag was decreasing during the experiment from 28 % to 15 % with $AgNO_3$, from 37 % to 16 % with uc-AgNP, and from 41 % to 14 % with PVP-AgNP. Also Ag associated on the Fe_{ox}/Mn_{ox} fraction decreased from 12 % to 6.8 %, from 6.2 % to 2.1 % and from 6.7 % to 3.2 % in $AgNO_3$, uc-AgNP and PVP-AgNP spiked sediment, respectively (Figure 1GHI). The coarse grain size of AS compared to natural sediments can explain the slower association kinetics, as discussed earlier.

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The carbonate fraction. The association of Ag on carbonates is reported to be low [17,18,38,39]. Our results compare well with those studies. In HS, the recoveries of dissolved Ag were 0.0-1.7 %, 0.0-1.8 % and 0.3-0.5 % spiked as $AgNO_3$, uc-AgNP and PVP-AgNP, respectively (Figure 1ABC). In KS, no Ag spiked as $AgNO_3$ was extracted from the carbonate fraction, but small proportion was recovered with uc-AgNP (0.0-2.3 %) and PVP-AgNP (1.2-3.4 %) (Figure 1DEF). In AS, 2.9 % of Ag spiked as $AgNO_3$ at 2 days was associated with the carbonate fraction but only marginal proportions in later sampling points or when spiked as AgNP (Figure 1GHI). More importantly, no Ag was recovered from the carbonate fraction in any of the Ag treatments at the end of the experiment.

The bioaccessible fraction. In the present study, more focus is given to the association of Ag on the bioaccesible fraction (sum of water-soluble and exchangeable Ag). The environmental relevance of this fraction is high, as bioaccesible Ag can potentially be bioavailable to benthic organisms.

In HS, the concentration of bioaccessible Ag spiked as $AgNO_3$ remained constant through the experiment; 2.9 % at the beginning and 3.8 % at the end of the experiment (Figure 1A). Uc-AgNP was not bioaccessible up to 9 days, but small amounts of bioaccessible Ag were extracted at 14 days (0.6 %) and at 28 days (0.4 %) (Figure 1B). In contrast to uc-AgNP, PVP-AgNP was more bioaccessible through the experiment; 1.5 % at the beginning and 0.9 % at the end of the experiment (Figure 1C).

In KS, at the 2-day sampling point, 3.0 % of Ag spiked as $AgNO_3$ was bioaccessible. The proportion decreased to under 0.5 % with longer contact times (Figure 1D). Of the Ag spiked as uc-AgNP, 0.5 % was bioaccessible at the beginning of the experiment, but no bioaccessible Ag could be recovered at the end of the experiment (Figure 1E). As in HS, the concentration of

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bioaccessible Ag originating from PVP-AgNP remained constant through the experiment; 3.0 % at the start and 2.7 % at the end of the experiment (Figure 1F).

In AS, 8.1 % of the $AgNO_3$-spiked Ag was bioaccesible at the beginning of the experiment. The proportion decreased with longer contact times, but still 0.8-4.6 % of Ag remained in the bioaccessible fraction throughout the experiment (Figure 1G). The proportion of bioaccessible Ag varied from 0.0 to 0.4 % with uc-AgNP and from 0.1 to 1.0 % with PVP-AgNP between 2-14 days, but no bioaccessible Ag was recovered at the end of the exposure from the AgNP-spiked AS sediment (Figure 1HI).

