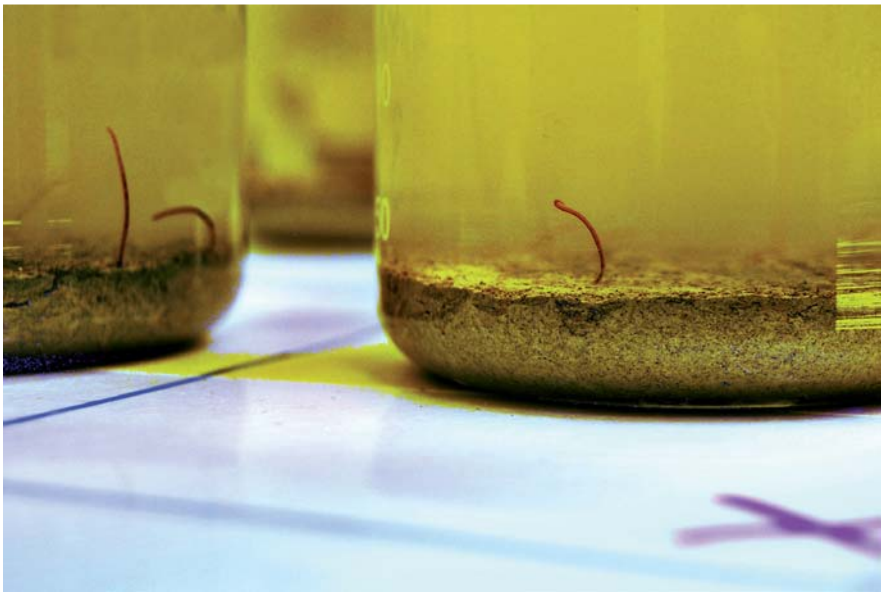
JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE

331

Juho Rajala

Behaviour of Sediment-Associated Silver Nanoparticles and Their Toxicity to *Lumbriculus variegatus*



JYVÄSKYLÄN YLIOPISTO

JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE 331

Juho Rajala

Behaviour of Sediment-Associated Silver Nanoparticles and Their Toxicity to *Lumbriculus variegatus*

Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston Ylistönrinteellä salissa YAA303,
kesäkuun 22. päivänä 2017 kello 12.

Academic dissertation to be publicly discussed, by permission of
the Faculty of Mathematics and Science of the University of Jyväskylä,
in Ylistönrinne, hall YAA303, on June 22, 2017 at 12 o'clock noon.

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2017

Behaviour of Sediment-Associated Silver Nanoparticles and Their Toxicity to *Lumbriculus variegatus*

JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE 331

Juho Rajala

Behaviour of Sediment-Associated Silver Nanoparticles and Their Toxicity to *Lumbriculus variegatus*

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2017

Editors
Anssi Lensu
Department of Biological and Environmental Science, University of Jyväskylä
Pekka Olsbo, Sini Tuikka
Publishing Unit, University Library of Jyväskylä

Permanent link to this publication: http://urn.fi/URN:ISBN:978-951-39-7105-2

URN:ISBN:978-951-39-7105-2
ISBN 978-951-39-7105-2 (PDF)

ISBN 978-951-39-7104-5 (nid.)
ISSN 1456-9701

Jyväskylä University Printing House, Jyväskylä 2017

ABSTRACT

Rajala, Juho
Behaviour of sediment-associated silver nanoparticles and their toxicity to *Lumbriculus variegatus*
Jyväskylä: University of Jyväskylä, 2017, 51 p.
(Jyväskylä Studies in Biological and Environmental Science
ISSN 1456-9701; 331)
ISBN 978-951-39-7104-5 (nid.)
ISBN 978-951-39-7105-2 (PDF)
Yhteenveto: Hopeananohiukkasten käyttäytyminen ja toksiset vaikutukset sedimentissä *Lumbriculus variegatus* -harvasukamadolle
Diss.

The increasing use of silver nanoparticles (AgNPs) in industry and consumer products results in the increasing environmental concentrations of AgNPs. In the aquatic environment, sediment is the major sink for the AgNPs. The behaviour and toxicity of AgNPs in sediments is largely unknown, and therefore the potential risk to the benthic ecosystems is difficult to predict. The aim of this thesis was to study the toxicity and uptake of uncoated AgNPs (uc-AgNPs) and polyvinylpyrrolidone-coated AgNPs (PVP-AgNPs) to *Lumbriculus variegatus* in sediment exposures. Also, the association of AgNP-originated dissolved Ag with sediment components was studied using a sequential extraction method. Studies were conducted in natural and artificial sediments using silver nitrate ($AgNO_3$) as a positive control for the toxicity and behaviour of silver ions (Ag^+). The toxicity of AgNPs was lower than toxicity of $AgNO_3$ in all the tested sediments. The properties of both the sediment and AgNPs were found to influence the environmental behaviour of AgNPs. The PVP-coating on AgNPs increased the bioaccumulation of Ag in alkaline artificial and natural sediments, whereas the bioaccumulation of uc-AgNPs was low. The toxicity of uc-AgNPs and PVP-AgNPs was comparably enhanced in acidic artificial sediment. Firstly, the dissolution of AgNPs was increased by low pH and secondly, the toxicity of Ag^+ was further enhanced in acidic sediment. AgNP-originated dissolved Ag was observed to associate strongly with the sediment components, and only a small proportion of Ag was bioaccessible. The bioaccessibility differed among the sediments and was higher with PVP-AgNPs than uc-AgNPs. The toxicity of AgNPs seemed to be a function of Ag^+ and no nano-specific toxicity was observed. According to our findings, the acute toxicity of AgNPs to *L. varigatus* is not expected to be higher than the toxicity of $AgNO_3$.

Keywords: Bioaccessibility; *Lumbriculus variegatus*; sediment; sequential extraction; silver nanoparticles; toxicity; silver nitrate.

Author's address Juho Rajala
Department of Biological and Environmental Science
P.O. Box 35
FI-40014 University of Jyväskylä
Finland
juho.e.rajala@jyu.fi

Supervisors Professor Jussi Kukkonen
Department of Biological and Environmental Science
P.O. Box 35
FI-40014 University of Jyväskylä
Finland

Docent Eeva-Riikka Vehniäinen
Department of Biological and Environmental Science
P.O. Box 35
FI-40014 University of Jyväskylä
Finland

Docent Ari Väisänen
Department of Chemistry
P.O. Box 35
FI-40014 University of Jyväskylä
Finland

Reviewers Dr. Markus Sillanpää
Finnish Environment Institute
Laboratory Center of Ecotoxicology and Risk Assessment
Hakuninmaantie 6
FI-00430 Helsinki
Finland

University Lecturer Olli-Pekka Penttinen
Department of Environmental Sciences
University of Helsinki
Niemenkatu 73
FI-15140 Lahti
Finland

Opponent Associate Professor Henriette Selck
Roskilde University
Department of Science and Environment
Universitetsvej 1
DK-4000 Roskilde
Denmark

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I-III.

I have planned the studies II and III with help from the co-authors. I participated in the planning of the first paper, mainly done by JJS-F and JVKK. JA and KM contributed to the writing of the paper I and E-RV to the writing of each study. The elemental analyses for each study were done by AV, who also contributed to the writing of the studies I and II. I have done all the other experimental work, data analyses and wrote the first draft for each study.

I Rajala J.E., Mäenpää K., Vehniäinen E.-R., Väisänen A., Scott-Fordsmand J.J., Akkanen J. & Kukkonen J.V.K. 2016. Toxicity testing of silver nanoparticles in artificial and natural sediments using the benthic organism *Lumbriculus variegatus*. *Archives of Environmental Contamination and Toxicology* 71(3): 405–414.

II Rajala J.E., Vehniäinen E.-R., Väisänen A. & Kukkonen J.V.K. 2017. Partitioning of nanoparticle–originated silver in natural and artificial sediments. *Environmental Toxicology and Chemistry*. In press.

III Rajala J.E., Vehniäinen E.-R., Väisänen A. & Kukkonen J.V.K. Toxicity of silver nanoparticles to *Lumbriculus variegatus* is promoted by low sediment pH. Submitted manuscript.

1 INTRODUCTION

1.1 Nanoparticles in general

The European Commission has defined nanomaterials as natural or artificial materials consisting of particles of which over 50 % in the number size distribution are in the nanoscale (1–100 nm) or materials that have at least one dimension in the nanoscale (Anon. 2011). These small particles are referred as nanoparticles (NPs). The definitions are under a criticism as they are always arbitrary and materials may have nano-specific properties without meeting the composition or size cutoffs (Maynard 2011). However, all NPs are small and have structures in the nanoscale. The small size of a NP is difficult to comprehend but can be illustrated with a simple comparison; the relative size of a tennis ball to the moon is similar to the size of a NP compared to a tennis ball (Fig. 1).

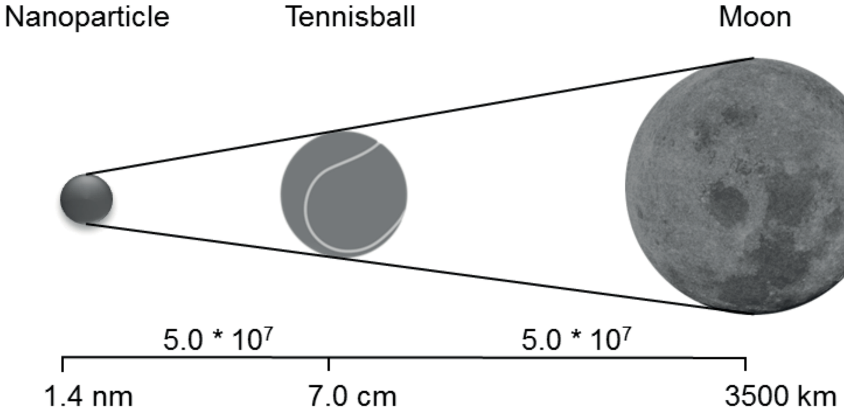


FIGURE 1 The diameter of a nanoparticle (1.4 nm) is 5.0 * 10^7 times smaller than the diameter of a tennis ball (7.0 cm) which is 5.0 x 10^7 times smaller than the diameter of the moon (3500 km).

Due to the small size, NPs have a relatively high amount of surface electrons and a large surface area. This special but common feature for all NPs makes them potentially very reactive and promising for industrial and commercial purposes. The use of NPs in commercial products has been increasing annually. The consumer products inventory lists 52, 1015 and 1814 products containing NPs in the years 2005, 2010 and 2015, respectively (Vance *et al.* 2015). The majority (42 %) of the products are categorized under the Health and Fitness group, containing for example, personal care items, clothing, cosmetics and sports goods (Vance *et al.* 2015).

The most used materials in NP production are: metals (Ti, Ag, Zn, Au), carbon and silicon (Vance *et al.* 2015). Nanoparticles can be produced in a variety of shapes (spheres, rods, triangular, tube, cubes etc.). The used material and shape depends on the application. For example, relatively long (10 µm) carbon nanotubes can be used to produce super durable fibers and small spherical gold nanoparticles are promising as drug delivery vectors (Dalton *et al.* 2003, Jewell *et al.* 2011). In addition to morphology and core material, the properties of NPs can derive from their surface chemistry. The surface chemistry is often modified during or after the synthesis by coating agents (Sperling and Parak 2010). Coatings are typically used to create electrostatic or steric forces between the particles. These forces prevent aggregation and agglomeration and allow the production of stable NP suspensions. It is also possible to add functional coatings on the NPs. The functional coatings are used on gold NPs, for example, to enhance the penetration of cell walls for medical drug delivery purposes (Jewell *et al.* 2011).

Considering the size, material, coatings, and morphology, the variety of NPs is almost infinite. All these parameters are important for the function of the NPs, which highlights the huge potential of nanotechnology in the future.

1.2 Challenging field of aquatic nanoecotoxicology

1.2.1 From ecotoxicology to nanoecotoxicology

The increasing use of nanomaterials leads to increasing release of NPs into the environment, raising concerns over possible harmful effects on ecosystems. Ecotoxicology is a relatively new brand of toxicology. It is defined as "the branch of toxicology with the study of toxic effects, caused by natural or synthetic pollutants, to all levels of biological organization from the molecular to whole communities and ecosystems, in an integral context" (Truhaut 1977). Adapting 'nano' with the term ecotoxicology is more recent. The first scientific papers concerning nanoecotoxicology came out in 2006 (Adams *et al.* 2006, Lovern and Klaper 2006). The special properties of nanomaterials pose multiple challenges for the nanoecotoxicological research (Cerillo *et al.* 2017, McGillicuddy *et al.* 2017). Although the number of publications addressing these challenges is increasing, many questions still remain.

Standardized test protocols have been developed for the use of regulatory ecotoxicity testing. The idea behind the standardization is to harmonise the test methods and conditions used. The protocols give detailed information from laboratory practises to data analysis methods. This way the environmental fate and safety of emerging chemicals can be evaluated under controlled laboratory conditions. Standardised test guidelines are provided by international organisations (Organization for Economic Co-operation and Development, OECD), institutions (American Society of Testing and Materials Committee on Biological Effects and Environmental Fate) and federal agencies (U.S. Environmental Protection Agency). Generally, these guidelines have also been proposed to be applicable in use with NPs (Crane *et al.* 2008, Kühnel and Nickel 2014, Petersen *et al.* 2015). All these protocols have however been designed for conventional chemicals, not considering the special properties of nanomaterials. Several modifications for the guidelines have been proposed and still need to be developed, especially considering the characterization, dosimetry and spiking of test media (Kühnel and Nickel 2014, Petersen *et al.* 2015).

1.2.2 The problem of the characterization

A lot of effort has gone into improving the characterization techniques of NPs in the last years. The solid state of NPs makes the characterization more demanding than with conventional chemicals, where the concentration and chemical speciation are usually adequate parameters. The comprehensive characterization of NPs should involve morphology, size distribution, surface chemistry, agglomeration status, possible dissolution rate and the speciation of the dissolved compounds (Laborda *et al.* 2016). All these factors may be important for the toxicity and behavior of NPs. Several techniques have been developed and applied in simple media. The most commonly used techniques are dynamic light scattering and electron microscopy coupled with energy-dispersive-X-ray spectroscopy to allow the determination of elemental composition (Murdock *et al.* 2008, Dudkiewicz *et al.* 2011). Transmission electron microscopy is often used to obtain size, shape, aggregation status and size number distribution of NPs (Dudkiewicz *et al.* 2011). Scanning electron microscopy produces 3D images and gives additional information about the morphology (Dudkiewicz *et al.* 2011). Dynamic light scattering gives also information about particle size distribution and aggregation status (Murdock *et al.* 2008). Advantage of the dynamic light scattering over the electron microscopy techniques is that it does not require dried samples but is done in suspension. The samples are thus more representative.

The problem arises when NPs are introduced into environmental media. The properties of the media, such as ionic strength, pH, concentration of organic matter and other complexing agents, have a great influence on NP behaviour (Lowry *et al.* 2012). In the worst case, the initial characterization does not necessarily represent the properties of media-associated NPs at all. For example, initially stable suspensions of NPs can rapidly aggregate and precipitate in aquatic media. Recently, the single particle-inductively coupled plasma-mass spectrometry has proven to be a promising new technique to simultaneously characterize size, size distribution, aggregation status and mass of NPs, also directly from environmental media (Kim *et al.* 2017).

To avoid drawing wrong conclusions, the characterization of NPs is recommended to be done in the test media at the beginning and at end of the exposure (Handy *et al.* 2012). In addition, the characteristics should preferably be studied during the test to ensure stability and consistent exposure (Handy *et al.* 2012). In environmental media, the characterization of NPs is more complicated and not always possible. As environmental media typically contains suspended solids and natural organic matter, the detection of NPs is difficult. Organic NPs in particular are difficult to separate from the organic background of the media. Highly sensitive techniques to detect AgNPs are also required, because environmental concentrations of AgNP are typically low (Handy *et al.* 2012).

When considering highly complex media, such as sediment, not much can be done with current methodology (Tiede *et al.* 2008, Petersen *et al.* 2015). The total concentration of inorganic NPs can be assessed with acid digestion. It does not tell us much about the NPs, as they dissolve during the digestion. The strong reactivity of NPs leads to interactions with the sediment components. Therefore, a more detailed characterization needs an additional separation step before the characterization is possible (Plathe *et al.* 2010, Von der Kammer *et al.* 2011). The separation step usually involves the use of strong solvents, which can alter the state of NPs and further influence the results (Tiede *et al.* 2008). The most promising techniques to characterize NPs from sediments without extensive modifications are chromatographic techniques and especially flow field-flow fractionation (Cumberland and Lead 2009, Tiede *et al*. 2009, Koopmans *et al.* 2015). Overall, there are no standard methods to characterize NPs in complex environmental media. The lack of proper characterization methods is a major step back in sediment studies.

1.2.3 Question of dosimetry

With most conventional chemical substances, mass concentration is typically used to express the exposure, as it reflects the number of molecules. Mass concentration is often used also with NPs, but it might not be the most suitable dose metrics. The problem arises from the insoluble or partly soluble nature of many NPs and the varying size distribution. Thus, the mass concentration does not reflect the number of NPs in the exposure media. Particle number (Wittmaack 2007), number size distribution (Kennedy *et al.* 2012, Rosário *et al.* 2016) and surface area (Schmid and Stoeger 2016), have been proposed to be better dose metrics over traditional mass concentration. However, it is unlikely that any of the suggested dose metrics are better than the mass concentration for all NPs in all systems (Petersen *et al.* 2015). Therefore, the mass concentration is still widely used. Sufficient characterization of NPs, including at least particle size, size number distribution, aggregation status and shape, in all environmental studies is recommended so that different dose metrics can be applied to the data later if one of them proves to be more suitable than mass concentration (Anon. 2012).

1.2.4 Spiking of the test media

Toxicity testing of NPs in aqueous media requires stable suspension of NPs in the test media. If particles tend to aggregate, they will eventually precipitate from the test media preventing the exposure (Handy *et al.* 2012). The stability of NPs can be increased with surface stabilizers (Sperling and Parak 2010). As these stabilizers are also used in commercial NPs, toxicity testing of surface stabilized particles is reasonable. Many of the particles however are not stable in aqueous media and external stabilizers need to be used. The use of organic solvents is not recommended due to the possible toxic effects involved (Petersen *et al.* 2014). Use of natural organic matter (NOM) as a surface stabilizer is promoted by the low toxicity and natural relevance, but NOM may also have a strong decreasing effect on the toxicity and dissolution of NPs (Fabrega *et al.* 2009, Liu and Hurt 2010). Overall, the use of dispersants must always be carefully considered and accompanied with the adequate control treatments (Handy *et al.* 2012, Petersen *et al.* 2014).

The most common way to spike NPs into sediment is direct addition as dry powder or in suspensions (Handy *et al.* 2012). If dry powder is used, aggregation status of NPs is expected to be high, but spiking of high concentrations is possible. In suspension, NPs are less aggregated, but the liquid media is transferred into the sediment together with NPs. This increases the liquid content of the sediment, and thus limits the possible concentration range.

Another strategy is to spike NPs indirectly into the overlying water and let them settle onto the sediment (Handy *et al.* 2012). This is a more environmentally realistic way to spike the NPs, as it mimics the natural route of NPs from overlying water to the sediment. The method is suitable for use with epibenthic organisms that feed at the surface of the sediment. If endobenthic sediment burrowing organisms are used, direct addition of NPs to the sediment should be preferred as these organisms feed below the sediment surface.

The general recommendation for the spiking of NPs into environmental media is to ensure that the exposure takes place in the most environmentally relevant way (Crane *et al.* 2008). Until more specific recommendations and standard protocols are available, the spiking procedure should always be carefully considered, taking into account the properties of the NPs, media, and the behaviour of the used test species.

1.3 Silver nanoparticles

1.3.1 From products to environment

Silver nanoparticles (AgNPs) are the most used of all NPs, when measured as the percent of manufactured commercial products (Vance *et al.* 2015). Due to the unique thermal, electrical, optical and antibacterial properties, AgNPs are used in paints, cleaning agents, textiles, electronics, cosmetics, anti-microbial coatings and medical applications (Piccinno *et al.* 2012, Hansen *et al.* 2016). The estimated annual production is 5.5–500 t (Aschberger *et al.* 2011, Piccinno *et al.* 2012, Windler *et al.* 2013, Sun *et al.* 2014). The increasing use of AgNPs in industry and consumer products leads to release of AgNPs into the environment. Sun et al. (2014) estimated that ~ 6.5 % of annually produced AgNPs will reach the surface waters. The release of AgNPs to surface waters takes place mainly via wastewater treatment plants and untreated waste waters (Sun *et al.* 2014). Majority of AgNPs is expected to precipitate from surface waters into sediments (Quik *et al.* 2014, Velzeboer *et al.* 2014).

1.3.2 AgNPs in the aquatic environment

The properties of AgNPs in the aquatic environment are likely to be greatly modified from the initial ones in stock suspensions or dry conditions. Aggregation, dissolution and surface modifications are the most important processes affecting AgNPs. Both the initial properties of the AgNPs and the properties of the surrounding media have major effects on the behavior of AgNPs in the environment.