The bioaccessibility of Ag among the treatments was compared by using the average bioaccessible proportion of dissolved Ag between 9 to 28 days. Two first sampling points (2 and 5 days) were excluded, as the relatively high concentrations at 2 days ($AgNO_3$ in KS and AS and PVP-AgNP in KS) and relatively low concentrations at 5 days ($AgNO_3$ in KS and AS and PVP-AgNP in KS) indicated that the Ag compounds were not in equilibrium. In the HS and AS sediment, Ag spiked as $AgNO_3$ was more bioaccessible than AgNP-spiked Ag ($p < 0.001$) (Figure 2). Interestingly in the KS sediment, there was significantly more bioaccessible Ag in the PVP-AgNP treatment than in $AgNO_3$ or uc-AgNP treatments ($p = 0.004$-0.018), and the Ag concentration was similar as in the $AgNO_3$-spiked HS and AS sediments ($p = 0.140$-1.000) (Figure 2). The bioaccessibility of PVP-AgNP-spiked Ag was constantly higher than uc-AgNP-spiked Ag also in the HS sediment, but the difference was not significant (Figure 2).

Our results indicate that dissolved Ag released from the PVP-AgNP is more bioaccessible in the KS sediment than $AgNO_3$-spiked Ag. This result is consistent with earlier findings of the toxicity test conducted with *L.variegatus* in the same sediments, where PVP-AgNP was found toxic only in KS but the toxicity of $AgNO_3$ was highest in AS followed by HS and KS [42].

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Increased bioaccessibility of dissolved Ag spiked as PVP-AgNP was unexpected, as released Ag from the AgNP was predicted to be bound by the sediment in a similar manner as Ag in the $AgNO_3$ treatment. Coutris el al. [18] observed similar increased bioaccessibility of Ag spiked as uc-AgNP compared to citrate-coated AgNP or $AgNO_3$ in organic and mineral soil. They proposed that larger and more easily aggregated uc-AgNP might release Ag in the form of smaller particles and relatively stable complexes. The association kinetics of these compounds is speculated to be slow, shifting the equilibrium in the soil media towards bioaccessible fractions during the 70-day exposure time. This might not be the case in our study, as the dissolution of AgNP was found to be immediate and bioaccessibility of Ag increased only with PVP-AgNP. The bioaccessibility of Ag spiked as uc-AgNP was in contrast decreasing to negligible at 28 days in AS and KS (Figure 1EH).

The binding of Ag^+ on the surface of PVP-AgNP could explain the elevated bioaccessibility of dissolved Ag [7,8,43]. The affinity of pristine PVP to the Ag^+ has been experimentally proven to be low [35], but the PVP-coating is expected to be partly replaced and covered by NOM in the sediment [34]. It is possible that formed PVP-NOM-AgNP complexes loosely bound dissolved Ag, thus increasing the bioaccessible Ag in the test system. The quality of NOM, such as the quantity of sulfur and nitrogen groups, has a strong effect on AgNP-NOM reactions, and could partly explain the differences among the sediments [35]. Although our experimental data fit this hypothesis, it is based on strong speculation and more research is needed to reveal the mechanism behind the increased bioaccessibility of dissolved Ag in the PVP-AgNP spiked KS sediment.

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Total body burdens of Ag in L. variegatus

The bioaccessible concentration of dissolved Ag determined by sequential extractions was generally in good agreement with the TBB of Ag measured from *L. variegatus* in natural sediments, despite the fact that the digested worm samples contain also AgNP attached on or taken up by the test organisms. Results suggest that most of the Ag is taken up as dissolved Ag rather than AgNP in natural sediments. No conclusions whether the differences in TBB are statistically significant could be considered because of the small sample size resulting from the unexpected outliers and mortality in the HS sediment (See SI for details).

In HS, the TBB of Ag for *L. variegatus* were very similar between $AgNO_3$ and PVP-AgNP (Figure 3A). The TBB was linearly increasing (r^2 = 0.91-0.93) as a function of time in both treatments. The sediment uptake coefficients determined from the slope were: $AgNO_3$ = 3.04 μg g^{-1} d^{-1} and PVP = 2.61 μg g^{-1} d^{-1}. In uc-AgNP spiked HS, the TBB were smaller compared to other Ag compounds. No statistically significant difference on the absolute extracted bioaccessible concentration of Ag among the treatments was found (Figure 3AB).

In KS, Ag spiked as PVP-AgNP produced a higher TBB than other Ag compounds, as also predicted by the higher amount of Ag in the bioaccessible fraction (Figure 3CD). Significantly more bioaccessible Ag was extracted with PVP-AgNP at the 2-day sampling point compared to uc-AgNP (p = 0.020), and at the 28-day sampling point compared to $AgNO_3$ (p = 0.016) and uc-AgNP (p = 0.010). The TBB of Ag was linearly increasing as a function of time in PVP-AgNP treatment (r^2 = 0.87) and $AgNO_3$ treatment (r^2=0.93). The sediment uptake coefficients determined from the slope were: $AgNO_3$ = 1.31 μg g^{-1} d^{-1} and PVP-AgNP = 3.41 μg g^{-1} d^{-1}. No Ag was detected in the worms exposed to uc-AgNP (Figure 3C).

In AS, the TBB in PVP-AgNP-treated sediment peaked during the early exposure but

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then decreased and reached a plateau at 9 days (Figure 3E). A similar peak was seen after a 9-day contact time in $AgNO_3$ spiked sediment (Figure 3E), but the TBBs decreased after that, possibly due to the growth of the test organisms (Supplemental data, Figure S5). The concentration of bioaccessible Ag was significantly higher in the beginning of the experiment with $AgNO_3$ when compared to uc-AgNP ($p < 0.001$) and PVP-AgNP ($p = 0.017$) or 5-9-day sampling points with $AgNO_3$ ($p = 0.001$-0.017) (Figure 3F).

Selection of the equilibrium time is essential in the laboratory dosed sediment studies. For AgNP, there is no information available on the proper contact time with the sediment before adding the organisms. Short equilibrium times may overestimate the bioavailability as the association with the sediment may be slow and high amounts of Ag can stay bioavailable during the early exposure. On the other hand, dissolution of AgNP can lead to increasing bioavailability of Ag during the long equilibrium periods [18]. In the natural sediments, the short 24 h equilibrium time did not lead to increased bioaccessibility or bioaccumulation of Ag in the early exposure. In AS, the bioaccessibility of $AgNO_3$ was highest at the beginning of experiment. The slower equilibrium in AS can be explained by the coarser grain size of the AS sediment, where the association of dissolved Ag and AgNP is slower compared to relatively finer grain sized natural sediments [44]. Most likely also PVP-AgNP were bioavailable at the early exposure in AS, as the highest TBB was observed at the beginning of the experiment despite the low bioaccessibility of dissolved Ag. The decreasing TBB at the latter sampling points can be explained by the growth of the test organisms and the stronger complexation of PVP-AgNP with the sediment (also supported by the increasing amount of Ag in the residual fraction). In the uc-AgNP spiked sediments this effect was not seen, probably due to lack of stabilizing PVP-coating, resulting in more rapid association with the sediment components. Coleman et al. [45] concluded

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in their study that neither the coating nor the size of AgNP influenced the bioaccumulation factors of the Ag from the sediment to *L. variegatus*. For marine benthic polychaete *Nereis virens* the bioaccumulation was influenced by the AgNP coating agent [46]. In the present experiment, the PVP-coating on AgNP was found to promote the bioaccumulation of AgNP originated Ag. Our results also highlight the importance of media properties, which seemed to have a varying effect on the behavior of uc-AgNP and PVP-AgNP. The sufficient equilibrium time was also dependent on the media and AgNP properties. According to our results, equilibrium time of 24 h was sufficient for AgNP in natural sediments but in artificial sediment equilibrium times up to 9 days should be used.

In waterborne exposures, the estimated tissue residues at the point, where 50 % of individuals died, have been reported to be 19.5 µg g^{-1} for $AgNO_3$ and 148.5-169.8 µg g^{-1} for AgNP with varying capping agents [47]. In our experiments, the TBB of Ag exceeded these values in all tested sediments for $AgNO_3$, but mortality (as missing worms) was only observed in the HS sediment after 28 days exposure. In waterborne exposures the ingestion of the Ag is minimal, and comparing the values is complicated by the different uptake route. However, the TBB were linearly increasing in the natural sediments spiked with $AgNO_3$ and PVP-AgNP. Considering this, it is possible that in natural sediments KS and HS these compounds pose toxic effects in chronic exposures longer than 28 days.