AgNPs are typically produced by reduction of Ag^+ with organic or inorganic reducing agents, followed by surface stabilization (Iravani *et al.* 2014). Stabilization makes AgNPs less prone to aggregation, dissolution and surface modifications. If surfactants are not used, the repulsive forces between the particles are typically weak and they are easily homoaggregated already in the stock solutions. The increasing ionic strength of the media enhances the aggregation of AgNPs, because the repulsive forces between the particles can be screened by the high electrolyte concentration (Chambers *et al.* 2013). Ionic strength of the media also promotes heteroaggregation of AgNPs with suspended solids, which is believed to be the main mechanism precipitating AgNPs from surface waters to sediments (Quik *et al.* 2014, Velzeboer *et al.* 2014).

Dissolution of AgNPs is an environmentally important process, as dissolved Ag is known to be very toxic for aquatic organisms (Ratte 1999). Dissolution takes place mainly under aerobic conditions when the surface of AgNP gets oxidized and Ag^+ is released. High ionic strength and low pH of the aqueous media promotes the dissolution of AgNPs (Liu and Hurt 2010, Chambers *et al.* 2013, Peretyazhko *et al.* 2014). In more complex media, the dissolution is reported to be independent of pH, possibly due to increased aggregation tendency (Fabrega *et al.* 2009, Cornelis *et al.* 2012). However, it is difficult to study the dissolution of AgNPs in the complex media, as the dissolved Ag species rapidly complex with the environmental media. As dissolution is enhanced by the low pH of aqueous media, it is likely that a similar process takes place also in the more complex media, such as sediment.

In the environmental media, where many complexing agents are present, the surface chemistry of AgNP can alter. Especially sulfides and chlorides have high affinity to Ag and both have been shown to passivate AgNPs by forming an insoluble layer of AgCl or Ag_2S on the surface of AgNPs (Kaegi *et al.* 2011, Levard *et al.* 2013a, Levard *et al.* 2013b). The passivation by sulfides is reversible, as under oxic conditions the Ag_2S is prone to oxidation (Levard *et al.* 2013a). Aggregation may be induced also, due to the formation of AgCl and Ag_2S bridges that link AgNPs together (Reinsch *et al.* 2012). If chloride concentration is high, though, the dissolution of AgNPs is enhanced due to the formation of soluble $AgCl_x$ complexes (Levard *et al.* 2013b, Quik *et al.* 2014). Also NOM increases the stability of AgNPs by forming organic coatings on the surface of AgNPs, thereby protecting the particles from aggregation and dissolution (Liu *et al.* 2010, Delay *et al.* 2011, Kennedy *et al.* 2012, Gunsolus *et al.* 2015). In particular, NOM rich in sulfur and nitrogen groups reacts strongly with AgNPs and has the potential to replace the initial surface coatings similar to chlorides and sulfides (Yang *et al.* 2014, Gunsolus *et al.* 2015).

Overall the behavior of AgNPs in the environmental media is highly varying and difficult to predict. As many environmental factors in addition to AgNP properties influence the behavior of AgNPs, it is essential to consider all the parameters carefully. The link between laboratory and in-situ studies can sometimes be difficult to establish. However, experiments under controlled laboratory conditions can give us information about the effects of specific parameters and add comparability between the studies. In addition, more in-situ research considering also site-specific effects and behaviour of AgNP is needed.

1.3.3 The mode of AgNP toxicity

The toxicity of Ag compounds has been studied for decades, as Ag^+ is one of the most toxic metal ions to aquatic organisms. In the review article by Ratte et al. (1999), Ag was concluded to be algicidal, bactericidal, fungicidal and toxic to aquatic invertebrates and vertebrates. Tendency of AgNPs to dissolve in the environmental media has raised questions regarding whether the toxicity of AgNPs is solely a function of Ag^+ or if there are nano-specific modes of toxicity.

Many studies support the theory of Ag^+ driven toxicity, as the toxic effects can be well related to the dissolved concentration of Ag (Yang *et al.* 2011, Newton *et al.* 2013). Inhibition of K, Na -ATPase leading to osmoregulatory failure has been reported to be the main toxic mechanisms in crustacean and fish (Hogstrand and Wood 1998, Bianchini and Wood 2003). In addition, DNA replication failure, inactivation of proteins, inhibition of respiratory processes, and oxidative stress have been reported to take place in bacteria, plants and benthic annelida (Feng *et al.* 2000, Pal *et al.* 2007, Cozzari *et al.* 2015, Homaee and Ehsanpour 2016).

An increasing number of papers indicate that toxicity of AgNPs can be higher than toxicity of Ag^+ alone, indicating nano-specific toxicity. One suggested mode of toxicity is referred to as "Trojan horse". It means that AgNPs are internalized into the test organism then dissolve and release toxic Ag^+ inside the organisms. The Trojan horse -type of toxicity has been seen in cell cultures and in algae (Park *et al.* 2010, Gliga *et al.* 2014, Hsiao *et al.* 2015, Wang *et al.* 2016). Intracellular release of Ag^+ can possibly take place also in animalia, as uptake of AgNPs has been observed in crustaceans, fish and benthic annelida (Lee *et al.* 2007, Zhao and Wang 2010, García-Alonso *et al.* 2011). In addition, genotoxicity, mitochondrial toxicity and oxidative stress responses have been observed in AgNP-exposed organisms and thus suggested as possible mechanisms for AgNP-specific toxicity (Kim and Ryu 2013, Stensberg *et al.* 2014, Buffet *et al.* 2014, Cozzari *et al.* 2015, Park *et al.* 2015, Li *et al.* 2017, Luo *et al.* 2017).

1.3.4 Toxicity of AgNPs in the sediment

Most of the toxicity studies with benthic organisms are done using waterborne exposures. Waterborne exposures are not environmentally realistic, as they do not consider the natural habitat of the benthic species. In addition, the behaviour of AgNPs in sediment is expected to be highly different from that in aqueous media. The toxicity of AgNPs is often overestimated in waterborne exposures compared to sediment exposures (Park *et al.* 2015, Lee *et al.* 2016).

For the benthic fauna, sediment-associated AgNPs have typically low toxicity when observed in acute tests using classical endpoints such as mortality, reproduction or growth of the test organisms. No toxic effects were seen in benthic annelida, arthropoda, crustacea or mollusca at the AgNP concentrations varying from 75 to 367 mg kg^{-1} dw (Coleman *et al.* 2013, Dai *et al.* 2013, Wang *et al.* 2014, Ramskov *et al.* 2015). However, the toxicity seems to be species-specific. In multispecies studies across three phyla, only the growth of annelida was affected at the concentration of 1 mg kg^{-1} dw (Chan and Chiu 2015). In addition, Ramskov et al. (2015) observed different toxicity among the annelida species.

When more sensitive molecular level endpoints are used, toxic effects are seen more often and in lower concentrations of AgNPs. DNA damage and activation of oxidative stress markers have been observed in benthic arthropoda and annelida at AgNP concentrations ranging from 1.0 to 50 mg kg^{-1} dw (Cong *et al.* 2011, Cong *et al.* 2014, Cozzari *et al.* 2015, Park *et al.* 2015).

Overall, the toxicity of sediment-associated AgNPs is poorly studied and more research is urgently needed. The environmental risk assessments in sediment are currently impossible to do, due to the scarcity of ecotoxicological data from the sediment assessments (Coll *et al.* 2016).

1.4 *Lumbriculus variegatus*

Endobenthic oligochaeta *Lumbriculus variegatus* Müller, 1774 is a common widespread freshwater sediment species that is easy to culture and handle in the laboratory (Phipps *et al.* 1993). The species has many desirable features for ecotoxicological testing. Thereby, it is commonly used in bioassays and as a model organism in standardized toxicity tests (Anon. 2002, Anon. 2007).

As endobenthic organisms, worms bury into the sediment, leaving the tail part over the sediment surface (Fig. 2). Respiration takes place mainly through posterior body wall (Thorp and Covich 1991). As the bright red color of species indicates, *L. variegatus* have red blood pigment to aid the oxygen uptake and transport (Thorp and Covich 1991). Worms ingest the sediment using organic matter and especially bacteria as a source of nutrition (Thorp and Covich 1991). Faeces are egested on the top of the sediment. Feeding behavior of the worms adds bioturbation to the sediment.

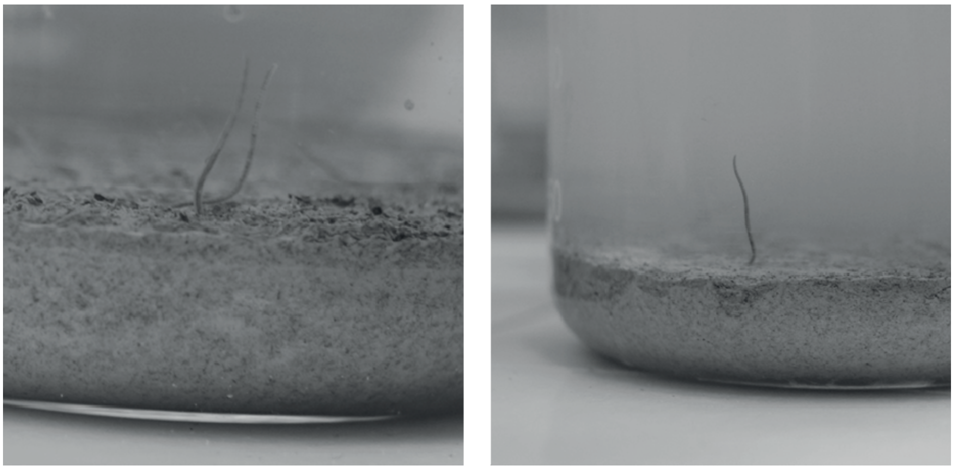


FIGURE 2 *Lumbriculus variegatus* worms partly buried into the artificial sediment in the laboratory test units. Egested faeces can be seen at the top of the sediment as a dark coloured layer.

L. variegatus reproduce mainly asexually by architomy. Fully grown individuals fragment into two parts and regenerate new posterior and anterior ends. Development of new segments takes 2 to 7 days, pausing the feeding of the organisms (Leppänen and Kukkonen 1998b). In laboratory cultures, the population size is reported to double every 10 to 14 days in 20 °C temperature (Phipps *et al.* 1993). Although the species can potentially reproduce sexually, this has not been observed in the laboratory cultures. Architomy reproduction has been proven to be useful in toxicological tests, as worms can be artificially cut in half to ensure the similar size and reproductive states. The procedure is referred to as synchronization of the test species and recommended to be done in the standard test protocol (Anon. 2007).

Fast growth and reproduction enables the use of these parameters as endpoints in the acute and sub-acute toxicity tests. Reproduction is a more sensitive endpoint than growth for oligochaetes in the metal toxicity assessments, and mortality has been observed to occur only in very high concentrations (Wiederholm *et al.* 1987). In general, it is difficult to observe the survival of the worms due to architomy reproduction, and it is often replaced by the total number of the worms after the test period (Phipps *et al.* 1993). Also behavioral endpoints, such as avoidance of sediment or feeding rate, have been proven to be simple and sensitive endpoints for toxicity (Leppänen and Kukkonen 1998b, West and Ankley 1998, Landrum *et al.* 2004).

As many species prey on *L. variegatus*, it is an excellent species in ecotoxicological studies concerning bioaccumulation and biomagnification of contaminants. Due to the partial burial behavior into the sediment, worms are susceptible to contaminant exposure via several pathways. Contaminants can enter the organism from the overlying water through the respiratory posterior part, from the sediment pore water through the skin contact or from the ingested sediment through the digestion channel.

2 OBJECTIVES

The main objective of the present thesis was to evaluate the toxicity of sediment-associated AgNPs to the benthic oligochaete *L. variegatus*. Toxicity of AgNPs to benthic fauna has been so far studied using mostly waterborne exposures. Only sediment exposures were utilized in this thesis, as sediment is the natural habitat of the test organisms. Artificial sediments and natural Finnish freshwater sediments were used to assess the effects of sediment properties on the toxicity of AgNPs. Also the effect of AgNP surface chemistry was studied by comparing the toxicity of uncoated-AgNPs (uc-AgNP) to polyvinylpyrrolidone-coated AgNPs (PVP-AgNP). Silver nitrate ($AgNO_3$) was used as a positive control for the toxicity of Ag^+. In addition, the toxicity of AgNPs was linked to the bioaccessible concentration of nanoparticle-originated dissolved Ag in the test sediments. More precisely, the objectives were:

1. To compare the toxicity of AgNPs with Ag^+ (spiked as $AgNO_3$) in sediment exposures using *L. variegatus* as a model species (I, III)
2. To assess the partitioning and bioaccessibility of dissolved Ag in AgNP and $AgNO_3$ spiked sediments (II, III)
3. To study the effect of sediment properties on the toxicity of AgNPs (I, III)
4. To evaluate the effect of the PVP-coating on AgNP to the toxicity and bioaccumulation of AgNPs in sediment (II, III)

3 MATERIAL AND METHODS

3.1 Test substances

Two types of AgNPs were purchased from the Nanostructured and Amorphous materials Inc. (Nanoamor, Houston, USA) and used throughout the studies. The main difference between the AgNPs was the surface chemistry. Uncoated AgNPs were prepared without any surface modifications and PVP-AgNPs were surface stabilized with polyvinylpyrrolidone (PVP, 0.2 % of the total mass). The PVP-polymers bind strongly on the surface of AgNPs through nitrogen atoms, adding steric repulsion between the particles. Increased repulsion further inhibits particle-particle contact and prevents reversible agglomeration and irreversible aggregation (Zhang *et al.* 1996). The Ag core of uc-AgNP is protected by Ag-oxide layer (Li *et al.* 2011). As no additional surface stabilizer was used, the AgNPs are referred as "uncoated". Generally uc-AgNPs are more prone to agglomeration and dissolution than surface stabilized particles in environmental media (Li *et al.* 2011). Both uc-AgNP and PVP-AgNP are widely used in scientific publications and were thus selected for the model particles in our study (Reidy *et al.* 2013).

The nominal diameter reported by the manufacturer was 25 nm for uc-AgNPs and 30–50 nm for PVP-AgNPs. For the characterization, a small amount of dry AgNP powder was suspended in 1 ml of sterile water and sonicated for 30 min in a water bath. The size of AgNPs was further analysed by measuring the diameter of individual spherical particles from the pictures taken by transmission electron microscope JOEL JEM-1400, using 25 000× magnification (Fig. 3). Observed diameters varied between 11.9–48.8 nm, resulting in a mean diameter of 39.4 nm (SD = 12.6 nm, n = 344) for uc-AgNPs and 7.5–74.8 nm, resulting in a mean diameter of 28.6 nm (SD = 5.80 nm, n = 205) for PVP-AgNPs (II, III) (Fig. 3 A, B). AgNPs formed large aggregates in highly concentrated stock suspensions (Fig. 3 C, D). A second batch of PVP-AgNPs from the same manufacturer was also used (I). The mean diameter was observed to be 81.8 nm (SD = 1.59 nm, n = 294) (Heckmann *et al.* 2011). Purity of PVP-AgNP was 99.9 %

and that of uc-AgNP 99.5 %, and all AgNPs were stored as dry powder prior to the use. Analytical grade $AgNO_3$ was used as a reference compound for Ag^+ toxicity and behaviour in sediment (I, II, III).

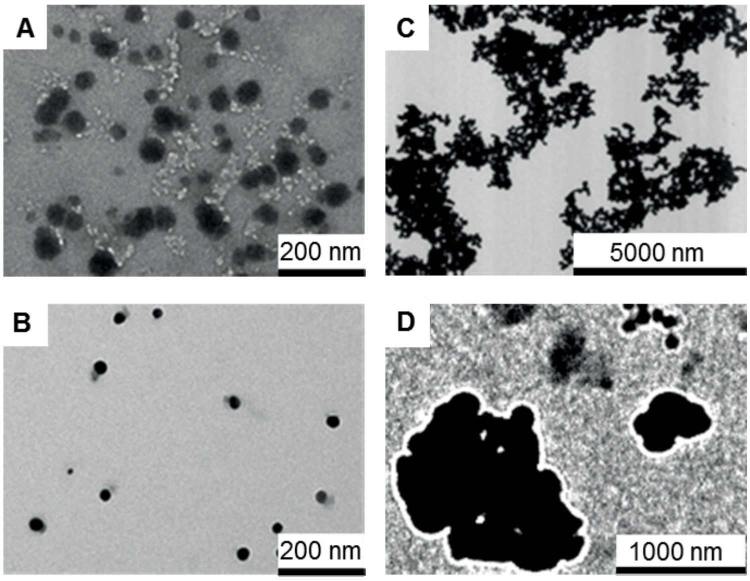


FIGURE 3 Transmission electron microscope pictures of A) individual uncoated silver nanoparticles (uc-AgNP), B) individual polyvinylpyrrolidone-coated silver nanoparticles (PVP-AgNP), C) agglomerated uc-AgNPs and D) agglomerated PVP-AgNPs.

3.2 Exposure media

3.2.1 Test sediments

Natural sediments were collected form Lake Höytiäinen (HS, 62°48´N, 29°41´E) and Lake Kuorinka (KS, 62°37´N, 29°24´E), situated in the eastern Finland. These sediments represent the typical geochemical background of Finnish lake sediments. The concentration of organic contaminants is minimal in HS and KS, and both sediments have been used as clean reference sediment in toxicity assessments (Ristola *et al.* 1996, Mäenpää *et al.* 2003). The organic carbon (OC) content and pH were higher in HS compared to KS (Table 1). Also the concentration of Fe and especially Mn oxides (Fe_{ox}, Mn_{ox}) was higher in HS compared to KS (Table 1).

Artificial sediments AS1 (I), AS2 (II), AS3 and AS4 (III) were constructed in the laboratory, following the standard guideline (Anon. 2007). All sediments consisted of 75 % combusted quartz sand, 5 % finely ground (< 500 µm) sphagnum peat and 20 % kaolin. Powder made out of leaves of *Urtica dioica* (0.4–0.5 % of total dw) was used as a food source. In AS2, the grain size of used quartz sand was relatively smaller (100–200 µm) compared to other artificial sediments (~60 % 100–200 µm, ~40 % 200–1200 µm). The pH of AS1, AS2, and AS3 was adjusted to ~7 and AS4 to ~5 with $CaCO_3$ (Table 1). More detailed characterization of the sediments can be found in each individual study (I, II, III).

TABLE 1 Characteristics of the artificial (AS1, AS2, AS3, AS4), Lake Höytiäinen (HS1, HS2) and Lake Kuorinka (KS1, KS2) sediments. The pH is reported as an average of the weekly measurements during the toxicity assessments. Organic carbon content (OC) was measured before the tests in three replicates and the concentration of Fe and Mn oxides (Fe_{ox}, Mn_{ox}) was measured in three replicates after the test period. Standard deviations are given in the brackets.

Parameters	Test sediments							
	AS1	AS2	AS3	AS4	HS1	HS2	KS1	KS2
pH	6.70 (0.26)	8.06 (0.29)	7.65 (0.32)	5.29 (0.23)	7.10 (0.21)	5.93 (0.17)	5.10 (0.11)	5.32 (0.61)
OC %	0.59 (0.15)	1.36 (0.07)	1.24 (0.02)	1.18 (0.02)	3.12 (0.31)	2.57 (0.04)	2.22 (0.14)	1.70 (0.00)
Fe_{ox} mg kg^{-1}		153 (2)				19700 (730)		9840 (340)
Mn_{ox} mg kg^{-1}		0.343 (0.020)				20400 (920)		313 (26)

3.2.2 Artificial fresh water

Artificial fresh water (AFW) used in the experiments was prepared according to the standard guideline by adding analytical grade calcium chloride, magnesium sulphate, sodium bicarbonate and potassium chloride to ultrapure water (Anon. 2007). The proportion of Ca : Mg ions was 1 : 4 and Na : K ions 10 : 1. The sum of Ca and Mg ions was 2.5 mmol l^{-1} in the first toxicity assessment (I) as recommended by the toxicity guideline, but reduced to 1.0 mmol l^{-1} for the further studies (II,III). The ionic strength was reduced to correspond to the culture conditions of the test organisms.

3.2.3 Spiking of the test sediments

Direct addition of AgNPs and $AgNO_3$ into the test sediments was used as a spiking method in all experiments (I, II, III). Dry AgNP powder was first mixed to a small subsample of AS1, HS1 and KS1 by hand with a metal spoon. The subsample was then mixed to the rest of the test sediment first by hand and then for 1 h with a rotating metal blade (I). Dry AgNP powder was directly added into AS2, HS2 and KS2, mixed with a rotating metal plate and placed in an overhead mixer (12 rpm) for 24 h (II). Due to the high agglomeration tendency of uc-AgNPs, dry AgNP powder was first moistened (1 ml of ultrapure water) and sonicated for 10 min to break down the largest agglomerates before spiking in AS3 and AS4 (III). The spiked sediments were mixed with a rotating blade and further placed in an overhead mixer (12 rpm) for 24 h. Spiking of $AgNO_3$ followed a similar protocol as used with AgNPs, except that $AgNO_3$ was dissolved in ultrapure water before introducing it into the sediment. The control treatments without Ag were treated in a similar way as the Ag-spiked sediments.