CONCLUSIONS

The hypothesis that aging of AgNP would lead to a higher bioaccessible concentration of Ag in sediment was rejected as the concentration of bioaccessible Ag did not increase in any treatment during the 28-day test period. The PVP-coating and sediment properties had an influence on the association of Ag in the sediments as hypothesized; the dissolved Ag that

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originated from PVP-AgNP was more bioaccessible than that from uc-AgNP or $AgNO_3$ in the natural sediment KS. The dissolution rates of uc-AgNP and PVP-AgNP were estimated to be similar, thus varying among the sediments. The measured sediment characteristics, such as OC content, Fe_{ox}/Mn_{ox} or pH, could not explain the observed differences. More research on the NOM(-PVP)-AgNP interactions should be conducted to reveal the mechanisms behind our observations. Higher bioaccessibility of dissolved Ag resulted also in higher TBB in *L. variegatus*, indicating that the uptake of Ag happens mainly as dissolved Ag species rather than AgNP. Overall, our results suggest that the possibility of hazardous effects in the environment is lower for uc-AgNP than $AgNO_3$ or PVP-AgNP.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

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Data Availability—Data and associated metadata are available from the corresponding author (juho.e.rajala@jyu.fi).

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Figure 1. Relative distributions of dissolved Ag (normalized to estimated total dissolved concentration of Ag in sediments) associated with the different sediment fractions, water-soluble, exchangeable fraction, carbonates Fe and Mn oxides and organic matter (NOM). The sum of water-soluble and exchangeable fractions is referred to as the bioaccessible fraction in the text. Association was measured by sequential extractions after 2, 5, 9, 14 and 28-day contact times. Ag was spiked to Höytäinen (A,B,C), Kuorinka (D,E,F) and artificial (G,H,I) sediments as $AgNO_3$ (A,D,G), uncoated silver nanoparticles (uc-AgNP: B,E,H) and polyvinylpyrrolidone-coated silver nanoparticles (PVP-AgNP: C,F,I). Results are given as a mean of 3 replicates + SD.

Figure 2. The percentage of bioaccessible Ag of the total dissolved Ag in Höytiäinen, Kuorinka and artificial sediment spiked as silver nitrate ($AgNO_3$), uncoated silver nanoparticles (uc-AgNP) and polyvinylpyrrolidone-coated silver nanoparticles (PVP-AgNP). Each bar represents an average % (+SD) of bioaccessible Ag between 9-28 days contact time (total $n = 9$). Letters A and B represent groups that significantly differ from each other ($p < 0.05$) according to the exact Wilcoxon Mann-Whitney rank sum test with Holm step-down correction.

Figure 3. The total body burdens of Ag in dry *Lumbriculus variegatus* tissue (µg g^{-1} dw) (A, C, E) and the concentration of Ag (mg kg^{-1} dw) associated with the bioaccessible fraction (sum of fractions 1 and 2) of the sediment (B,D,F). Ag was spiked to Höytäinen (A,B), Kuorinka (C,D) and artificial (E,F) sediments as silver nitrate ($AgNO_3$), uncoated silver nanoparticles (uc-AgNP) and polyvinylpyrrolidone-coated silver nanoparticles (PVP-AgNP) and measured after 2, 5, 9, 14 and 28 days exposure. Results are given as a mean of 3 replicates (±SD).

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Table 1. Characteristics of the test sediments

	Höytiäinen	Kuorinka	Artificial
dw (%)[a]	18.7 (0.7)	43.6 (1.0)	68.8 (1.2)
LOI (%)[a]	10.6 (0.2)	4.20 (0.07)	4.73 (0.08)
OC (%)[b]	2.57 (0.04)	1.70 (0.00)	1.36 (0.07)
AVS (µmol g^{-1})[b]	ND	2.83 (0.68)	ND
SEM (µmol g^{-1})[b]	2.60 (0.32)	1.26 (0.06)	0.126 (0.064)
AVS-SEM[b]	-2.60 (0.32)	1.57 (0.70)	-0.126 (0.064)
Fe_{ox}[c]	19700 (730)	9840 (340)	153 (2)
Mn_{ox}[c]	20400 (920)	313 (26)	0.343 (0.020)
Cd[d]	2.37 (0.24)	1.07 (0.14)	ND
Cr[d]	67.4 (7.6)	33.4 (4.6)	9.27 (1.63)
Cu[d]	55.9 (10.8)	41.7 (8.2)	20.5 (13.1)
Ni[d]	51.6 (4.8)	35.6 (3.0)	4.81 (0.75)
Pb[d]	32.7 (2.7)	12.4 (1.1)	ND
Zn[d]	124 (13)	68.0 (7.0)	3.00 (1.14)
$AgNO_3$[d]	21.5 (4.0)	19.4 (0.7)	17.9 (2.2)
uc-AgNP[d]	178 (112)	171 (137)	315 (122)
PVP-AgNP[d]	196 (45)	262 (33)	278 (42)

a at the end of exposure, n = 3 (±SD)

b before the exposure, n = 2 (±SD)

c at the end of exposure, n = 3, mg kg^{-1} dry weight (±SD)

d at the end of the exposure, n = 6, mg kg^{-1} dry weight (±SD)

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dw = dry weight, LOI = loss of ignition, OC = organic carbon, AVS = acid volatile sulfides, SEM = simultaneously extracted metals, Fe_{ox} = easily reductable iron oxides, Mn_{ox} = easily reductable manganese oxides, $AgNO_3$ = silver nitrate, uc-AgNP = uncoated silver nanoparticles, PVP-AgNP = polyvinylpyrrolidone-coated silver nanoparticles , ND = not detected

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Table 2 Estimated dissolution of silver nanoparticles in the test sediments

	Höytiäinen		Kuorinka		Artificial	
	uc-AgNP (%)	PVP-AgNP (%)	uc-AgNP (%)	PVP-AgNP (%)	uc-AgNP (%)	PVP-AgNP (%)
2 d[a]	41.0 (2.8)	39.3 (3.6)	31.8 (3.0)	21.7 (2.8)	16.8 (4.8)	20.7 (1.0)
5 d[a]	28.8 (5.0)	40.5 (5.7)	38.8 (7.4)	27.6 (4.0)	20.3 (7.1)	23.0 (3.1)
9 d[a]	35.9 (8.3)	38.0 (5.4)	42.8 (38.5)	32.3 (6.0)	20.6 (6.4)	21.2 (1.8)
14 d[a]	91.2 (10.1)[b]	66.4 (10.3)b	31.0 (12.5)	19.7 (4.1)	25.9 (3.3)	29.3 (3.0)
28 d[a]	29.3 (2.5)	30.2 (1.9)	11.3 (2.2)	14.4 (1.9)	14.1 (0.6)	14.5 (0.7)
Average	33.8 (6.8)	37.0 (5.7)	31.1 (19.2)	23.1 (7.3)	19.8 (5.8)	21.7 (5.2)

a mean of 3 replicates ± SD

b excluded from the average

uc-AgNP = uncoated silver nanoparticles, PVP-AgNP = polyvinylpyrrolidone-coated silver nanoparticles