3.3 Test organisms

Two laboratory cultures of endobenthic oligochaeta *L. variegatus* were used in the experiments. First culture was maintained at the Department of Biology, University of Eastern Finland, Joensuu, Finland (I) and the second culture at the Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland (II, III). Both cultures were maintained in constantly aerated large aquariums filled with AFW (1.0 mmol as sum of Ca and Mg ions). Cellulose paper tissues were used as a substrate and worms were fed with Tetramin fish food (Tetrawerke). The light regime of the culture rooms was 16 h light and 8 h dark, and temperature 20 ± 2 °C. An acclimation phase of a minimum of 24 h was used to adapt the organisms to test water. The test organisms were synchronized by cutting them in half 13–14 days before the exposures (II, III). This was done to ensure the similar size and reproductive states of the organisms. If synchronization was not done, individually selected similar sized worms with no signs of recent reproduction were used in the experiments (I).

3.4 Performed experiments

3.4.1 Summary of the experiments

A brief description on each performed experiment is given under this section (Table 2). The concentrations used in the toxicity tests were selected according to the preliminary experiments (I, III). The lowest concentrations used were considered to be environmentally realistic. For the second study, the concentrations were selected to be high enough to ensure the analytical precision but low enough for *L. variegatus* to survive (II). The concentration of AgNP was over what is expected to be found in the environment, but the processes studied are considered environmentally realistic. More detailed information on the experiments is given in the individual part studies referred in the text (I, II, III).

TABLE 2 Overview of the used endpoints for toxicity, sediments (AS = artificial, HS = lake Höytiäinen, KS = lake Kuorinka), Ag substances (PVP-AgNP = polyvinylpyrrolidone-coated silver nanoparticles, uc-AgNP = uncoated silver nanoparticles, $AgNO_3$ = silver nitrate) and nominal Ag concentrations in each individual part study.

Study	Endpoints	Sediments	Ag substances and concentrations (mg kg^{-1})
I	No. of individuals	AS1	$AgNO_3$:
	Biomass	HS1	10, 40, 100, 200, 400
	Reproduction	KS1	PVP-AgNP:
	Feeding rate		100, 300, 600, 900, 1200
II	Partitioning of Ag	AS2	$AgNO_3$:
	Bioaccessibility	HS2	20
	Total body burden	KS2	PVP-AgNP, uc-AgNP:
			300
III	No. of individuals	AS3	$AgNO_3$:
	Biomass	AS4	10, 20, 40, 100, 300
	Bioaccessibility		PVP-AgNP and uc-AgNP, AS3:
	Total body burden		40, 100, 300, 700, 1400
			PVP-AgNP and uc-AgNP, AS4:
			10, 40, 100, 300, 1000

3.4.2 Toxicity test (I, III).

The standard toxicity test guideline 225 for the effects of sediment-associated chemicals on the reproduction and the biomass of the *L. variegatus* provided by the OECD was followed with slight modifications (Anon. 2007.)

The Ag-spiked test and control sediments were added to the bottom of 250 ml glass beakers and topped with AFW (Table 2). The amount of sediment was calculated to exceed the 1 : 50 ratio of dry biomass of the test organisms to the total OC of the sediment. A separate control group with 3 replicates was used for each Ag compound, and the number of replicates for the Ag exposures was 4 for each concentration. In each unit, 10 *L. varigatus* were introduced as a model organism for the toxicity of Ag substances. Worms were exposed to the Ag spiked sediments for 23 (III) or 28 days (I). The test units were constantly aerated and pH (I, III) and ammonium concentration (III) measured from the overlying water once a week. The temperature of the test room was 20 ± 2°C and the light regime adjusted to 16 h light and 8 h dark. At the end of the exposure, worms were sieved out of the sediment, counted and dried at 105 °C overnight. The total dry weight of the worms was measured and the dried samples were stored in the dark at 4 °C for the analysis of total body burden (TBB) of Ag by inductively coupled plasma atomic emission spectrometry (ICP-OES). Number of individuals, biomass and reproduction were used as endpoints for the toxicity (Table 2). Three parameter log-logistic models were used to estimate the effective concentrations where the response was 50 % of that in the control group (EC_{50}) (I, III). The no-observed-effect concentrations (NOEC) and the lowest-observed-effect concentrations (LOEC) were defined using the stepwise step-down Jonckheere-Terpstra test. The Kruskal-Wallis rank sum test, with multiple comparisons between groups according to Siegel and Castellan (1988), was used to compare the responses among the different Ag concentrations ($p < 0.05$) (I).

3.4.3 Feeding rate (I)

The feeding behaviour of *L. variegatus* makes it possible to observe the amount of sediment egested. Worms feed below the sediment surface egesting the faeces on the top of the sediment. Faeces can be separated from the bulk sediment by adding a thin quartz sand layer at the top of the sediment. Egested faeces can be collected from the top of the quartz sand layer. Feeding rate of *L. variegatus* is proven to be a sensitive indicator of response to contaminant concentrations (Leppänen and Kukkonen 1998a, Leppänen and Kukkonen 1998b, Landrum *et al.* 2002).

Feeding rate of the worms was observed in a separate assessment using PVP-AgNP spiked AS1, HS1 and KS1 sediments (I). The test was done in 50 ml beakers with 3 replicates for each Ag concentration (Table 2). For each of the test sediments, 2 control groups with 3 replicates without added Ag substances were used. Each unit received 23 g of wet sediment and was topped with AFW. The egestion rate of 5 individually selected similar sized *L. variegatus* worms was followed over the 14-day exposure period. The faecal pellets were collected from the top of quartz sand every second day and the dry weight of the pellets measured after overnight incubation at 105 °C. The test water was renewed, and pH and oxygen saturation measured in 2-day intervals. After 14 days, the worms were sieved out of the sediment, counted and the dry weight measured after overnight incubation at 105 °C. The amount of faeces produced was normalized to the total dry biomass of the worms after the exposure (mg mg^{-1} dw). The differences on the feeding rate among Ag concentrations were studied using one-way ANOVA followed with Tukey's honestly significant difference post-hoc test ($p < 0.05$). Normality of the data was tested with Shapiro-Wilk normality test and the homogeneity of variances with Levene's test ($p < 0.05$).

3.4.4 Partitioning and bioaccessibility of dissolved Ag (II, III)

Partitioning of dissolved Ag spiked as $AgNO_3$, uc-AgNPs and PVP-AgNPs were assessed in a separate experiments using AS2, HS2 and KS2 sediments

(Table 2) (II). The experiment was done in 50 ml glass beakers using 3 replicates. Spiked test sediment was added at the bottom of the beaker and topped with AFW. Test units were aerated, and 3 synchronized *L. variegatus* were introduced into each unit. The amount of sediment was calculated to match 1 : 50 ratio of dry biomass of the test organisms to total OC in the sediment. Partitioning of Ag was studied after 2, 5, 9, 14 and 28 days contact time (II).

Sequential extraction method was used to evaluate the partitioning of Ag with the sediment components (II). The procedure followed classical Tessiers method accompanied with ultrasound-assisted extraction (Tessier *et al.* 1979, Väisänen *et al.* 2002). Although the method does not separate the nanoparticulate Ag from other Ag species, the amount of AgNPs in the samples was theoretically and experimentally proven to be negligible (II, III). All the Ag analysed from the AgNP spiked sediment samples was thus assumed to derive from the dissolved AgNPs (II, III).

Sediment-associated dissolved Ag was analysed from 5 sediment fractions: 1) Ag in the water-soluble fraction, 2) bound to the exchangeable fraction, 3) bound to carbonates, 4) bound to Fe_{ox} and Mn_{ox}, and 5) bound to NOM. The sum of water-soluble and exchangeable fraction is referred as the bioaccessible fraction. In addition, Ag associated with the residual fraction was calculated by extracting the total amount of extractable Ag from the total dissolved Ag in the sediment. The residual fraction consists of Ag-sulfides, Ag trapped into cavities of amorphous clays and reduced metallic Ag, and is considered to be highly stable (Tessier *et al.* 1979, Yamada *et al.* 2001, Akaighe *et al.* 2011). The dissolution of AgNPs was estimated by relating the partitioning of AgNP spiked Ag to $AgNO_3$ spiked Ag (II). The extraction results were normalized to the estimated total dissolved concentration of Ag in the sediment (II).

The bioaccessibility of Ag was also assessed in the toxicity test using AS3 and AS4 (III). Only the first two steps of sequential extraction were performed to the sediment samples to evaluate the water-soluble and exchangeable Ag concentrations. The toxicity of Ag, observed as biomass decrease, was related to the bioaccessible concentration of dissolved Ag in the test sediments using three parameter log-logistic models (III).

3.4.5 Total body burdens (II, III)

The dried worm samples from the Ag exposures were digested with aqua regia (3:1 HCl : HNO_3) and analysed by ICP-OES to assess the TBBs of Ag (II, III). Aqua regia -digestion dissolves all forms of Ag in the samples, thus the speciation of Ag could not be assessed. The bioaccumulation of Ag was observed as TBB of Ag after 2, 5, 9, 14 and 28 days exposure (II). In the toxicity assessment, the TBBs were related to the biomass of the worms (III). Critical body burdens (CBB_{50}), where the biomass of the worms was decreased to 50 % of that in the control treatment were estimated using three parameter log-logistic model and compared with z-test ($p < 0.05$) using *drc* package in R version 3.0.1 (R Core Team 2017) (III).

4 RESULTS & DISCUSSION

4.1 Toxicity

4.1.1 Toxicity of AgNPs compared to $AgNO_3$ (I, III)

The $AgNO_3$-spiked sediments were generally more toxic to *L. variegatus* than uc-AgNP or PVP-AgNP spiked sediments (Fig. 4). The only exception was the acidic AS4 sediment, where the toxicity, observed as number of survived individuals, did not differ among the Ag s (Fig. 5). Overall, the toxicity of AgNPs in the alkaline AS1, AS3 and natural KS1 and HS1 sediments was low. LOEC was found only for uc-AgNPs (number of individuals) in AS3 and for PVP-AgNPs (biomass decrease) in KS1 (Fig. 4). Both of these concentrations were several times higher than observed with $AgNO_3$ in the same sediments (Fig. 4).

Our results compare well with the literature. In the meta-analysis (42 articles concerning terrestrial and waterborne toxicity), Notter et al. (2014) found that in 93.8 % of the studies Ag^+ was observed more toxic than AgNPs. In sediment assessments, often no toxic effects in AgNP exposure have been observed using classical endpoints such as mortality and growth (Coleman *et al.* 2013, Dai *et al.* 2013, Wang *et al.* 2014). However, AgNPs have been reported to be more toxic than Ag^+ in sediment exposures when more sensitive endpoints, such as genotoxicity or gene markers for oxidative stress, were studied (Cong *et al.* 2011, Buffet *et al.* 2014, Cong *et al.* 2014, Park *et al.* 2015). It is possible that molecular level toxicity was triggered by AgNP exposure also in our experiments but they did not translate to higher-level effects. According to our experiments, acute toxicity of sediment-associated AgNPs to *L. variegatus* is not expected to be greater than toxicity of $AgNO_3$.

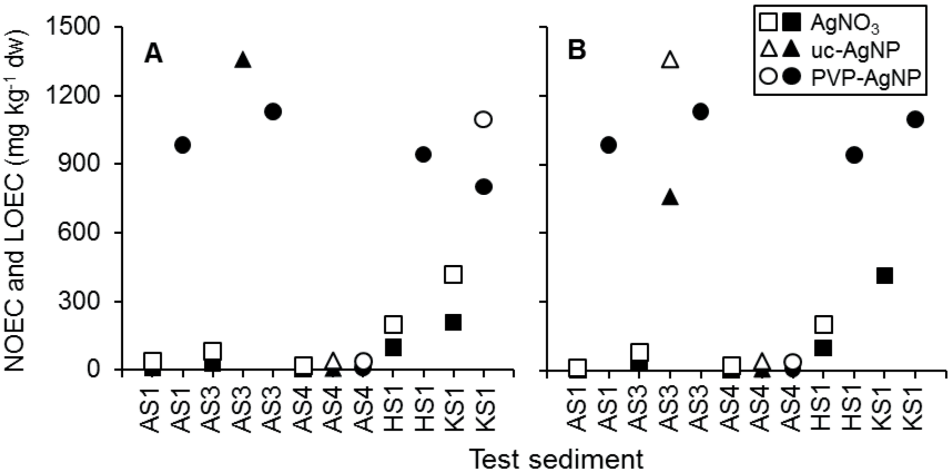


FIGURE 4 No-observed-effect (NOEC; filled symbols) and lowest-observed-effect (LOEC; open symbols) concentrations for silver nitrate ($AgNO_3$), uncoated silver nanoparticles (uc-AgNP) and polyvinylpyrrolidone-coated silver nanoparticles (PVP-AgNP) in artificial (AS1, AS3, AS4), Höytiäinen (HS1) and Kuorinka (KS1) sediments. Two endpoints for the toxicity were used, A) the number of survived *L. variegatus* and B) the biomass of *L. variegatus*.

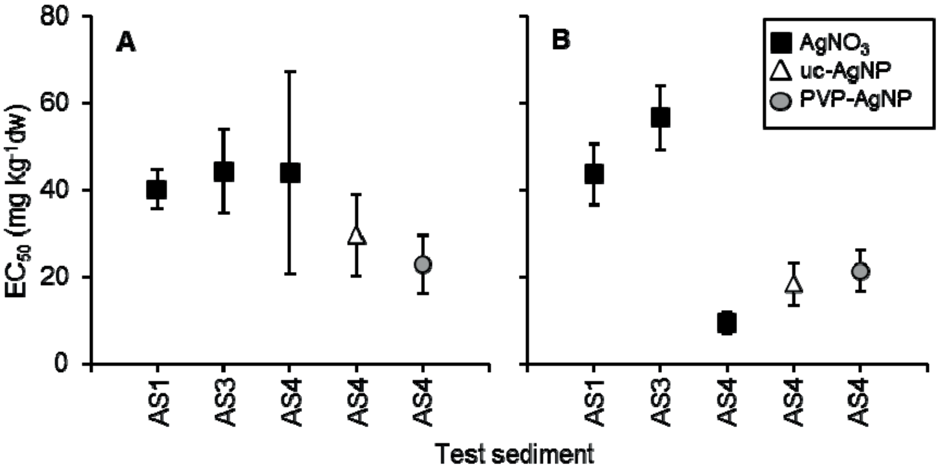


FIGURE 5 The concentration where A) the number of survived *L.variegatus* and B) the biomass of survived *L. variegatus* was 50 % of that in the control group (EC_{50}). Error bars indicate 95 % confidence intervals. Silver nitrate ($AgNO_3$), uncoated silver nanoparticles (uc-AgNP) and polyvinylpyrrolidone-coated silver nanoparticles (PVP-AgNP) were spiked to artificial sediments (AS1, AS3, AS4).

4.1.2 The effect of sediment pH (I, III)

Toxicity of uc-AgNPs, PVP-AgNPs and $AgNO_3$ were promoted by the low sediment pH. In the $AgNO_3$ spiked AS sediments, the EC_{50} for the biomass decrease were 4.5–5.9 times lower in acidic AS4 than in neutral AS1 or AS3 (Fig. 5 B). The promoting effect of pH on the toxicity of uc-AgNPs and PVP-AgNPs was greater than observed with $AgNO_3$. Comparison of the LOEC in AS4 to NOEC in AS1 and AS3 emphasizes this effect. With uc-AgNP spiked AS4, LOEC were 19–34 times lower than NOEC in AS3 and with PVP-AgNP spiked AS3 26–30 times lower than in AS1 or AS3 (Fig. 4A). In natural sediments, PVP-AgNPs were more toxic in the acidic KS1 sediment than in HS1, despite opposite observation with $AgNO_3$ spiked sediments (Fig. 4). The effect of pH can thus be concluded to be more important factor for the toxicity of AgNPs than for $AgNO_3$.

The increased toxicity of AgNPs seemed to be a two-step process. Firstly, the dissolution of AgNPs was promoted by the low sediment pH and secondly, the toxicity of released Ag^+ was further increased in acidic media (Fig. 5 B). The increased dissolution of AgNPs by the low pH has been also reported in aquatic media (Liu and Hurt 2010, Peretyazhko *et al.* 2014). In soil studies, the dissolution of AgNPs was independent from the soil pH, possibly due to increased aggregation of particles in acidic soil (Fabrega *et al.* 2009, Cornelis *et al.* 2012). Contrary results have been reported in the study conducted by Hedberg *et al.* (2015), where the dissolution of AgNPs was promoted by the low pH, leading to rapid and complete dissolution. Also higher microbial toxicity of AgNPs in acidic soils was explained by the higher dissolution rate in low pH (Schlich and Hund-Rinke 2015). In our experiment, toxicity of AgNPs was promoted by the low pH of the AS4. The toxicity of $AgNO_3$ in the artificial sediments was also higher in the low pH, but the effect was more pronounced with AgNPs. Thus the increase of toxicity derives most likely from the higher dissolution rate of AgNPs in the acidic sediment.

4.1.3 Feeding rate in AgNP spiked sediments (I)

In AS1 and HS1, the egested amount of sediment was similar to control in PVP-AgNP spiked sediment through the 14-day assessment (Fig. 6). Interestingly in KS1, *L. variegatus* egested more sediment at higher PVP-AgNP concentrations, except in the highest treatment (1098 mg kg^{-1}) where worms were avoiding the sediment (Fig. 6). Increasing feeding rate in KS1 at higher AgNP concentrations was unexpected. Typically the feeding rate decreases with increasing contaminant concentration. Induced feeding has been also seen in fluoranthene-spiked sediment but the observation remains unexplained (Landrum *et al.* 2002). The antibacterial properties of Ag may explain the increased ingestion of sediment, as microbes serve as a food source for *L. variegatus*. Organisms may compensate the decreased nutritional value of the sediment by feeding more. Microbes may be a more important source of nutrients in KS1 than in AS1 or HS1, explaining why this effect was only observed in KS1. The low nutritional value of KS is reported in literature (Mäenpää *et al.* 2008) and also supported by the higher egestion rate of sediment in the control groups of KS1 compared to control groups of HS1 and AS1 (Fig. 6). Avoidance of the sediment and decreased feeding rate were observed at a similar concentration as defined LOEC in the toxicity assessment (Fig. 4 A).

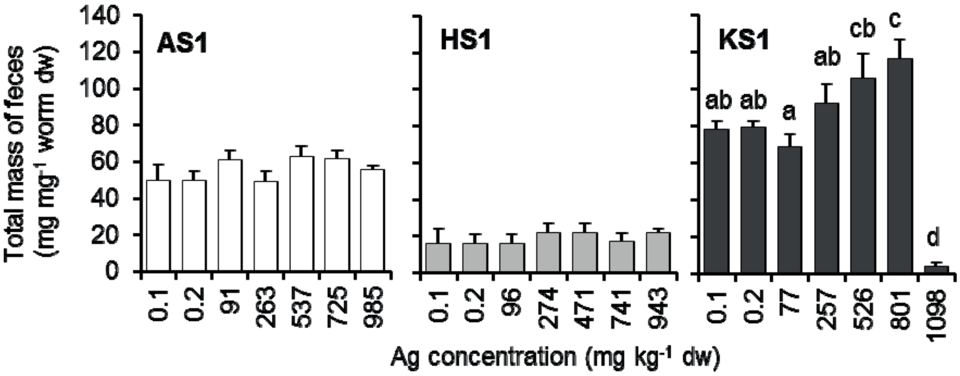


FIGURE 6 Amount of egested sediment (mg mg^{-1} of worm dw) in the 14-day feeding rate test in PVP-AgNP spiked artificial (AS1), Höytiäinen (HS1) and Kuorinka (KS1) sediments. Letters a,b,c,d indicate groups that do not significantly differ from each other according to one-way ANOVA with Tukeys HSD post-hoc test ($p < 0.05$).

4.2 Bioaccumulation (II, III)

The bioaccumulation of Ag was observed as TBB of Ag in one exposure concentration of $AgNO_3$ (20 mg kg^{-1} dw), uc-AgNPs (300 mg kg^{-1} dw) and PVP-AgNPs (200 mg kg^{-1} dw) using 5 time points in AS2, HS2 and KS2 sediments. The speciation of Ag was not considered and results are given as total Ag in the dry worm tissue. The TBBs were increasing linearly; 3.04 µg g^{-1} d^{-1} in $AgNO_3$ spiked HS2 ($R^2 = 0.91$) and 1.31 µg g^{-1} d^{-1} in $AgNO_3$ spiked KS2 ($R^2 = 0.93$) (Fig. 7). In AS, the highest TBBs were measured after 9 days of exposure (Fig. 7). In the PVP-AgNP spiked sediments, the TBBs were increasing linearly; 2.61 µg g^{-1} d^{-1} ($R^2 = 0.91$) in HS and 3.41 µg g^{-1} d^{-1} ($R^2 = 0.87$) in KS (Fig. 7). In AS, the TBBs were highest at the beginning of the experiment and decreased to plateau at day 9 (Fig. 7). In the uc-AgNP spiked sediments, Ag did not accumulate into the worms (Fig. 7).