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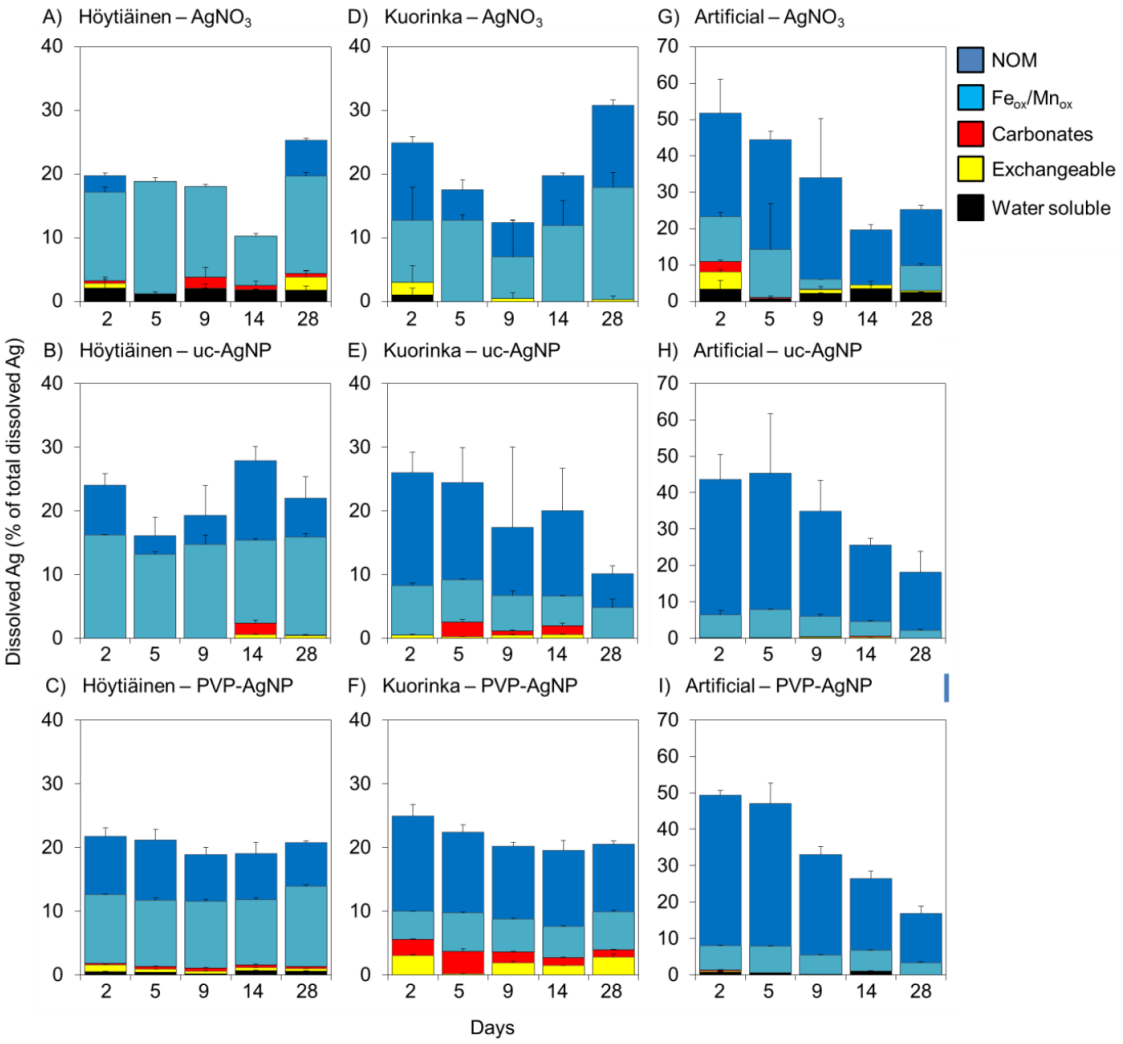