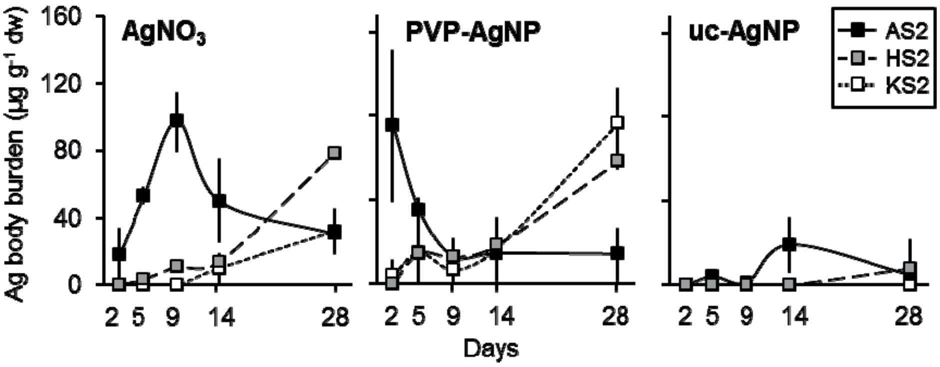


FIGURE 7 The total body burdens of Ag in dry *L. variegatus* tissue after 2, 5, 9, 14 and 28 days exposure. Silver nitrate (AgNO$_3$), polyvinylpyrrolidone-coated silver nanoparticles (PVP-AgNP) and uncoated silver nanoparticles (uc-AgNP) were spiked in the artificial (AS2), Höytiäinen (HS2) and Kuorinka (KS2) sediments.

The decreasing TBBs in the AS2 sediment were promoted by the increased biomass of the test organisms in AS2 (II). In natural sediments the test organisms did not gain weight during the experiment (II). The equilibrium time of Ag in the sediments before the exposure was not sufficient in AS2, as the highest TBBs were observed at the beginning of the experiment. In natural sediments the equilibrium was reached faster. The HS and KS sediments are reported to consist mostly fine sized material (HS = 79.0 % and KS = 77.9 % under 65 µm) (Mäenpää *et al.* 2003). The relatively coarser grain size (75 % between 100–200 µm) and thus smaller surface area of AS2 sediment compared to natural sediments may explain the slower equilibrium (Simpson *et al.* 2004). This slow equilibrium may also lead to an increased concentration of unbound AgNPs in the AS2 sediment. Thus the bioaccumulation may have been overestimated by attachment of AgNPs on the worm surface at the early exposure in PVP-AgNP spiked AS2.

As the Ag concentration was different among the treatments, accurate comparisons of the uptake rates are difficult to make. However the results clearly show the lower bioaccumulation of uc-AgNPs compared to AgNO$_3$ or PVP-AgNPs in all tested sediments (Fig. 7). Surface chemistry of AgNPs was also found to be important on the bioaccumulation of Ag in marine sediments (Wang *et al.* 2014), but Coleman et al. (2013) reported similar uptake rates among differently coated AgNPs and AgNO$_3$ in freshwater sediment. Contrary results indicate that the properties of both the sediment and AgNP together influence the bioaccumulation of sediment-associated AgNPs. Under environmental conditions, the uptake can thus be difficult to predict and site-specific differences should be taken into account in risk assessments.

In the toxicity assessment conducted in AS3 and AS4, the TBBs of Ag were related to the decreased biomass of the test organisms in varying concentrations of AgNO$_3$, uc-AgNPs and PVP-AgNPs. The modelled CBB$_{50}$ (95 % confidence intervals) were 83.7 µg g^{-1} (58.2–109 µg g^{-1}) for AgNO$_3$ in AS3 and 133.2 µg g^{-1}

(69.0–197 µg g^{-1}), 98.9 µg g^{-1} (38.3–160 µg g^{-1}) and 94.7 µg g^{-1} (2.28–187 µg g^{-1}) for $AgNO_3$, uc-AgNPs and PVP-AgNPs, respectively in AS4. No statistically significant differences were observed among CBB_{50} values (p = 0.15–0.94). The CBB_{50} for AgNPs in AS3 could not be calculated due to the low toxicity of AgNPs in the sediment (Fig. 4).

Interestingly, in the high AgNP concentration the TBBs in AS3 were higher than modelled CBB_{50} but no toxicity was observed (Fig. 8). This indicates that Ag was taken up in non-toxic form, most likely as AgNPs. It is also possible that AgNPs were attached on the surface of test organisms or retained in the gut due to the incomplete gut clearance also observed in soil studies (Shoults-Wilson *et al.* 2011, Coutris *et al.* 2012b). Uptake of AgNPs in AS4 was assumed to be low, as toxicity was observed already at the low Ag concentrations. The increase of TBBs in AS4 was intensified by the decreased biomass of the worms in the higher total Ag concentrations (Fig. 8). The similar CBB_{50} among $AgNO_3$, uc-AgNPs and PVP-AgNPs indicate similar toxic mechanisms of Ag. Uptake of Ag is thus suggested to happen mainly in the form of Ag^+. Our results support the theory of Ag^+ driven toxicity of AgNPs (Yang *et al.* 2011, Newton *et al.* 2013).

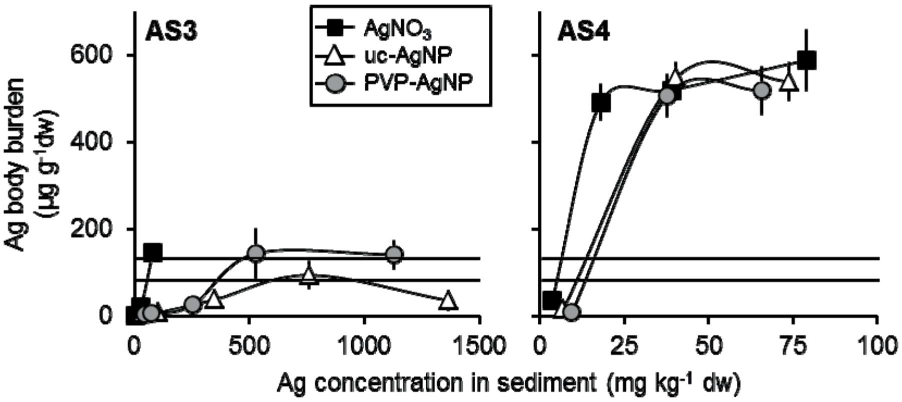


FIGURE 8 Total body burdens of Ag in the artificial sediments (AS3, AS4) spiked with silver nitrate ($AgNO_3$), uncoated silver nanoparticles (uc-AgNP) and polyvinylpyrrolidone-coated silver nanoparticles (PVP-AgNP). Horizontal lines indicate the lowest (83.7 µg g^{-1}) and the highest (133 µg g^{-1}) estimates of critical body burdens, where the biomass was decreased to 50 % of that in the control treatment.

4.3 Partitioning and bioaccessibility of dissolved Ag (II, III)

The estimated total dissolution rates of AgNPs with standard deviations in brackets were 33.8 % (6.8 %), 31.1 % (19.2 %), 19.8 % (5.8 %) for uc-AgNPs and 37.0 % (5.7 %), 23.1 % (7.3 %), 21.7 % (5.2 %) for PVP-AgNPs in HS2, KS2 and AS2, respectively (II). Results compare well with the estimated 3–30 % dissolution of PVP-AgNPs in soil (Cornelis *et al.* 2012). In all treatments, the majority of dissolved Ag was associated on the residual fraction of the sediments, followed by the NOM or Fe_{ox}/Mn_{ox} fractions (Fig. 9**)**. Ag bound on these fractions is stable under environmental conditions and not considered to be bioavailable for the organisms (Tessier *et al.* 1979). The association of dissolved Ag on the carbonate fraction was low in all treatments (Fig. 9). Observations compare well with earlier sequential extraction studies using AgNP spiked soils (Coutris *et al.* 2012a, Coutris *et al.* 2012b).

In HS2 and AS2, $AgNO_3$ was more bioaccessible than AgNPs (Fig. 9). Interestingly in KS2, PVP-AgNPs were significantly more bioaccessible than $AgNO_3$ or uc-AgNPs (II). This result is supported by the toxicity test, where PVP-AgNPs were found to be more toxic in the KS1 sediment compared to the HS1 or AS1 sediments (Fig. 4 A). Similar increased bioaccessibility has also been observed in uc-AgNP-spiked organic and mineral soils (Coutris *et al.* 2012a). One proposed mechanism for this involves the release of small particles or relatively stable Ag-complexes from uc-AgNPs, leading to slow association and shifted equilibrium towards the bioaccessible (Coutris *et al.* 2012a). In our study, this hypothesis does not explain the increased bioaccessibility, as it was only observed with PVP-AgNPs in the KS sediment. More likely, released Ag^+ from the PVP-AgNPs was loosely bound on the surface of the particles, increasing the concentration of Ag in the bioaccessible fraction (Liu *et al.* 2010, Liu and Hurt 2010, Klitzke *et al.* 2014). Although, the affinity of PVP towards Ag^+ is reportedly low (Gunsolus *et al.* 2015), the PVP-coating is expected to be partly replaced by the NOM in the sediment (Yang *et al.* 2014). Formed PVP-NOM-AgNP complexes may have increased affinity towards Ag^+. As the increased bioaccessibility was observed only in KS2, the quality of NOM seems to be important in this process. For example, the affinity of NOM towards Ag is known to be increased by the quantity of sulphur and nitrogen groups in the NOM-molecules (Gunsolus *et al.* 2015). Our experimental data fits this hypothesis, but more research is needed to confirm the mechanism behind the increased bioaccessibility of PVP-AgNPs in the KS sediment.

In alkaline artificial sediments AS2 and AS3, the bioaccessibility of dissolved Ag spiked as uc-AgNPs and PVP-AgNPs was low (Fig. 9, Fig. 10 B). In AS3, only marginal concentrations of bioaccessible Ag was observed in the high total Ag concentration (Fig. 10 B). In acidic AS4, the bioaccessible concentration of dissolved Ag was modelled using the Freundlich equation (III). Bioaccessibility of dissolved Ag observed was almost similar between uc-AgNPs and PVP-AgNPs and overlapped with $AgNO_3$ in the low range of total Ag concentration (Fig. 10 A). The bioaccessibility of $AgNO_3$ was significantly higher in AS4 than in AS3 (Fig. 10 A). This indicates higher bioaccessibility of Ag^+ in acidic sediment. This alone did not explain the differences in the AgNP spiked sediments, thus also the dissolution of AgNP is assumed be higher in AS4 than in AS3. This supports the hypothesis of higher dissolution of AgNP in more acidic media (Liu and Hurt 2010, Peretyazhko *et al.* 2014, Wang *et al.* 2014, Hedberg *et al.* 2015).

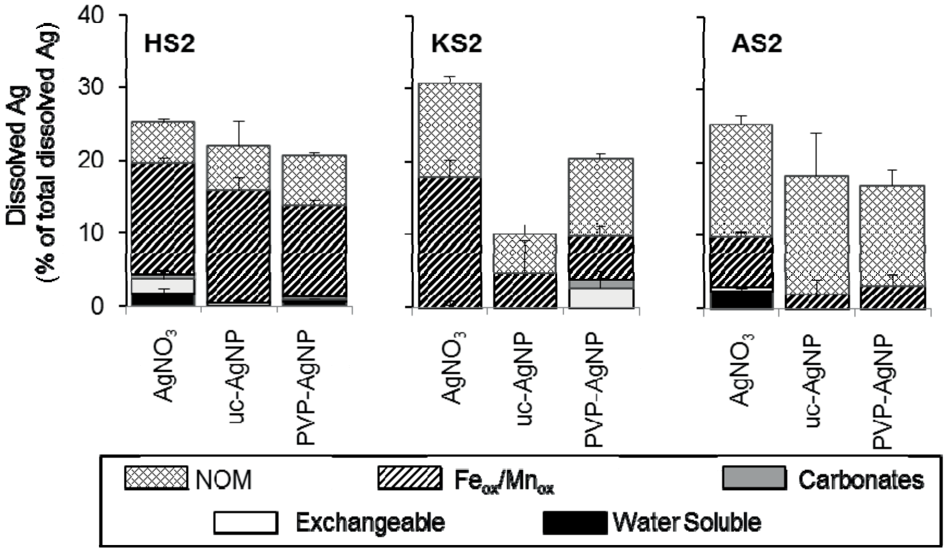


FIGURE 9 Association of dissolved Ag with the natural organic matter (NOM), iron and manganese oxides (Fe$_{ox}$/Mn$_{ox}$), carbonates, exchangeable and water soluble fractions in Höytiäinen (HS2), Kuorinka (KS2) and Artificial (AS2) sediments spiked as silver nitrate (AgNO$_3$), uncoated silver nanoparticles (uc-AgNP) and polyvinylpyrrolidone-coated silver nanoparticles (PVP-AgNP). Bars (+standard deviation of 3 replicates) indicate the proportion of dissolved Ag associated with each factor from the estimated total dissolved Ag in the test sediments after a 28-day contact time.

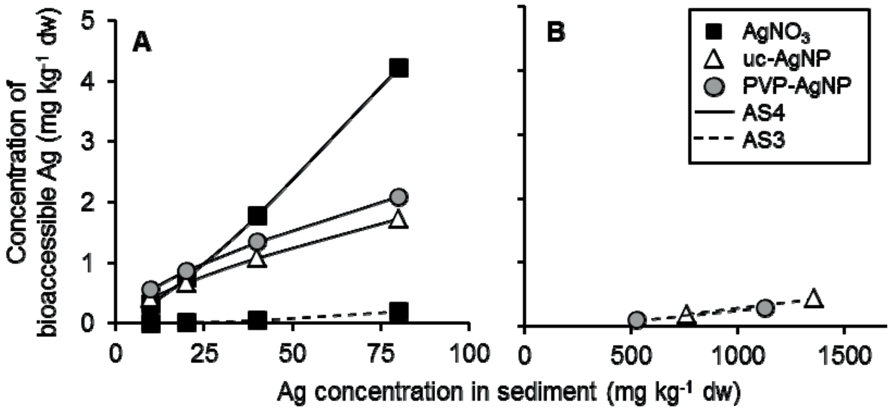


FIGURE 10 Concentration of bioaccessible dissolved Ag in artificial sediments (AS3, AS4) spiked with silver nitrate (AgNO$_3$), uncoated silver nanoparticles (uc-AgNP) and polyvinylpyrrolisone-coated silver nanoparticles (PVP-AgNP). In part A, the bioaccessible concentrations are modelled using the Freundlich equation (II). In part B, the markers indicate direct observations of bioaccessible Ag in the sediment.

4.4 Toxic mechanism of AgNPs

Our results indicate that the toxicity of AgNPs to *L. variegatus* is a function of dissolved Ag rather than nano-specific mode of toxicity. The low toxicity of AgNPs in AS3 was explained by the low bioaccessible concentration of dissolved Ag in the sediment (Fig. 4, Fig. 10 B). When the toxic responses in AS4 were plotted against the bioaccessible dissolved Ag concentrations, no differences in the EC_{50} values were observed (III). Similar mode of toxicity between $AgNO_3$ and AgNPs is also supported by the CBB_{50}, which did not differ among $AgNO_3$ (AS3 and AS4), uc-AgNPs (AS4) and PVP-AgNPs (AS4). In addition, the TBBs of Ag were higher than estimated CBB_{50} in AgNP spiked AS3 (Fig. 8). That indicates uptake of Ag, most likely as AgNPs, without any observed toxic effects.

In majority of toxicity studies, Ag^+ is found to be more toxic than AgNPs, similar to our observations (Notter *et al.* 2014). In our experiments, no nano-specific toxicity was observed, and the toxic effects were related to the dissolved Ag in the sediment. Similar relationship between toxicity and dissolved concentration of Ag has been established in some studies (Yang *et al.* 2011, Newton *et al.* 2013). However, the toxicity of sediment-associated AgNPs is found to be species-specific (Chan and Chiu 2015, Ramskov *et al.* 2015). In addition, nano-specific toxicity to benthic fauna is observed in molecular level, which was not considered in this thesis (Cong *et al.* 2011, Cong *et al.* 2014, Cozzari *et al.* 2015, Park *et al.* 2015). In conclusion, the Ag^+ driven toxicity of AgNPs observed in our study cannot be generalized. In addition, it is possible that AgNPs pose chronic or molecular level toxicity over Ag^+. However, the acute toxicity of sediment-associated AgNPs to *L. variegatus* was observed to derive from dissolved Ag and no nano-specific toxicity was confirmed.

4.5 Future research needs

The use of NPs is annually increasing in industry and consumer products leading to increasing concentrations in the environment. As sediment is believed to be a major sink for the NPs, it is important to study the effects of the NPs using sediment-assessment. The availability of ecotoxicological data is not currently sufficient to do environmental risk assessments in sediment for even the most studied NPs (Coll *et al.* 2016).

The lack of methodology to characterize NPs in sediment is the major step back in sediment studies. The development of new cost effective and easily achievable characterization methods is maybe the most important field of research in the near future. However, the present thesis demonstrates that much can be learned about the environmental effects of NPs in quite simple sediment assessments, despite the fact that methods to characterize NPs in sediment are limited.

The acute toxicity of AgNPs to *L. variegatus* was proven to be a function of dissolved Ag, and no nano-specific toxicity was observed in our experiments. However, also AgNP-specific modes of toxicity are reported in the literature, especially when more sensitive endpoints for toxicity were used (Cong *et al.* 2011, Buffet *et al.* 2014, Cong *et al.* 2014, Park *et al.* 2015). Alkaline artificial sediment proved to be good media to study the AgNP-specific toxicity, as the dissolution of AgNP and the bioaccessibility of Ag^+ were observed low. As the toxicity of Ag^+ is already well known, future research should focus on revealing the mechanisms of AgNP-specific toxicity.

High TBBs of Ag in *L. variegatus* suggests that AgNPs may have been taken up by the test organisms in our experiments. It is also possible that AgNP were attached on the surface of the test organisms or retained in the gut with sediment particles. The uptake of AgNP by *L. variegatus* should be confirmed in future studies. Also the possible chronic or molecular toxicity due to AgNP exposure should be considered.

The binding of Ag^+ on the surface of AgNPs may increase the bioavailability of Ag and lead to higher toxicity. We observed that sediment properties, possibly the quality of NOM, have an effect on the surface binding. More research is needed on AgNP-NOM-Ag^+ interactions taking account the role of surface stabilizer used on the AgNPs.

Considering also the species-specific nature of AgNP toxicity, more nanoecotoxicological studies with varying AgNPs, model species and experimental conditions are needed to make universal generalizations about the toxicity of AgNPs in sediments.

5 CONCLUSIONS

The main findings of the present thesis referring to the study objectives were:

1. The toxicity of $AgNO_3$ to *L. variegatus* was found to be higher than the toxicity of uc-AgNPs or PVP-AgNPs in all tested sediments. The toxic effects of AgNPs seemed to derive from the dissolved Ag^+ and no nano-specific toxicity was observed. According to our experiments, the acute toxicity of sediment-associated AgNPs to *L. variegatus* is not expected to be higher than toxicity of $AgNO_3$.
2. In all treatments, the majority of dissolved Ag was associated with the residual fraction of the sediment, followed by the NOM or Fe_{ox}/Mn_{ox} fractions. The Ag associated with these fractions is considered to be stable and not bioavailable for benthic organisms under environmental conditions. The bioaccessibility of dissolved Ag was increased by the PVP-coating, but only in the natural KS sediment. Formation of PVP-NOM-Ag^+ complexes is a suggested process behind the increased bioaccessibility, but more research is needed to confirm this hypothesis. In the artificial sediments, the low pH significantly promoted the bioaccessibility of dissolved Ag in similar manner with uc-AgNP and PVP-AgNP.
3. As predicted by the increased bioaccessibility of dissolved Ag, the low sediment pH also promoted the toxicity of uc-AgNPs and PVP-AgNPs in artificial sediment, likely due to the increased dissolution of AgNPs. Despite the low pH in natural sediments, the toxicity of AgNP was low. Other sediment properties, such as high surface area, content of NOM and Fe_{ox}/Mn_{ox}, are proposed to mitigate the toxicity of AgNP, as dissolved Ag species are efficiently bound by the sediment components. Overall, in alkaline artificial sediments and natural sediments the toxic effects were seen only in extreme concentrations, not expected to be found in the environment.
4. The PVP-coating on the particles increased the bioaccumulation of Ag in alkaline artificial and natural sediments. Due to the low bioaccessibility

of dissolved Ag in these sediments, the uptake is believed to take place mainly in the form of AgNP. In acidic artificial sediment, the toxicity and bioaccumulation of PVP-AgNP and uc-AgNP was similar. The uptake of Ag is suggested to take place in the form of Ag^+, as the bioaccessibility of dissolved Ag was high in artificial acidic sediment.

Acknowledgements

This study was funded by The Doctoral Programme in Biological and Environmental Science as a part of University of Jyväskylä Graduate School for Doctoral Studies. In addition, I would like to acknowledge the Finnish Doctoral Programme in Environmental Science and Technology (EnSTe) and the Society of Environmental Toxicology and Chemistry (SETAC) for additional travel grants.

First of all, I would like to thank my supervisors, Jussi Kukkonen, Eeva-Riikka Vehniäinen and Ari Väisänen. Without Jussi, I would never have started on my postgraduate studies. He has been a great mentor for me. Thank you for this opportunity and for believing in me. Eeva-Riikka is one of the most positive and friendly people I have met. After each meeting with her, I would gain new confidence that everything would work out. This is something not to be taken for granted in academia. Thank you for everything, you were the best supervisor one could have. Ari joined as my supervisor in the second year of my studies, when I was spending most of my time in his laboratory at the Department of Chemistry (not necessarily a favourite place to be for someone with a background in Biology). Ari took me in with a warm welcome, and was an irreplaceable help with the elemental analysis done. I enjoyed working with you and am glad to have gotten to know you better in the process.

Besides my supervisors, I would like to thank my follow-up group members, Silke Krol and Jarkko Akkanen, who were always very motivational and supportive. The collaboration with Silke resulted in two research visits to Milan, Italy. It was a great joy to work with all the wonderful people in Silke's research group. As for Jarkko, we had many unofficial support meetings (often involving a fishing rod) in conference trips all around the world. These trips and moments shared were one of the best experiences during my studies. I was definitely very lucky to have you two as my follow-up group members, thank you for everything.

I would also like to thank Varpu Marjomäki, who was originally named as one of my supervisors. We cooperated on a promising project that unfortunately did not work out. Nonetheless, I learned a number of new methods and techniques while working in Varpu's laboratory. Thank you for giving me the opportunity to be part of your research group.

I owe thanks to my co-authors, Kimmo Mäenpää and Janeck Scott-Fordsmand, for all the help granted with the first article. I am also grateful for for comments of Olli-Pekka Penttinen and Markus Sillanpää, pre-examiners of this thesis. Special thanks goes to the most supportive laboratory personnel one could imagine, Leena Siitonen and Mervi Koistinen. In addition, thanks to Riku Sinervo, Clara Forner, and Emmi Ikäheimo for your help in the laboratory, to Marriah Ellington for the proofreading of the thesis and to Lasse Kaaos for the cover art.

To all my colleagues, especially Hanna Arola, Anna Suuronen, Elijah Ngumba, Hanna Aarnos, Jaana Wallin, Yufei Gu and Sebastian Abel, a big thank you for the peer support. With Yufei, we shared the same office for four years. Thank you for bearing with my bad sense of humor (and coffee cup collection) over these years. With Hanna, we had numerous, not always academic, conversations that made even the worst days look brighter. Thank you for listening and sharing the good and bad. Finally, I would like to thank everyone from Jyväskylän Liitokiekkoilijat for getting me out of the office once in a while. Without your support, I would probably have graduated many years sooner (or more likely not at all).

YHTEENVETO (RÉSUMÉ IN FINNISH)

Hopeananohiukkasten käyttäytyminen ja toksiset vaikutukset sedimentissä *Lumbriculus variegatus* -harvasukamadolle

Nanotiede on kasvava tieteenala, joka hyödyntää nanomateriaaleja ja jolla alkaa olla teollisen mittakaavan teknologisia sovelluksia. Nanomateriaalit sisältävät pieniä nanohiukkasia. Euroopan komissio on määritellyt nanohiukkasten kokoluokaksi 1–100 nm mutta tarkkojen ylä- ja alarajojen vetäminen on aina keinotekoista. Nanohiukkaset ovat joka tapauksessa erittäin pieniä ja omaavat erityisiä ominaisuuksia nanorakenteensa ansiosta. Kaupallisten sovellusten määrässä mitattuna hopeasta koostuvat nanohiukkaset ovat yleisimmin käytettyjä. Niillä on erityisiä sähkö-, lämpö- ja antibakteerisia ominaisuuksia, joita hyödynnetään esimerkiksi tekstiileissä, maaleissa, kosmetiikassa ja lääketieteellisissä sovelluksissa. Nanomateriaalien tullessa yhä yleisempään käyttöön kaupallisissa tuotteissa ja teollisuudessa kasvaa väistämättä myös niiden pitoisuus ympäristössä. Vesistöihin päätyvät hopeananohiukkaset kertyvät lopulta pohjasedimentteihin. Vesifaasissa hopeananohiukkaset ovat erittäin myrkyllisiä vesieliöille, mutta niiden ympäristövaikutuksista pohjasedimenteissä ei vielä tiedetä paljoa.

Väitöskirjan päätavoitteena oli tutkia sedimenttiin lisättyjen hopeananohiukkasten käyttäytymistä ja toksisia vaikutuksia laboratorio-olosuhteissa. Kokeissa käytettiin polyvinyylipyrrolidonilla (PVP) pinnoitettuja ja pinnoittamattomia hopeananohiukkasia sekä runsasliukoista hopeanitraattia positiivisena hopeaionitoksisuuskontrollina. Kahta pintakemialtaan eroavaa hiukkastyyppiä käyttämällä pyrittiin selvittämään, vaikuttaako hiukkasten pinnoitus myös niiden ympäristökäyttäytymiseen. Sedimentin ominaisuuksien vaikutusta hopeananohiukkasiin tutkittiin käyttämällä sekä luonnollisia että keinotekoisesti valmistettuja sedimenttejä. Luonnolliset sedimentit nostettiin Höytiäisestä ja Kuoringasta, jotka ovat Itä-Suomessa sijaitsevia järviä. Toksisuuskokeissa mallieliönä käytettiin pohjasedimenttiin kaivautuvia *Lumbriculus variegatus* -harvasukamatoja. Toksisina vasteina tarkkailtiin altistettujen eliöiden biomassan, syödyn sedimentin määrän sekä yksilömäärän suhdetta altistamattomiin kontrollieliöihin. Analysoimalla mallieliöiden hopeapitoisuudet koejakson päätyttyä tarkasteltiin eri hopeamateriaalien biokertyvyyttä eliöihin. Toksisuuskokeiden lisäksi tutkittiin hopeananohiukkasista vapautuneen hopean sitoutumista sedimentin eri komponentteihin sekä biosaatavan liukoisen hopean konsentraatiota sedimentissä.

Hopeaionien toksisuus tutkituissa sedimenteissä havaittiin pääsääntöisesti suuremmaksi kuin hopeananohiukkasten. Poikkeuksen teki keinosedimentti, jonka pH oli säädetty happamaksi (pH = 5). Tässä sedimentissä hopeananohiukkasten toksisuus oli samaa luokkaa hopeaionien kanssa. Sedimentin happamuuden havaittiin nostavan sekä hopeaionien että hopeananohiukkasten toksisuutta keinosedimentissä. Happamuuden vaikutus hopeananohiukkasiin oli moninkertainen hopeaioneihin verrattuna, todennäköisesti hiukkasten suuremman liukoisuuden takia. Hopeananohiukkasten toksisuuden todettiin johtuvan pääasiassa hopeaioneista, sillä toksiset vasteet pystyttiin yhdistämään sedimentistä analysoituun biosaatavan liukoisen hopean määrään. Hiukkasten pintakemialla ei havaittu olevan merkittävää vaikutusta toksisuuteen keinosedimenteissä. Myös happamassa Kuoringan luonnonsedimentissä hopeananohiukkaset olivat toksisempia kuin Höytiäisen sedimentissä. Nanohiukkasten liukoisuus arvioitiin kuitenkin molemmissa sedimenteissä samalle tasolle, ja toksisuuden oletettiin johtuvan pääasiassa liukoisen hopean biosaatavuuden noususta. Biosaatavuus oli korkeampaa vain päällystetyillä hopeananohiukkasilla Kuoringan sedimentissä, joten hiukkasten pintakemia vaikutti hopean biosaatavuuteen. Todennäköisesti PVP:lla päällystetyt nanohiukkaset sitoivat liukoisia hopeaioneja itseensä pinnoittamattomia paremmin. Liukoinen hopea hiukkasten pinnalta kertyi koe-eliöihin nostaen siten toksisuutta. Todennäköisesti myös sedimentin sisältämä orgaaninen aines ja sen koostumus vaikuttivat hopeaionien ja hopeananohiukkasten välisiin reaktioihin, sillä biosaatavuus kasvoi vain Kuoringan sedimentissä. Tätä teoriaa tukee pohjatieto siitä, että PVP-molekyylit sitovat heikosti hopeaioneja.

Väitöskirjan tulokset korostavat sedimentin ominaisuuksien vaikutusta hopeananohiukkasten toksisuuteen ja ympäristökäyttäytymiseen. Etenkin sedimentin happamuus havaittiin toksisuutta ja biosaatavuutta nostavaksi tekijäksi. Sedimentin sisältämä orgaaninen aines ja sen laatu näytti myös vaikuttavan hopeananohiukkasten ympäristökäyttäytymiseen, mutta jatkotutkimuksia tulisi tehdä tämän teorian varmistamiseksi. Sedimentin ominaisuuksien lisäksi myös nanohiukkasten pintakemialla havaittiin olevan vaikutusta hopean biosaatavuuteen ja siten hopean kertymiseen eliöihin. Kokonaisuudessaan hopeananohiukkasten toksisuus sedimentissä oli samalla tasolla tai heikompaa kuin hopeaionien. Näiden tulosten perusteella näyttäisi, etteivät hopeananohiukkasten aiheuttamat ympäristövaikutukset olisi tavanomaisia hopeayhdisteitä suurempia. Hopeananohiukkasten toksisuutta sedimentissä on kuitenkin tutkittu hyvin vähän. Tulosten varmistamiseksi tulisi vastaavia toksisuuskokeita tehdä useammilla lajeilla

REFERENCES

Adams L.K, Lyon D.Y. & Alvarez P.J.J. 2006, Comparative eco-toxicity of nanoscale TiO_2, SiO_2, and ZnO water suspensions. *Water Res* 40: 3527–3532.

Akaighe N., MacCuspie R.I., Navarro D.A., Aga D.S., Banerjee S., Sohn M. & Sharma V.K. 2011. Humic acid-induced silver nanoparticle formation under environmentally relevant conditions. *Environ Sci Technol* 45: 3895–3901.

Anon. 2002. *Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates*. United States Environmental Protection Agency, Washington DC.

Anon. 2007. *Test No. 225: Sediment-water Lumbriculus toxicity test using spiked sediment*. Organisation for Economic Co-operation and Development, Paris, France.

Anon. 2011. *Commission Recommendation of 18 October 2011 on the definition of nanomaterial (2011/696/EU)*. European Commission, Brussels, Belgium.

Anon. 2012. *Guidance on sample preparation and dosimetry for the safety testing of manufactured nanomaterials.* Organisation for Economic Co-operation and Development, Paris, France.

Aschberger K., Micheletti C., Sokull-Klüttgen B. & Christensen F.M. 2011. Analysis of currently available data for characterising the risk of engineered nanomaterials to the environment and human health—lessons learned from four case studies. *Environ Int* 37: 1143–1156.

Bianchini A. & Wood C.M. 2003. Mechanism of acute silver toxicity in *Daphnia magna*. *Environ Toxicol Chem* 22: 1361–1367.

Buffet P.E., Zalouk-Vergnoux A., Chatel A., Berthet B., Metais I., Perrein-Ettajani H., Poirier L., Luna-Acosta A., Thomas-Guyon H., Risso-de Faverney C., Guibbolini M., Gilliland D., Valsami-Jones E. & Mouneyrac C. 2014. A marine mesocosm study on the environmental fate of silver nanoparticles and toxicity effects on two endobenthic species: the ragworm *Hediste diversicolor* and the bivalve mollusc *Scrobicularia plana*. *Sci Total Environ* 470–471: 1151–1159.

Cerillo C., Barandika G., Igartua A., Areitioaurtena O. & Mendoza G. 2017. Key challenges for nanotechnology: Standardization of ecotoxicity testing. *J Envrion Sci Health C* [in press]

Chambers B.A., Afrooz A.N., Bae S., Aich N., Katz L., Saleh N.B. & Kirisits M.J. 2013. Effects of chloride and ionic strength on physical morphology, dissolution, and bacterial toxicity of silver nanoparticles. *Environ Sci Technol* 48: 761–769.

Chan C.Y.S. & Chiu J.M.Y. 2015. Chronic Effects of Coated Silver Nanoparticles on Marine Invertebrate Larvae: A Proof of Concept Study. *PLoS ONE* 10: e0132457.

Coleman J.G., Kennedy A.J., Bednar A.J., Ranville J.F., Laird J.G., Harmon A.R., Hayes C.A., Gray E.P., Higgins C.P., Lotufo G. & Steevens J.A. 2013. Comparing the effects of nanosilver size and coating variations on bioavailability, internalization, and elimination, using *Lumbriculus variegatus*. *Environ Toxicol Chem* 32: 2069–2077.

Coll C., Notter D., Gottschalk F., Sun T., Som C. & Nowack B. 2016. Probabilistic environmental risk assessment of five nanomaterials (nano-TiO_2, nano-Ag, nano-ZnO, CNT, and fullerenes). *Nanotoxicology* 10: 436–444.

Cong Y., Banta G.T., Selck H., Berhanu D., Valsami-Jones E. & Forbes V.E. 2011. Toxic effects and bioaccumulation of nano-, micron-and ionic-Ag in the polychaete, *Nereis diversicolor*. *Aquat Toxicol* 105: 403–411.

Cong Y., Banta G.T., Selck H., Berhanu D., Valsami-Jones E. & Forbes V.E. 2014. Toxicity and bioaccumulation of sediment-associated silver nanoparticles in the estuarine polychaete, *Nereis* (Hediste) *diversicolor*. *Aquat Toxicol* 156: 106–115.

Cornelis G., Doolette M., Thomas C., McLaughlin M.J., Kirby J.K., Beak D.G. & Chittleborough D. 2012. Retention and dissolution of engineered silver nanoparticles in natural soils. *Soil Sci Soc Am J* 76: 891–902.

Coutris C., Joner E.J. & Oughton D.H. 2012a. Aging and soil organic matter content affect the fate of silver nanoparticles in soil. *Sci Total Environ* 420: 327–333.

Coutris C., Hertel-Aas T., Lapied E., Joner E.J. & Oughton D.H. 2012b. Bioavailability of cobalt and silver nanoparticles to the earthworm *Eisenia fetida*. *Nanotoxicology* 6: 186–195.

Cozzari M., Elia A.C., Pacini N., Smith B.D., Boyle D., Rainbow P.S. & Khan F.R. 2015. Bioaccumulation and oxidative stress responses measured in the estuarine ragworm (*Nereis diversicolor*) exposed to dissolved, nano-and bulk-sized silver. *Environ Pollut* 198: 32–40.

Crane M., Handy R.D., Garrod J. & Owen R. 2008. Ecotoxicity test methods and environmental hazard assessment for engineered nanoparticles. *Ecotoxicology* 17: 421.

Cumberland S.A. & Lead J.R. 2009: Particle size distributions of silver nanoparticles at environmentally relevant conditions. *J Chromatogr A* 1216: 9099–9105.

Dai L., Syberg K., Banta G.T., Selck H. & Forbes V.E. 2013. Effects, uptake, and depuration kinetics of silver oxide and copper oxide nanoparticles in a marine deposit feeder, *Macoma balthica*. *ACS Sustain Chem Eng* 1: 760–767.

Dalton A.B., Collins S., Munoz E., Razal J.M., Ebron V.H., Ferraris J.P., Coleman J.N., Kim B.G. & Baughman R.H. 2003. Super-tough carbon-nanotube fibres. *Nature* 423: 703–703.

Delay M., Dolt T., Woellhaf A., Sembritzki R. & Frimmel F.H. 2011. Interactions and stability of silver nanoparticles in the aqueous phase: Influence of natural organic matter (NOM) and ionic strength. *J Chromatogr A* 1218: 4206–4212.

Dudkiewicz A., Tiede K., Loeschner K., Jensen L.H.S., Jensen E., Wierzbicki R., Coxali A.B.A. & Molhave K. 2011. Characterization of nanomaterials in food by electron microscopy. *Trends Analyt Chem* 30: 28–43.

Fabrega J., Fawcett S.R., Renshaw J.C. & Lead J.R. 2009. Silver nanoparticle impact on bacterial growth: effect of pH, concentration, and organic matter. *Environ Sci Technol* 43: 7285–7290.

Feng Q., Wu J., Chen G., Cui F., Kim T. & Kim J. 2000. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *J Biomed Mater Res* 52: 662–668.

García-Alonso J., Khan F.R., Misra S.K., Turmaine M., Smith B.D., Rainbow P.S., Luoma S.N. & Valsami-Jones E. 2011. Cellular internalization of silver nanoparticles in gut epithelia of the estuarine polychaete *Nereis diversicolor*. *Environ Sci Technol* 45: 4630–4636.

Gliga A.R., Skoglund S., Wallinder I.O., Fadeel B. & Karlsson H.L. 2014. Size-dependent cytotoxicity of silver nanoparticles in human lung cells: the role of cellular uptake, agglomeration and Ag release. *Part Fibre Toxicol* 11: 11.

Gunsolus I.L., Mousavi M.P., Hussein K., Bühlmann P. & Haynes C.L. 2015. Effects of humic and fulvic acids on silver nanoparticle stability, dissolution, and toxicity. *Environ Sci Technol* 49: 8078–8086.

Handy R.D., Cornelis G., Fernandes T., Tsyusko O., Decho A., Sabo-Attwood T., Metcalfe C., Steevens J.A., Klaine S.J. & Koelmans A.A. 2012. Ecotoxicity test methods for engineered nanomaterials: practical experiences and recommendations from the bench. *Environ Toxicol Chem* 31: 15–31.

Hansen S.F., Heggelund L.R., Besora P.R., Mackevica A., Boldrin A. & Baun A. 2016. Nanoproducts–what is actually available to European consumers? *Environ Sci Nano* 3: 169–180.

Heckmann L., Hovgaard M.B., Sutherland D.S., Autrup H., Besenbacher F. & Scott-Fordsmand J.J. 2011. Limit-test toxicity screening of selected inorganic nanoparticles to the earthworm *Eisenia fetida*. *Ecotoxicology* 20: 226–233.

Hedberg J., Oromieh A.G., Kleja D.B. & Wallinder I.O. 2015. Sorption and dissolution of bare and coated silver nanoparticles in soil suspensions—Influence of soil and particle characteristics. *J Environ Sci Health A* 50: 891–900.

Hogstrand C. & Wood C.M. 1998. Toward a better understanding of the bioavailability, physiology, and toxicity of silver in fish: implications for water quality criteria. *Environ Toxicol Chem* 17: 547–561.

Homaee M.B. & Ehsanpour A.A. 2016. Silver nanoparticles and silver ions: Oxidative stress responses and toxicity in potato (*Solanum tuberosum* L) grown in vitro. *Hortic Environ Biotechol* 57: 544–553.

Hsiao I., Hsieh Y., Wang C., Chen I. & Huang Y. 2015. Trojan-horse mechanism in the cellular uptake of silver nanoparticles verified by direct intra-and extracellular silver speciation analysis. *Environ Sci Technol* 49: 3813–3821.

Iravani S., Korbekandi H., Mirmohammadi S.V. & Zolfaghari B. 2014. Synthesis of silver nanoparticles: chemical, physical and biological methods. *Res Pharm Sci* 9: 385–406.

Jewell C.M., Jung J., Atukorale P.U., Carney R.P., Stellacci F. & Irvine D.J. 2011. Oligonucleotide Delivery by Cell-Penetrating "Striped" Nanoparticles. *Angew Chem Int Ed* 50: 12312–12315.

Kaegi R., Voegelin A., Sinnet B., Zuleeg S., Hagendorfer H., Burkhardt M. & Siegrist H. 2011. Behavior of metallic silver nanoparticles in a pilot wastewater treatment plant. *Environ Sci Technol* 45: 3902–3908.

Kennedy A.J., Chappell M.A., Bednar A.J., Ryan A.C., Laird J.G., Stanley J.K. & Steevens J.A. 2012. Impact of organic carbon on the stability and toxicity of fresh and stored silver nanoparticles. *Environ Sci Technol* 46: 10772–10780.

Kim S. & Ryu D. 2013. Silver nanoparticle-induced oxidative stress, genotoxicity and apoptosis in cultured cells and animal tissues. *J Appl Toxicol* 33: 78–89.

Kim H.-A., Lee B.-T., Na S.-Y., Kim K.-W., Ranville J.F., Kim S.-O., Jo E. & Eom I.-C. 2017: Characterization of silver nanoparticle aggregates using single particle-inductively coupled plasma-mass spectrometry (spICP-MS). *Chemosphere* 171: 468–475.

Klitzke S., Metreveli G., Peters A., Schaumann G.E. & Lang F. 2014. The fate of silver nanoparticles in soil solution—Sorption of solutes and aggregation. *Sci Total Environ* 535: 54–60.