Figure 1

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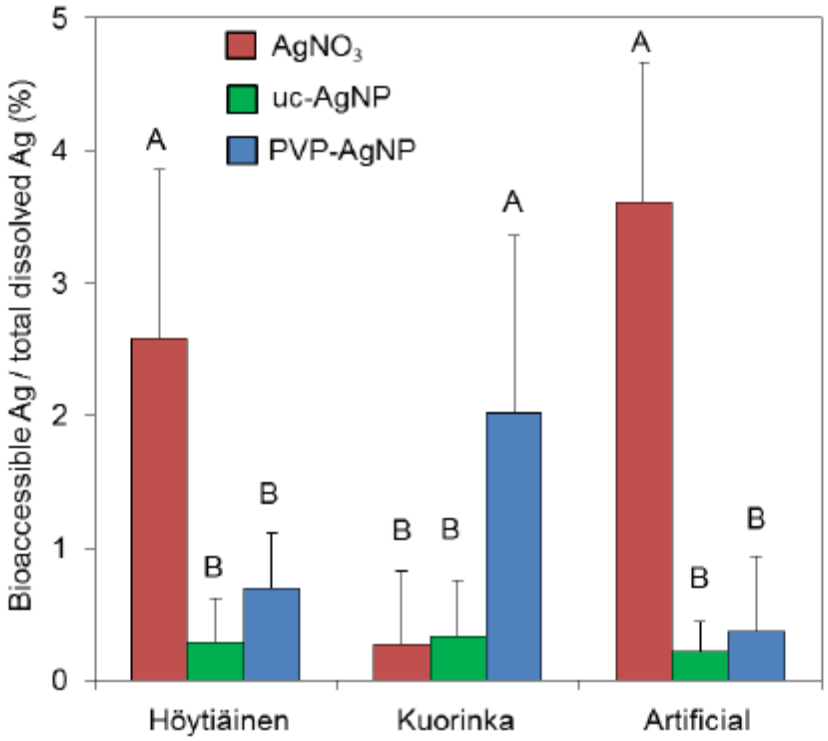

Figure 2

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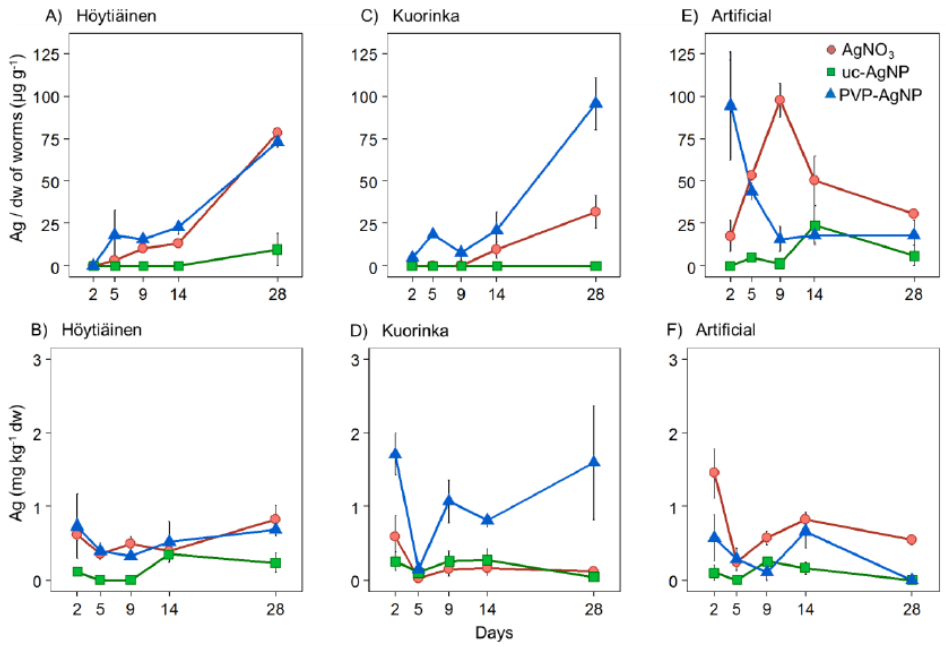

Figure 3

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