Koopmans G.F., Hiemstra T., Regelink I.C., Molleman B. & Comans R.N.J. 2015: Asymmetric flow field-flow fractionation of manufactured silver nanoparticles spiked into soil solution. *J Chromatogr A* 1392: 100–109.

Kühnel D. & Nickel C. 2014. The OECD expert meeting on ecotoxicology and environmental fate—towards the development of improved OECD guidelines for the testing of nanomaterials. *Sci Total Environ* 472: 347–353.

Laborda F., Bolea E., Cepriá G., Gómez M.T., Jiménez M.S., Pérez-Arantegui J. & Castillo J.R. 2016. Detection, characterization and quantification of inorganic engineered nanomaterials: a review of techniques and methodological approaches for the analysis of complex samples. *Anal Chim Acta* 904: 10–32.

Landrum P.F., Gedeon M.L., Burton G.A., Greenberg M.S. & Rowland C.D. 2002. Biological responses of *Lumbriculus variegatus* exposed to fluoranthene-spiked sediment. *Arch Environ Contam Toxicol* 42: 292–302.

Landrum P.F., Leppänen M., Robinson S.D., Gossiaux D.C., Burton G.A., Greenberg M., Kukkonen J.V., Eadie B.J. & Lansing M.B. 2004. Comparing behavioral and chronic endpoints to evaluate the response of Lumbric*ulus variegatus* to 3, 4, 3′, 4′-tetrachlorobiphenyl sediment exposures. *Environ Toxicol Chem* 23: 187–194.

Lee K.J., Nallathamby P.D., Browning L.M., Osgood C.J. & Xu X.N. 2007. In vivo imaging of transport and biocompatibility of single silver nanoparticles in early development of zebrafish embryos. *ACS nano* 1: 133–143.

Lee S., Park S., Kim Y., Im H. & Choi J. 2016. Effect of sulfidation and dissolved organic matters on toxicity of silver nanoparticles in sediment dwelling organism, *Chironomus riparius*. *Sci Total Environ* 553: 565–573.

Leppänen M.T. & Kukkonen J.V.K. 1998a. Factors affecting feeding rate, reproduction and growth of an oligochaete *Lumbriculus variegatus* (Müller). *Hydrobiologia* 377: 183–194.

Leppänen M.T. & Kukkonen J.V.K. 1998b. Relationship between reproduction, sediment type, and feeding activity of *Lumbriculus variegatus* (Müller): implications for sediment toxicity testing. *Environ Toxicol Chem* 17: 2196–2202.

Levard C., Hotze E.M., Colman B.P., Dale A.L., Truong L., Yang X., Bone A.J., Brown Jr G.E., Tanguay R.L., Di Giulio R.T., Bernhardt E.S., Meyer J.N., Wiesner M.R. & Lowry G.V. 2013a. Sulfidation of silver nanoparticles: Natural antidote to their toxicity. *Environ Sci Technol* 47: 13440–13448.

Levard C., Mitra S., Yang T., Jew A.D., Badireddy A.R., Lowry G.V. & Brown Jr G.E. 2013b. Effect of chloride on the dissolution rate of silver nanoparticles and toxicity to *E. coli*. *Environ Sci Technol* 47: 5738–5745.

Li X., Lenhart J.J. & Walker H.W. 2011. Aggregation kinetics and dissolution of coated silver nanoparticles. *Langmuir* 28: 1095–1104.

Li Y., Qin T., Ingle T., Yan J., He W., Yin J.-J. & Chen T. 2017. Differential genotoxicity mechanisms of silver nanoparticles and silver ions. *Arch Toxicol* 91: 509–519.

Liu J. & Hurt R.H. 2010. Ion release kinetics and particle persistence in aqueous nano-silver colloids. *Environ Sci Technol* 44: 2169–2175.

Liu J., Sonshine D.A., Shervani S. & Hurt R.H. 2010. Controlled release of biologically active silver from nanosilver surfaces. *ACS nano* 4: 6903–6913.

Lovern S.B. & Klaper R. 2006. *Daphnia magna* mortality when exposed to titanium dioxide and fullerene (C60) nanoparticles. *Environ Toxicol Chem* 25: 1132–1137.

Lowry G.V., Gregory K.B., Apte S.C. & Lead J.R. 2012. Transformations of nanomaterials in the environment. *Environ Sci Technol* 46: 6893–6899.

Luo X., Xu S., Yang Y., Zhang Y., Wang S., Chen S., Xu A. & Wu L. 2017. A novel method for assessing the toxicity of silver nanoparticles in *Caenorhabditis elegans. Chemosphere* 168: 648–657.

MacDonald D.D., Ingersoll C.G. & Berger T.A. 2000. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. *Arch Environ Contam Toxicol* 39: 20–31.

Mäenpää K.A., Sormunen A.J. & Kukkonen J.V.K. 2003. Bioaccumulation and toxicity of sediment associated herbicides (ioxynil, pendimethalin, and bentazone) in *Lumbriculus variegatus* (Oligochaeta) *Chironomus riparius* (Insecta). *Ecotoxicol Environ Saf* 56: 398–410.

Mäenpää K., Sorsa K., Lyytikäinen M., Leppänen M. & Kukkonen J.V.K. 2008. Bioaccumulation, sublethal toxicity, and biotransformation of sediment-associated pentachlorophenol in *Lumbriculus variegatus* (Oligochaeta). *Ecotoxicol Environ Saf* 69: 121–129.

Maynard A.D. 2011. Don't define nanomaterials. *Nature* 475: 31.

McGillicuddy E., Murray I., Kavanagh S., Morrison L., Fogarty A., Cormican M., Dockery P., Prendergast M., Rowan N. & Morris D. 2017. Silver nanoparticles in the environment: Sources, detection and ecotoxicology. *Sci Total Envrion* 575: 231–246.

Murdock R.C., Braydich-Stolle L., Schrand A.M., Schlager J.J. & Hussain S.M. 2008. Characterization of nanomaterial dispersion in solution prior to *in vitro* exposure using dynamic light scattering technique. *Toxicol Sci* 101: 239–253.

Newton K.M., Puppala H.L., Kitchens C.L., Colvin V.L. & Klaine S.J. 2013. Silver nanoparticle toxicity to *Daphnia magna* is a function of dissolved silver concentration. *Environ Toxicol Chem* 32: 2356–2364.

Notter D.A., Mitrano D.M. & Nowack B. 2014. Are nanosized or dissolved metals more toxic in the environment? A meta-analysis. *Environ Sci Technol* 33: 2733–2739.

Pal S., Tak Y.K. & Song J.M. 2007. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium *Escherichia coli*. *Appl Environ Microbiol* 73: 1712–1720.

Park E., Yi J., Kim Y., Choi K. & Park K. 2010. Silver nanoparticles induce cytotoxicity by a Trojan-horse type mechanism. *Toxicol in Vitro* 24: 872–878.

Park S., Chung J., Colman B.P., Matson C.W., Kim Y., Lee B., Kim P., Choi K. & Choi J. 2015. Ecotoxicity of bare and coated silver nanoparticles in the aquatic midge, *Chironomus riparius*. *Environ Toxicol Chem* 34: 2023–2032.

Peretyazhko T.S., Zhang Q. & Colvin V.L. 2014. Size-controlled dissolution of silver nanoparticles at neutral and acidic pH conditions: kinetics and size changes. *Environ Sci Technol* 48: 11954–11961.

Petersen E.J., Henry T.B., Zhao J., MacCuspie R.I., Kirschling T.L., Dobrovolskaia M.A., Hackley V., Xing B. & White J.C. 2014. Identification and avoidance of potential artifacts and misinterpretations in nanomaterial ecotoxicity measurements. *Environ Sci Technol* 48: 4226–4246.

Petersen E.J., Diamond S.A., Kennedy A.J., Goss G.G., Ho K., Lead J., Hanna S.K., Hartmann N.B., Hund-Rinke K., Mader B., Manier N., Pandard P., Salinas E.R. & Sayre P. 2015. Adapting OECD aquatic toxicity tests for use with manufactured nanomaterials: key issues and consensus recommendations. *Environ Sci Technol* 49: 9532–9547.

Phipps G.L., Ankley G.T., Benoit D.A. & Mattson V.R. 1993. Use of the aquatic oligochaete *Lumbriculus variegatus* for assessing the toxicity and bioaccumulation of sediment-associated contaminants. *Environ Toxicol Chem* 12: 269–279.

Piccinno F., Gottschalk F., Seeger S. & Nowack B. 2012. Industrial production quantities and uses of ten engineered nanomaterials in Europe and the world. *J Nanopart Res* 14: 1109.

Plathe K., Von der Kammer F., Hassellöv M., Moore J., Murayama M., Hofmann T. & Hochella M. 2010. Using FlFFF and aTEM to determine trace metal–nanoparticle associations in riverbed sediment. *Envir Chem* 7: 82–93.

Quik J., Velzeboer I., Wouterse M., Koelmans A. & Van de Meent D. 2014. Heteroaggregation and sedimentation rates for nanomaterials in natural waters. *Water Res* 48: 269–279.

Ramskov T., Forbes V.E., Gilliland D. & Selck H. 2015. Accumulation and effects of sediment-associated silver nanoparticles to sediment-dwelling invertebrates. *Aquat Toxicol* 166: 96–105.

Ratte H.T. 1999. Bioaccumulation and toxicity of silver compounds: a review. *Environ Toxicol Chem* 18: 89–108.

Reidy B., Haase A., Luch A., Dawson K.A. & Lynch I. 2013. Mechanisms of silver nanoparticle release, transformation and toxicity: a critical review of current knowledge and recommendations for future studies and applications. *Materials* 6: 2295–2350.

Reinsch B., Levard C., Li Z., Ma R., Wise A., Gregory K., Brown Jr G. & Lowry G. 2012. Sulfidation of silver nanoparticles decreases *Escherichia coli* growth inhibition. *Environ Sci Technol* 46: 6992–7000.

Ristola T., Pellinen J., Van Hoof P.L., Leppänen M. & Kukkonen J. 1996. Characterization of Lake Ladoga sediments. II. Toxic chemicals. *Chemosphere* 32: 1179–1192.

Rosário F., Hoet P., Santos C. & Oliveira H. 2016. Death and cell cycle progression are differently conditioned by the AgNP size in osteoblast-like cells. *Toxicology* 368: 103–115.

Schlich K. & Hund-Rinke K. 2015. Influence of soil properties on the effect of silver nanomaterials on microbial activity in five soils. *Environ Pollut* 196: 321–330.

Schmid O. & Stoeger T. 2016. Surface area is the biologically most effective dose metric for acute nanoparticle toxicity in the lung. *J Aerosol Sci* 99: 133–143.

Shoults-Wilson W.A., Reinsch B.C., Tsyusko O.V., Bertsch P.M., Lowry G.V. & Unrine J.M. 2011. Role of particle size and soil type in toxicity of silver nanoparticles to earthworms. *Soil Sci Soc Am J* 75: 365–377.

Siegel S. & Castellan J. 1988. *Non parametric statistics for the behavioural sciences.* McGraw-Hill, London.

Simpson S.L., Angel B.M. & Jolley D.F. 2004. Metal equilibration in laboratory-contaminated (spiked) sediments used for the development of whole-sediment toxicity tests. *Chemosphere* 54: 597–609.

Sperling R.A. & Parak W.J. 2010. Surface modification, functionalization and bioconjugation of colloidal inorganic nanoparticles. *Philos Trans A Math Phys Eng Sci* 368: 1333–1383.

Stensberg M.C., Madangopal R., Yale G., Wei Q., Ochoa-Acuña H., Wei A., Mclamore E.S., Rickus J., Porterfield D.M. & Sepúlveda M.S. 2014. Silver nanoparticle-specific mitotoxicity in *Daphnia magna*. *Nanotoxicology* 8: 833–842.

Sun T.Y., Gottschalk F., Hungerbühler K. & Nowack B. 2014. Comprehensive probabilistic modelling of environmental emissions of engineered nanomaterials. *Environ Pollut* 185: 69–76.

Tessier A., Campbell P.G. & Bisson M. 1979. Sequential extraction procedure for the speciation of particulate trace metals. *Anal Chem* 51: 844–851.

Thorp J.H. & Covich A.P. 1991. *Ecology and classification of North American freshwater invertebrates.* Academic press, San Diego.

Tiede K., Boxall A.B., Tear S.P., Lewis J., David H. & Hassellöv M. 2008. Detection and characterization of engineered nanoparticles in food and the environment. *Food Addit Contam* 25: 795–821.

Tiede K., Boxall A.B.A., Tiede D., Tear S.P., David H. & Lewis J. 2009. A robust size-characterisation methodology for studying nanoparticle behavior in 'real' environmental samples, using hydrodynamic chromatography coupled to ICP-MS. *J Anal At Spectrom* 24: 964–972.

Truhaut R. 1977. Ecotoxicology: objectives, principles and perspectives. *Ecotoxicol Environ Saf* 1: 151–173.

Väisänen A., Suontamo R., Silvonen J. & Rintala J. 2002. Ultrasound-assisted extraction in the determination of arsenic, cadmium, copper, lead, and silver in contaminated soil samples by inductively coupled plasma atomic emission spectrometry. *Anal Bioanal Chem* 373: 93–97.

Vance M.E., Kuiken T., Vejerano E.P., McGinnis S.P., Hochella Jr M.F., Rejeski D. & Hull M.S. 2015. Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory. *Beilstein J Nanotech* 6: 1769–1780.

Velzeboer I., Quik J., van de Meent D. & Koelmans A. 2014. Rapid settling of nanoparticles due to heteroaggregation with suspended sediment. *Environ Sci Technol* 33: 1766–1773.

Von der Kammer F., Legros S., Hofmann T., Larsen E.H. & Loeschner K. 2011. Separation and characterization of nanoparticles in complex food and environmental samples by field-flow fractionation. *Trends Anal Chem* 30: 425–436.

Wang H., Ho K.T., Scheckel K.G., Wu F., Cantwell M.G., Katz D.R., Horowitz D.B., Boothman W.S. & Burgess R.M. 2014. Toxicity, bioaccumulation, and biotransformation of silver nanoparticles in marine organisms. *Environ Sci Technol* 48: 13711–13717.

Wang S., Lv J., Ma J. & Zhang S. 2016. Cellular internalization and intracellular biotransformation of silver nanoparticles in *Chlamydomonas reinhardtii*. *Nanotoxicology* 10: 1129–1135.

West C. & Ankley G. 1998. A laboratory assay to assess avoidance of contaminated sediments by the freshwater oligochaete *Lumbriculus variegatus*. *Arch Environ Contam Toxicol* 35: 20–24.

Wiederholm T., Wiederholm A. & Milbrink G. 1987. Bulk sediment bioassays with five species of fresh-water oligochaetes. *Water Air Soil Poll* 36: 131–154.

Windler L., Height M. & Nowack B. 2013. Comparative evaluation of antimicrobials for textile applications. *Environ Int* 53: 62–73.

Wittmaack K. 2007. In search of the most relevant parameter for quantifying lung inflammatory response to nanoparticle exposure: particle number, surface area, or what? *Environ Health Perspect* : 187–194.

Yamada H., Michalik J., Sadlo J., Perlinska J., Takenouchi S., Shimomura S. & Uchida Y. 2001. Electron spin resonance studies on silver atoms in imogolite fibers. *Appl Clay Sci* 19: 173–178.

Yang X., Gondikas A.P., Marinakos S.M., Auffan M., Liu J., Hsu-Kim H. & Meyer J.N. 2011. Mechanism of silver nanoparticle toxicity is dependent on dissolved silver and surface coating in *Caenorhabditis elegans*. *Environ Sci Technol* 46: 1119–1127.

Yang X., Lin S. & Wiesner M.R. 2014. Influence of natural organic matter on transport and retention of polymer coated silver nanoparticles in porous media. *J Hazard Mater* 264: 161–168.

Zhang Z., Zhao B. & Hu L. 1996. PVP protective mechanism of ultrafine silver powder synthesized by chemical reduction processes. *J Solid State Chem* 121: 105–110.

Zhao C. & Wang W. 2010. Biokinetic uptake and efflux of silver nanoparticles in *Daphnia magna*. *Environ Sci Technol* 44: 7699–7704.

ORIGINAL PAPERS

I

TOXICITY TESTING OF SILVER NANOPARTICLES IN ARTIFICIAL AND NATURAL SEDIMENTS USING THE BENTHIC ORGANISM *LUMBRICULUS VARIEGATUS*

by

Juho Elias Rajala, Kimmo Mäenpää, Eeva-Riikka Vehniäinen, Ari Väisänen,
Janeck James Scott-Fordsmand, Jarkko Akkanen & Jussi Vilho Kalevi Kukkonen
2016

Archives of Environmental Contamination and Toxicology 71: 405–414.

Arch Environ Contam Toxicol (2016) 71:405–414
DOI 10.1007/s00244-016-0294-4

Toxicity Testing of Silver Nanoparticles in Artificial and Natural Sediments Using the Benthic Organism *Lumbriculus variegatus*

Juho Elias Rajala[1] · **Kimmo Mäenpää**[2] · **Eeva-Riikka Vehniäinen**[1] ·
Ari Väisänen[3] · **Janeck James Scott-Fordsmand**[4] · **Jarkko Akkanen**[2] ·
Jussi Vilho Kalevi Kukkonen[1]

Received: 11 March 2016 / Accepted: 22 June 2016

Abstract The increased use of silver nanoparticles (AgNP) in industrial and consumer products worldwide has resulted in their release to aquatic environments. Previous studies have mainly focused on the effects of AgNP on pelagic species, whereas few studies have assessed the risks to benthic invertebrates despite the fact that the sediments act as a large potential sink for NPs. In this study, the toxicity of sediment-associated AgNP was evaluated using the standard sediment toxicity test for chemicals provided by the Organization of Economic Cooperation and Development. The freshwater benthic oligochaete worm *Lumbriculus variegatus* was exposed to sediment-associated AgNP in artificial and natural sediments at concentrations ranging from 91 to 1098 mg Ag/kg sediment dry weight. Silver nitrate ($AgNO_3$) was used as a reference compound for Ag toxicity. The measured end points of toxicity were mortality, reproduction, and total biomass. In addition, the impact of sediment-associated AgNP on the feeding rate of *L. variegatus* was studied in a similar test set-up as mentioned previously. The addition of AgNP into the sediment significantly affected the feeding rate and reproduction of the test species only at the highest concentration (1098 mg/kg) of Ag in the natural sediment with the lowest pH. In comparison, the addition of $AgNO_3$ resulted in reproductive toxicity in every tested sediment, and Ag was more toxic when spiked as $AgNO_3$ than AgNP. In general, sediments were observed to have a high capacity to eliminate the AgNP-derived toxicity. However, the capacity of sediments to eliminate the toxicity of Ag follows a different pattern when spiked as AgNP than $AgNO_{3.}$ The results of this study emphasize the importance of sediment-toxicity testing and the role of sediment properties when evaluating the environmental effects and behavior of AgNP in sediments.

Silver nanoparticles (AgNP), used, e.g., in healthcare, textiles, paints, cosmetics, and cleaning agents, have the highest degree of commercialization (as a number of products) of all nanoscale materials due to their unique optical and antibacterial properties (Vance et al. 2015). In surface waters, AgNP are mainly released through wastewater-treatment plants and untreated wastewater (Gottschalk et al. 2009). Sediment is the final sink for the AgNP, and the modeled annual increase of sediment concentrations varies between 0.15 and 10.18 µg/kg/y resulting in a possible hazard for aquatic organisms (Gottschalk et al. 2009).

In environmental media, AgNP may be oxidized, which leads to dissolution and release of Ag ions (Ag^+) (Loza et al. 2014). Ionic Ag is highly toxic to aquatic organisms, and thus the toxicity of AgNP may be related to the concentration of dissolved Ag^+ (Navarro et al. 2008; van Aerle et al. 2013). However, the concentration of freely dissolved Ag^+ in environmental media is typically low due to strong complexation with chloride, sulfide, and natural organic matter (Levard et al. 2013; Loza et al. 2014). AgNP also pose NP-specific toxicity (Chan and Chiu 2015; Cozzari

✉ Juho Elias Rajala
juho.e.rajala@jyu.fi

[1] Department of Biological and Environmental Science, University of Jyväskylä, 40014 Jyväskylä, Finland

[2] Department of Biology, University of Eastern Finland, 80101 Joensuu, Finland

[3] Department of Chemistry, University of Jyväskylä, 40014 Jyväskylä, Finland

[4] Department of Bioscience, Århus University, 8600 Silkeborg, Denmark

et al. 2015; García-Alonso et al. 2014). One of the primary identified toxic mechanisms at the molecular level is the generation of reactive-oxygen species resulting in oxidative stress (Cozzari et al. 2015; Roh et al. 2009).

The behavior and toxicity of AgNP in sediment is still poorly understood, and there is an urgent need for studies and standardized test methods. The biggest challenge in studies with nanomaterials in sediment and other complex environmental media is the lack of proper characterization methods. Because most of the nanomaterials are not stable in water, sediment studies are still considered to be relevant and sometimes even more representative of environmental exposure than aqueous tests (Petersen et al. 2015). In water-only exposure tests of AgNP with varying coatings resulted in LC_{50} (lethal concentration to kill 50 % of the test organisms in 96 h) values of 0.07–0.33 mg/L to the benthic organism *Lumbriculus variegatus* (Khan et al. 2015). When the same species was exposed by way of sediment, AgNP showed no mortality on exposure at 367 mg/kg (Coleman et al. 2013). However, in sediment exposure, AgNP induces oxidative stress in *Nereis diversicolor* at concentrations >10 mg/kg (Cozzari et al. 2015). The results indicate that AgNP-induced toxicity is decreased when particles are introduced into sediment, but the role of sediment properties has not yet been studied.

The aims of this study were (1) to examine how sediment properties influence the toxicity of AgNP; (2) to compare the toxicity of Ag spiked as AgNP with dissolved Ag spiked as silver nitrate ($AgNO_3$); and (3) to evaluate the suitability of the Organisation for Economic Co-operation and Development (OECD) standard test method guideline 225 for use with nanomaterials. Artificial and two natural sediments that differed in their characteristics were selected and spiked with polyvinylpyrrolidone-coated AgNP and $AgNO_3$. OECD standard test guideline 225, Sediment–Water Lumbriculus Toxicity Test Using Spiked Sediment (OECD 2007), was followed, and mortality, reproduction, and changes in biomass were used as indicators of toxicity to the endobenthic aquatic Oligochaeta *L. variegatus*. In addition, the feeding rate of *L. variegatus* was used as an end point of toxicity for AgNP.

Materials and Methods

AgNP

AgNP (polyvinylpyrrolidone coating 0.2 %; NanoAmor) had a nominal reported surface area of 5–10 m^2/g and a diameter of 30–50 nm with a purity of 99.9 %. The characterization of the particles was published in the same year as the experimental part of this study was performed (Heckmann et al. 2011). Particles were stored as dry powder and kept away from direct sunlight, as recommended by the manufacturer, to minimize possible changes in particle properties during storage. The characterization of AgNP included transmission electron microscopy (Phillips CM20; Phillips/FEI), dynamic light scattering, and zeta-potential measurements (Malvern Zetasizer Nano; Malvern Instruments). The characterization of AgNP was performed in deionized-water suspension due to the lack of methods to characterize the particles in complex environmental media. Characterization in the test water was not considered to be relevant because particles were never introduced into the test water. The mean diameter of AgNP has been reported to be 82 ± 2 nm ($n = 294$) as measured from transmission electron microscope images and 235 ± 4 nm ($n = 4$) with a zeta potential of -28.6 ± 0.6 mV ($n = 8$) by dynamic light scattering (Heckmann et al. 2011). Agglomeration of the AgNP in water suspension explains the larger diameter of the particles as measured by dynamic light scattering. For further details of the characterization, see Heckmann et al. (2011).

Test Organisms

Endobenthic oligochaeta *L. variegatus* originated from the laboratory culture maintained at the Department of Biology, University of Eastern Finland, Joensuu, Finland. Worms were cultured in 5-L tanks containing artificial freshwater (AFW; pH 7, hardness 1.0 mM/L as calcium [Ca] + magnesium [Mg]) with constant aeration. The light regime was adjusted to 16 h light and 8 h dark, and temperature was held constant at 20 ± 2 °C. A layer of paper towels was used as a substrate. Worms were fed twice a week with a Tetramin fish food (Tetrawerke) and water was renewed once a week. An acclimation phase of 24 h was used to adapt the worms to the test water.

Sediments

One artificial sediment (AS) and two natural sediments collected from Lake Höytiäinen (HS) and Lake Kuorinka (KS) were used in this study. Both natural sediments have been used as clean reference sediments in similar experiments, and possible trace amounts of organic chemicals are low and not believed to have an influence on the outcome of current experiments (Mäenpää et al. 2008; Ristola et al. 1996). The sediment AS was prepared using OECD guideline 225 (OECD 2007). The exact constituent composition was 5 % peat, 74 % quartz sand (60 % <0.2 mm, 40 % 0.2–1.0 mm), 20 % kaolin, and 51 % water (of total dw). *Urtica dioica* powder (0.5 %) was added as a food source to AS, and the pH was adjusted to 6.7 with $CaCO_3$.

For analyses, natural sediments were sieved through a 1-mm sieve to remove large particles and debris. Subsamples of the sediments were dried at 105 °C overnight to measure dry weight (dw). The determination of organic carbon (OC), inorganic carbon (IC), and black carbon (BC) was performed with Analytik Jena TOC analyzer with a solid sample module (Analytik Jena N/C 2100). Furthermore, subsamples of the sediments were heated for 2 h at 550 °C in a muffle furnace oven (Naber 2804 L47) to obtain the percent loss of ignition (LOI). All analyses were performed in three replicates.

The heavy-metal concentrations of sediments were measured from two different test vessels for each treatment, and the total Ag concentrations were determined in triplicate for each treatment. The sediment samples were stored frozen at −20 °C before extraction. The extraction was as follows: A subsample of approximately 200 mg (500 mg for total Ag) was taken from dry sediment and digested in 1:3 (v/v) solution nitric acid and hydrochloric acid for 9 min in 3-minute intervals in an ultrasound water bath (650 W, 35 kHz; ELMA Transsonic T820/H) at 60 °C. The sample tubes were shaken between each 3-minute step. The digested sediment samples were filtered (Whatman no. 41) and diluted to a volume of 20 mL (50 mL for total Ag) with ultrapure water before analysis. The samples were analyzed with a Perkin-Elmer model Optima 8300 inductively coupled plasma optical emission spectrometer. A cyclonic spray chamber equipped with the GemCone Low-Flow nebulizer was used throughout. The plasma power of 1500 W and a nebulizer flow of 0.6 L/min were used to obtain robust plasma conditions for the accurate analysis of the elements. Reagent blank samples were used in between the samples to ensure the analytical procedure. The accepted relative SD of three replicate measurements was <10 %, and the detection limit was 1.9 µg/L. All of the used reagents were of analytical grade and supplied by Merck.

Spiking of the Sediments

Direct addition of dry AgNP powder to the sediment was chosen as the spiking method due to the unstable behavior of the particles in the water suspension. The final Ag concentrations were selected based on the preliminary test (Table 1). The sediments were spiked with AgNP by first mixing the NP powder with a small subsample of the sediment using a metal spoon. The subsample was then mixed with the rest of the sediment. To ensure the homogenous distribution of the compounds, the sediment was mixed with a rotating metal blade for 1 h. Silver nitrate (high-grade, 99.5 % purity; J. T. Baker) was used as a source of dissolved Ag, and added to the sediment in a stock solution dissolved in water (400 g/L). The sediment was treated in a similar way as the AgNP-spiked sediment.

Toxicity Test

The toxicity of AgNP was tested according to the OECD guideline 225 using $AgNO_3$ as a reference for Ag^+ toxicity (OECD 2007). The test was performed in 250-mL beakers (diameter 6 cm) with four replicates for each treatment and six replicates for the control treatment. The amount of the sediment was adjusted to the ratio of 1:50 (dry biomass of worms and total OC of the sediment [w:w]). The sediment-overlying water ratio was adjusted to approximately 1:3 (v:v). The water hardness of AFW was 2.5 mM/L ([Ca] + [Mg] concentration), and the pH was 7.5 (OECD 2007). The sediments were allowed to settle for 7 days with gentle aeration before adding 10 similar-sized *L. variegatus* into the test vessels. The worms were not synchronized for the toxicity test based on the consistent results with only low variation in reproduction and biomass of the worms in the preliminary test (data not shown). During the incubation, the temperature was kept constant at 20 ± 2 °C, and the light regime was 16 h light to 8 h dark. Oxygen and pH were measured once a week during the test. After the 28-day exposure, the worms were removed from the sediment, counted, and placed on a Petri dish with a small amount of AFW. A depuration time of 4 h was used to let the worms empty their gut before placing the worms in an oven at 105 °C for overnight. The dry weight was measured with a microbalance (Sartorius 4503). Missing worms from the test vessels after the exposure period were interpreted as mortality and extra worms as reproduction.

Feeding-Rate Test

The feeding rate test was performed according to Leppänen and Kukkonen (1998) under similar conditions as described previously for the toxicity test. Three replicates were used for each treatment, and two control treatments were performed for each sediment (total $n = 6$). A portion of 23 g of wet test sediment was added on the bottom of the 50-mL beakers, which were then filled with 2.5 mM/L ([Ca] + [Mg] concentration) AFW (OECD 2007). The oxygen level and pH in the overlying water were measured during the test, and the water was renewed using aerated AFW every 2 days. Before adding organisms into the beakers, the sediments were allowed to settle for 2 days. Each beaker received five worms of a similar size. Immediately after the worms buried themselves into the sediment, a layer of a few millimeters of combusted quartz sand (grain size 1–2 mm) was added on the top of the sediment. The egestion rate of the worms was followed by collecting fecal pellets every second day for 14 days. The fecal pellets were dried overnight at 105 °C, and the dry weight was measured with a microbalance. On the last day of the experiment, the worms were removed from the

Table 1 Nominal and determined silver concentrations (mg/kg dw) spiked as $AgNO_3$ and AgNP in test sediments

Compound	Nominal concentration	Determined concentration in sediments (mg/kg)[a]		
		AS	HS	KS
$AgNO_3$	10	11.7 (0.6)	10.8 (0.0)	10.1 (0.0)
	40	36.9 (1.0)	42.8 (0.4)	41.9 (0.3)
	100	92.7 (1.7)	98.9 (0.9)	103.1 (3.6)
	200	180.6 (1.6)	199.3 (1.2)	208.9 (2.9)
	400	360.4 (7.1)	394.8 (5.2)	415.9 (4.0)
AgNP	100	90.6 (1.9)	95.7 (10.8)	77.1 (5.0)
	300	262.5 (2.3)	273.8 (18.1)	256.7 (23.5)
	600	537.4 (12.3)	471.1 (9.8)	525.8 (27.4)
	900	725.4 (23.5)	741.2 (20.1)	801.3 (10.5)
	1200	985.1 (17.8)	943.4 (164.0)	1097.9 (7.5)

[a] Mean and SD of three to five replicates

sediment. After a 4 h' depuration time in clean AFW, the worms were counted and dried at 105 °C overnight to measure their dry weight.

Statistical Testing

The effective (EC) and inhibition (IC) concentrations were estimated using a three-parameter log-logistic model. The normality of data was tested with Shapiro–Wilk normality test and the homogeneity of variances with Bartlett's test. Normally distributed data with equal variances between groups was studied with one-way analysis of variance (ANOVA) followed by pairwise Student *t* test. When the data were not normally distributed, Kruskal–Wallis rank-sum test was used, and multiple comparisons between groups were performed according to Siegel and Castellan (1988).

One-way ANOVA with Tukey's honestly significant difference post hoc test ($p < 0.05$) was used to compare the amount of fecal pellets in the feeding-rate test. The normality of the data were tested with Shapiro–Wilk normality test and the homogeneity of variances with Levene's test. Due to the small sample size ($n = 3$), the normality of the treatment groups was assumed from the normally distributed control groups ($n = 6$) in all sediments. Statistical analyses and graphical illustrations were performed with R version 3.0.1.

Results

Sediment Characteristics

The HS sediment had the highest pH (7.10) and OC percent (OC % = 3.12 %) of the tested sediments (Table 2). In the KS sediment, the pH was low (5.10) and OC % (2.22) lower than in HS, but it was greater than in the AS sediment (0.59 %), which had also greater pH (6.70) than the KS sediment (Table 2). The visual detection and smaller dw % indicated that the natural sediments KS and HS consisted of finer material compared with AS (Table 2). The AS contained only low levels of heavy metals. The natural sediments had greater concentrations with the HS sediment containing approximately twofold to threefold greater concentrations compared with the KS sediment (Table 2). The determined Ag concentrations were in good agreement with the nominal concentrations, and the SD

Table 2 Characteristics of the test sediments

	Sediment		
	AS	HS	KS
pH[a]	6.70 (0.26)	7.10 (0.21)	5.10 (0.11)
LOI %[b]	6.30 (0.06)	10.6 (0.1)	6.03 (0.03)
OC %[b]	0.59 (0.15)	3.12 (0.31)	2.22 (0.14)
BC %[b]	0.04 (0.01)	0.05 (0.00)	0.14 (0.00)
IC %[b]	0.75 (0.37)	–	–
dw %[c]	60.0 (0.2)	18.2 (0.1)	33.2 (0.3)
Cd[d]	0.15 (0.08)	3.34 (0.18)	1.07 (0.07)
Cr[d]	3.79 (0.81)	51.9 (4.8)	20.1 (1.7)
Cu[d]	3.56 (3.15)	53.0 (4.8)	35.8 (3.5)
Ni[d]	2.49 (0.41)	44.2 (3.8)	25.0 (1.7)
Pb[d]	0.77 (0.14)	44.4 (1.9)	18.8 (1.8)
Zn[d]	3.19 (1.60)	130 (7)	56.6 (5.4)

[a] Mean (SD) of weekly measures during the 28-day toxicity test ($n > 55$)

[b] Percent of the sediment (dw); mean (SD) of 3 replicates

[c] Percent of the sediment total weight; mean (SD) of 3 replicates

[d] Metal concentration as dry sediment (mk/kg); mean (SD) of 24–26 replicates

among the replicates was relatively small, which indicates homogenous distribution of Ag in the sediments (Table 1).

Toxicity Test

The pH of the overlying water was at acceptable levels (6–9) for *L. variegatus* in AS and HS, but in the KS sediment the pH was lower than the recommended guideline (OECD 2007). The oxygen saturation was >90 % throughout the experiment in all sediments, but the validity criteria of an 1.8-fold increase in the number of individuals was only fulfilled in the AS sediment (OECD 2007).

Exposure to AgNP-spiked sediments caused no mortality in any sediment type or exposure concentration, but reproduction was significantly decreased compared with the control at the highest concentration in the KS sediment (pairwise Student *t* test, $p < 0.001$) (Fig. 1a). In this treatment, the worms were also avoiding the sediment. The $AgNO_3$-spiked AS sediment was the most toxic to *L. variegatus* and the only sediment where mortality was observed (Fig. 1b). Reproduction decreased with increasing $AgNO_3$ concentration in all of the tested sediments (Fig. 1b). The calculated IC_{50} values for reproduction and EC_{50} values for number of worms (compared with the control) indicate that the Ag spiked as AgNP was only toxic in KS, that Ag spiked as $AgNO_3$ showed the highest toxicity in AS followed by HS, and that the lowest toxicity was observed in KS (Table 3).

No statistically significant differences in total dry biomass of the worms were observed among the exposure concentrations in any of the test sediments spiked with AgNP (Fig. 1c). The total dry biomass decreased with increasing $AgNO_3$ concentration in AS, but it stayed constant in KS and HS (Fig. 1d). Furthermore, sediment properties affected the total biomass of the test species among the test sediments. In the beginning of the experiment, the total dry biomass of the worms was 15.69 mg (SD 0.17) in AS and HS and 19.04 mg (SD 1.78) in KS. After the 28-day test period, the total dry biomass of worms increased in the control groups by, on average, 49 % (23.31 mg SD 1.89) in AS but decreased by, on average, 7 % (14.56 mg SD 1.03) in HS and 16 % (16.01 mg SD 1.60) in KS.

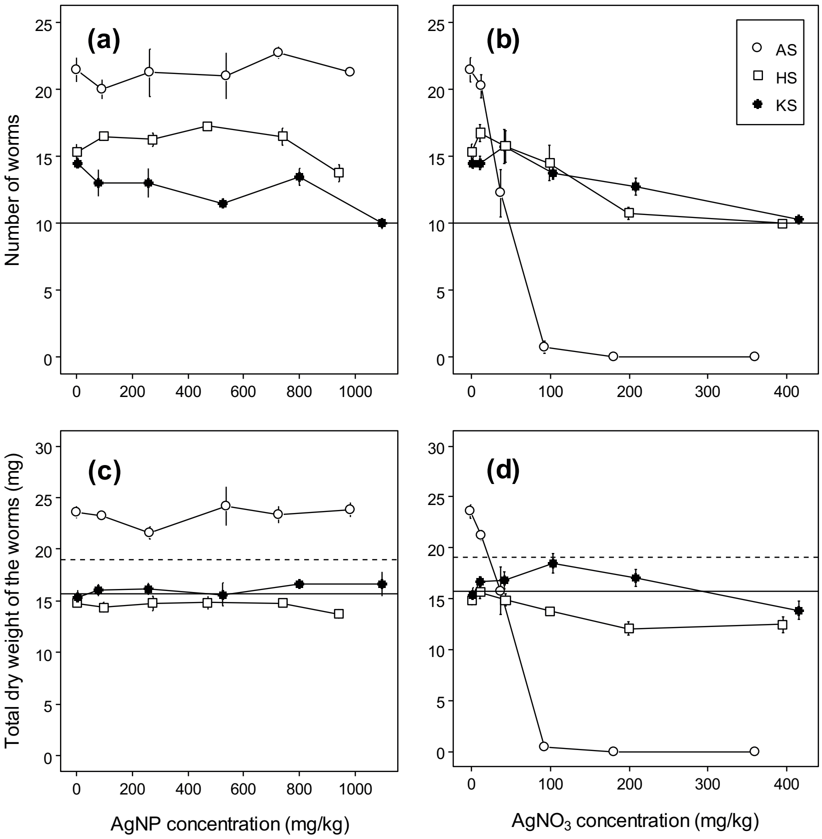


Fig. 1 Toxicity of Ag spiked as silver nanoparticles (AgNP) and silver nitrate ($AgNO_3$) to *Lumbriculus variegatus* after a 28-day exposure in spiked artificial (AS), Höytiäinen (HS) and Kuorinka (KS) sediments at various Ag concentrations (mg/kg dry weight). Number of worms after a 28-day exposure to AgNP (**a**) and $AgNO_3$ (**b**). Dry biomass of worms after exposure to AgNP (**c**) and $AgNO_3$ (**d**). *Each symbol* indicates mean and SD of four replicate samples, except for control exposure that had six replicates. *Solid line* in (**a**) and (**b**) indicates the number of worms (10) at the beginning of the experiment. *Solid line* in (**c**) and (**d**) indicates the starting dry biomass of the worms in the AS and HS sediments (15.69 mg), *dashed line* in the KS sediment (19.04 mg)

Table 3 Calculated IC_{50} and EC_{50} values for a decrease in the number of worms compared with control for $AgNO_3$- and AgNP-exposed *L. variegatus*

	End point	Compound	Sediment		
			AS	HS	KS
Reproduction[a]	IC_{50}	$AgNO_3$	23.9 (3.6)	129 (20)	214 (48)
	IC_{50}	AgNP	–	–	443 (317)
No. of worms[a]	EC_{50}	$AgNO_3$	38.0 (2.0)	525 (107)	688 (187)

[a] Based on three-parameter log-logistic model, Ag (determined concentration [kg/dry sediment])

Feeding Rate

AgNP exposure had no effect on the fecal pellet production of the worms in HS and AS (Fig. 2a). In the KS sediment, however, fecal-pellet production increased with increasing exposure concentration of AgNP with one exception: At the highest exposure concentration (1098 mg/kg), the worms avoided the sediment, and thus the pellet production decreased (Fig. 2a).

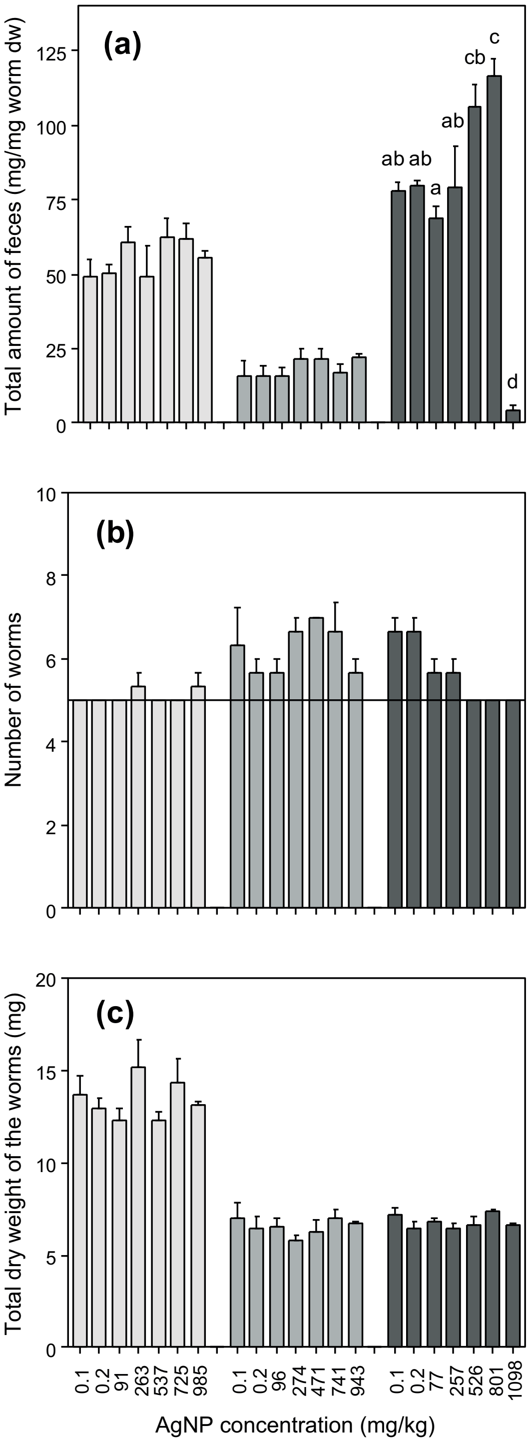
In the natural sediments HS and KS, the worms reproduced during the 14-day exposure period (Fig. 2b). In the HS sediment, the worms reproduced in each concentration somewhat evenly, but in the KS sediment reproduction was observed only in the controls and in the two lowest-exposure concentrations (Fig. 2b). In the AS sediment, only a few extra worms were occasionally found in test vessels. No significant differences were found in total dry biomass of the worms between the different Ag concentrations. The biomass gain was different among the sediments as was also observed in the toxicity test. The biomass of the worms increased during the 14-day exposure period in AS (71 %), stayed constant in KS, and decreased in HS (17 %) (Fig. 2c).

Discussion

Silver nitrate and AgNP are known to be extremely toxic to benthic organisms (Khan et al. 2015; Nair et al. 2013). However, the majority of toxicity studies have been performed using waterborne exposures which does not consider the natural environment of benthic organisms. In waterborne exposures, the uptake of Ag occurs primarily over the respiratory-body surface. Sediment exposures are more environmentally realistic because organisms feed on the sediment, and g is also internalized into the organisms through the gut epithelium. Dietary uptake is especially important when Ag is spiked as AgNP because particles can be internalized directly by way of endocytosis (García-Alonso et al. 2011). Endocytic uptake can lead to NP-specific modes of toxicity, which cannot be considered in water-only exposures.

In this study, the toxicity of Ag spiked as AgNP and $AgNO_3$ to *L. variegatus* in sediment exposures was remarkably lower compared with waterborne exposures in the literature. Khan et al. (2015) reported LC_{50} concentrations in a 96-hour acute toxicity test to be 64.6 µg/L for polyvinylpyrrolidone-coated AgNP and 4.4 µg/L for $AgNO_3$ in OECD 225 standard AFW. In the present study, no mortality was observed in any of the tested sediments even at the highest 1098 mg/kg (dw) concentration of sediment-associated AgNP. The EC_{50} value for $AgNO_3$ was 38 mg/kg (dw) in AS sediment, but no mortality was observed in other test sediments. The decrease of Ag toxicity in sediments compared with the waterborne exposures was dramatic, especially when spiked as AgNP, despite the possible direct uptake of AgNP by endocytosis. The capacity of sediment to decrease Ag toxicity emphasizes the need for sediment-toxicity tests when evaluating the environmental effects of AgNP. Our results indicate that the toxicity to benthic fauna may be highly overestimated if only waterborne exposures are used.

Dissolved Ag spiked as $AgNO_3$ was more toxic to *L. variegatus* in artificial sediment than in the natural sediments. This suggests that Ag^+-binding capacity is greater in the natural sediments compared with the AS sediment. The greater OC content of the HS and KS sediments compared with the AS sediment partly explains the lower toxicity of $AgNO_3$ because Ag is known to form complexes with OC (Erickson et al. 1998). In addition, the grain size of the natural sediments is small; 79.0 % (HS) and 77.9 % (KS) of the particles are under 63-µm diameter (Mäenpää et al. 2003). The high dw % in the AS sediment indicates that the sediment was mainly reconstructed from coarse quartz sand resulting in a smaller surface area in the AS sediment components to bind Ag. The concentration of acid-volatile sulfides (AVS) in sediment is often considered to be the most important individual factor in anoxic sediments because Ag has a strong affinity toward organic and inorganic sulfur groups (Bell and Kramer 1999; Berry et al. 1999). In this study, the tested sediments were treated under oxidized conditions where the concentration of AVS can be considered negligible (Di Toro et al. 1990). Ag also has high affinity toward Cl^- anions (Wingert-Runge and Andren 1993). In our test set-up, the amount of Cl anions in the overlying AFW was theoretically high enough to complex all Ag^+; however, because the Ag compounds were spiked directly to the sediment, the effect of Cl and



◀**Fig. 2** Effects of Ag spiked as silver nanoparticles (AgNP) on *Lumbriculus variegatus* in 14-days feeding rate test in artificial (AS), Höytiäinen (HS) and Kuorinka (KS) sediments at various Ag concentrations (mg/kg dry weight). Two control groups without AgNP are marked as 0.1 and 0.2. Mass of dry feces produced during the experiment normalized to the total dry biomass of the worms (**a**). *Identical letters* (**a**–**d**) indicate groups that do not significantly differ from each other ($p < 0.05$). Number of worms in the end of the experiment; the *solid line* indicates the number of worms in the beginning of the experiment (**b**). Total dry biomass of the worms after the experiment (**c**). *Each bar* indicates mean and SD of three replicate samples

other anions is considered small. This is proved by the toxicity of $AgNO_3$ in the test sediments despite the complexing anions in the overlying water.

The toxicity of Ag increases when pH decreases due to the increased free Ag^+ concentration in the media (Erickson et al. 1998). Low pH also increases the dissolution of AgNP, which leads to a greater free Ag^+ concentration and increased toxicity (Navarro et al. 2008; Peretyazhko et al. 2014; van Aerle et al. 2013). The natural sediment KS had the lowest pH of the tested sediments. Although the toxicity of Ag spiked as $AgNO_3$ was the lowest in KS, it was the only sediment in which the addition of AgNP resulted in reproductive toxicity. This indicates that low pH may increase the toxicity of AgNP more than that of $AgNO_3$. The IC_{50} values for reproduction were approximately 2 times greater for AgNP than for $AgNO_3$ in KS sediment. If toxicity is proposed to be solely a function of Ag^+, approximately 50 % of the particles would be dissolved. The partitioning studies performed in soil, however, show that the bioavailable concentration of Ag^+ in soil is greater when added as AgNP than when added as $AgNO_3$ (Coutris et al. 2012). Therefore, direct comparisons between the toxicity data and the dissolution of AgNP cannot be made. AgNP can also pose NP-specific toxicity over Ag^+ (Chan and Chiu 2015; Cozzari et al. 2015; García-Alonso et al. 2014) or “Trojan horse”-type of behavior, thus leading to the intracellular release of Ag^+ (Moore 2006; Park et al. 2010; Wang et al. 2013). If these NP-specific modes of toxicity explained the toxicity of AgNP in the KS sediment, the bioavailability of AgNP should be greater in the KS sediment compared with the other tested sediments because no toxicity was observed in the HS or AS sediments. This is unlikely because the relatively low pH in KS actually suggests lower bioavailability of AgNP compared with the other more alkaline sediments due to a stronger electrostatic attraction between the negatively charged particles and positively charged matrix (Cornelis et al. 2014). Considering these facts, we suggest that the AgNP toxicity in KS was mainly caused by dissolved Ag^+

released from the particles and that dissolution is promoted by the low pH of the sediment.

The nutritional value of sediment to *L. variegatus* varied between the tested sediments. The AS sediment was the only sediment in which worms gained weight. The total biomass of the worms decreased in the HS and KS sediments despite the fact that the worms ingested both natural sediments. This indicates poor nutritional value of the natural sediments compared with the AS sediment. Especially the KS sediment seems to have a poor nutrient content because the biomass-normalized ingestion rate was highest among the test sediments but the biomass loss was the largest. No significant difference in the total biomass was found between the treatments in toxicity or feeding-rate test despite the significant decrease in the ingested amount of sediment in the highest concentration of AgNP in KS. The biomass change does not seem to be an applicable end point for the acute-toxicity tests in the natural sediments with poor nutritional value because the worms also lost weight also in the control groups for the HS and KS sediments.

The feeding behavior of *L. variegatus* has been shown to give an immediate response to exposure, and it is considered to be a more sensitive end point than mortality, biomass gain, or reproduction (Leppänen and Kukkonen 1998). Generally the ingestion rate tends to decrease with increasing concentration of contaminant, but in the KS sediment *L. variegatus* ingested more sediment with increasing AgNP concentration. We suggest that the antibacterial properties of AgNP disturbed the microbial growth in the sediment, which impeded adequate nutrition of *L. variegatus*, and thus the worms had to compensate for the nutrient-poor food by ingesting more sediment. In the highest-exposure concentration, however, the AgNP-induced stress seemed to become too high for the *L. variegatus* because the worms avoided the sediment throughout the test period, and thus the feeding rate was minimal. The increase in the feeding rate was only observed in the KS sediment. Microbes can be a more important food source in KS compared with the other test sediments due to the poor nutritional value. In addition, the low pH of KS is believed to be an intensifying factor for AgNP toxicity as discussed previously.

The natural sediments HS and KS used in this study were selected to represent typical unpolluted Finnish lake sediments from a watershed without industrial influence and have been used in studies as clean reference sediments (Mäenpää et al. 2003; Ristola et al. 1996). The geochemical background level of metals is slightly increased compared with the consensus-based threshold-effect concentrations (TEC), thus meaning that these metals possibly cause toxic effects in a freshwater ecosystem (MacDonald et al. 2000). In HS, the cadmium (Cd), chromium, copper (Cu), nickel (Ni), lead, and zinc (Zn) concentrations are greater than the TEC. In KS, the metal concentrations were also increased, but they were somewhat lower compared with HS, and only Cd, Cu, and Ni are greater than the TEC values. However, the background metal concentrations are typical for sediments in this area (Ristola et al. 1996). When a test sediment is amended with Ag, it is possible that Ag^+ and AgNP displace sediment-bound metals and release them into the sediment porewater. Especially Zn and Ni are known to be displaced by Ag (Call et al. 1999). The measured toxicity in the natural sediments may therefore be a mixture effect of metals with Ag being the predominant active substance. Greater concentration of background metals may therefore explain the greater toxicity of $AgNO_3$ in HS sediment compared with KS sediment. In the AgNP treatments, this effect is not pronounced because the dissolution of NPs is believed to be promoted more by the lower pH of KS compared with HS, thus leading to the greater toxicity in KS. The environmental relevance is often a key factor when considering the behavior of NPs in the aquatic environment. Because the properties of the natural sediments differ greatly from the artificially prepared standard sediment, we consider testing in the natural sediments to be highly important despite the fact that the environmental factors, apart from the NP exposure, may complicate interpretation of the results.

The OECD standard test guideline 225 was successfully applied for use with nanomaterials. The AS sediment prepared according to the OECD standard guideline was the only sediment that fulfilled the validity criteria of an 1.8-fold increase in the number of individuals, and thus only this part of the study can be considered as a standardized toxicity test. The low reproduction rate and pH-related problems in natural sediments advocate the use of AS in standard testing. The results of the OECD toxicity tests are in line with the feeding-rate test, which increases the reliability of the test. However, the following concerns may have significant effect on the results of the test and should be properly addressed in the future:

1. The spiking method of the nanomaterial may have an influence on the outcome of the test. We chose to add the dry-powder AgNP directly into the sediment because the amount of NPs was high, and the particles were unstable in water suspension at such a high concentration. More stable NP suspensions could also be spiked as suspension to avoid clumping of the material. Decreased clumping leads to a greater total surface area of the spiked component and may possibly lead to increased toxicity. Indirect addition of NPs to the overlying water would be an environmentally more relevant way to spike the nanomaterial, but it could

decrease the oral uptake of the substance because *L. variegatus* burrow into the sediment and feed below the sediment surface.

2. The characterization of nanomaterial should be carefully considered. Because we do not currently have proper methods to characterize the sediment-associated nanomaterial, characterization in this study was performed in deionized water before spiking the NPs into the test media. Despite the fact that the characterization in water does not correspond to the experimental conditions in the sediment, it is essential to assess the primary structure and properties of the particles in standard conditions to add comparability between the studies. The characterization of NPs in the overlying water was not considered relevant because AgNP were spiked to the sediment by direct addition and were never present in the water phase. If indirect addition is used, characterization in the overlying water should also be considered because the aggregation and dissolution of coated AgNP in the water phase is differently affected by the presence of sediment (Bone et al. 2012; Unrine et al. 2012). In conclusion, there is an urgent need to develop reliable and easily achievable methods for the characterization of the nanomaterials in sediment media.

Conclusions

The acute toxicity of Ag spiked as AgNP to *L. variegatus* was greatly decreased in sediments compared with literature-reported waterborne toxicity. Silver nitrate was significantly more toxic than AgNP in all of the test sediments, but sediment properties had a different effect on the toxicity of the two compounds. The toxicity of $AgNO_3$ was lower in the sediments with fine grain size and a relatively high amount of OC. The low pH of the sediment seemed to overcome these factors when Ag was spiked as AgNP, and toxicity in terms of reproductive failure, changes in feeding behavior, and sediment avoidance was only observed in the natural KS sediment with the lowest pH value of the tested sediments. We suggest that the low pH of the KS sediment enhances the release of Ag^+ from AgNP and thus promotes the toxicity. However, NP-specific toxicity, or the synergistic effect of both Ag^+ and AgNP and natural heavy metals, cannot be excluded. Finally, we conclude that OECD guideline 225 can be used for evaluating the toxicity of nanomaterials in sediments. Further studies considering AgNP in sediments should concentrate on the dissolution kinetics and the effects of sediment pH on the AgNP toxicity. In general with NPs, the development of characterization methods in complex environmental media is the most essential issue. However, the lack of characterization methods should not hinder the toxicity testing of NPs in complex environmental media because NPs are constantly released into the environment where they are likely to pose a risk to the benthic ecosystems.

Acknowledgments The work was funded by the Academy of Finland (Projects Nos. 214545 and 21800). Kimmo Mäenpää and Eeva-Riikka Vehniäinen acknowledge the Academy of Finland for funding (Project No. 258120 to K. M. and 285296 to E-RV). The responsible author also acknowledges the support of the Finnish Doctoral Programme in Environmental Science Technology and the University of Jyväskylä Graduate School for Doctoral Studies.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Bell RA, Kramer JR (1999) Structural chemistry and geochemistry of silver-sulfur compounds: critical review. Environ Toxicol Chem 18:9–22

Berry WJ, Cantwell MG, Edwards PA, Serbst JR, Hansen DJ (1999) Predicting toxicity of sediments spiked with silver. Environ Toxicol Chem 18:40–48

Bone AJ, Colman BP, Gondikas AP, Newton KM, Harrold KH, Cory RM et al (2012) Biotic and abiotic interactions in aquatic microcosms determine fate and toxicity of Ag nanoparticles: part 2—toxicity and Ag speciation. Environ Sci Technol 46:6925–6933

Call DJ, Polkinghorne CN, Markee TP, Brooke LT, Geiger DL, Gorsuch JW et al (1999) Silver toxicity to *Chironomus tentans* in two freshwater sediments. Environ Toxicol Chem 18:30–39

Chan CYS, Chiu JMY (2015) Chronic effects of coated silver nanoparticles on marine invertebrate larvae: a proof of concept study. PLoS One 10:e0132457

Coleman JG, Kennedy AJ, Bednar AJ, Ranville JF, Laird JG, Harmon AR et al (2013) Comparing the effects of nanosilver size and coating variations on bioavailability, internalization, and elimination, using *Lumbriculus variegatus*. Environ Toxicol Chem 32:2069–2077

Cornelis G, Hund-Rinke K, Kuhlbusch T, Van den Brink N, Nickel C (2014) Fate and bioavailability of engineered nanoparticles in soils: a review. Crit Rev Environ Sci Technol 44:2720–2764

Coutris C, Joner EJ, Oughton DH (2012) Aging and soil organic matter content affect the fate of silver nanoparticles in soil. Sci Total Environ 420:327–333

Cozzari M, Elia AC, Pacini N, Smith BD, Boyle D, Rainbow PS et al (2015) Bioaccumulation and oxidative stress responses measured in the estuarine ragworm (*Nereis diversicolor*) exposed to dissolved, nano-, and bulk-sized silver. Environ Pollut 198:32–40

Di Toro DM, Mahony JD, Hansen DJ, Scott KJ, Hicks MB, Mayr SM et al (1990) Toxicity of cadmium in sediments: the role of acid volatile sulfide. Environ Toxicol Chem 9:1487–1502

Erickson RJ, Brooke LT, Kahl MD, Venter FV, Harting SL, Markee TP et al (1998) Effects of laboratory test conditions on the toxicity of silver to aquatic organisms. Environ Toxicol Chem 17:572–578

García-Alonso J, Khan FR, Misra SK, Turmaine M, Smith BD, Rainbow PS et al (2011) Cellular internalization of silver nanoparticles in gut epithelia of the estuarine polychaete *Nereis diversicolor*. Environ Sci Technol 45:4630–4636

García-Alonso J, Rodriguez-Sanchez N, Misra SK, Valsami-Jones E, Croteau M, Luoma SN et al (2014) Toxicity and accumulation of silver nanoparticles during development of the marine polychaete *Platynereis dumerilii*. Sci Total Environ 476:688–695

Gottschalk F, Sonderer T, Scholz RW, Nowack B (2009) Modeled environmental concentrations of engineered nanomaterials (TiO_2, ZnO, Ag, CNT, fullerenes) for different regions. Environ Sci Technol 43:9216–9222

Heckmann L, Hovgaard MB, Sutherland DS, Autrup H, Besenbacher F, Scott-Fordsmand JJ (2011) Limit-test toxicity screening of selected inorganic nanoparticles to the earthworm *Eisenia fetida*. Ecotoxicology 20:226–233

Khan FR, Paul KB, Dybowska AD, Valsami-Jones E, Lead JR, Stone V et al (2015) Accumulation dynamics and acute toxicity of silver nanoparticles to *Daphnia magna* and *Lumbriculus variegatus:* implications for metal modelling approaches. Environ Sci Technol 49:4389–4397

Leppänen MT, Kukkonen JVK (1998) Relationship between reproduction, sediment type, and feeding activity of *Lumbriculus variegatus* (Müller): implications for sediment toxicity testing. Environ Toxicol Chem 17:2196–2202

Levard C, Hotze EM, Colman BP, Dale AL, Truong L, Yang X et al (2013) Sulfidation of silver nanoparticles: natural antidote to their toxicity. Environ Sci Technol 47:13440–13448

Loza K, Diendorf J, Sengstock C, Ruiz-Gonzales L, Gonzalez-Calbet JM, Vallet-Regi M et al (2014) The dissolution and biological effects of silver nanoparticles in biological media. J Mater Chem B 2:1634–1643

MacDonald DD, Ingersoll CG, Berger TA (2000) Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. Arch Environ Contam Toxicol 39:20–31

Mäenpää KA, Sormunen AJ, Kukkonen JVK (2003) Bioaccumulation and toxicity of sediment associated herbicides (ioxynil, pendimethalin, and bentazone) in *Lumbriculus variegatus* (Oligochaeta) *Chironomus riparius* (Insecta). Ecotoxicol Environ Saf 56:398–410

Mäenpää K, Sorsa K, Lyytikäinen M, Leppänen M, Kukkonen JVK (2008) Bioaccumulation, sublethal toxicity, and biotransformation of sediment-associated pentachlorophenol in *Lumbriculus variegatus* (Oligochaeta). Ecotoxicol Environ Saf 69:121–129

Moore M (2006) Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? Environ Int 32:967–976

Nair PMG, Park SY, Choi J (2013) Evaluation of the effect of silver nanoparticles and silver ions using stress responsive gene expression in *Chironomus riparius*. Chemosphere 92:592–599

Navarro E, Piccapietra F, Wagner B, Marconi F, Kaegi R, Odzak N et al (2008) Toxicity of silver nanoparticles to *Chlamydomonas reinhardtii*. Environ Sci Technol 42:8959–8964

Organization for Economic Cooperation and Development (2007) Test no. 225: sediment-water Lumbriculus toxicity test using spiked sediment. OECD, Paris, France

Park E, Yi J, Kim Y, Choi K, Park K (2010) Silver nanoparticles induce cytotoxicity by a Trojan-horse type mechanism. Toxicol In Vitro 24:872–878

Peretyazhko TS, Zhang Q, Colvin VL (2014) Size-controlled dissolution of silver nanoparticles at neutral and acidic pH conditions: kinetics and size changes. Environ Sci Technol 48:11954–11961

Petersen EJ, Diamond SA, Kennedy AJ, Goss GG, Ho K, Lead J et al (2015) Adapting OECD aquatic toxicity tests for use with manufactured nanomaterials: key issues and consensus recommendations. Environ Sci Technol 49:9532–9547

Ristola T, Pellinen J, Van Hoof PL, Leppänen M, Kukkonen J (1996) Characterization of Lake Ladoga sediments. II: toxic chemicals. Chemosphere 32:1179–1192

Roh J, Sim SJ, Yi J, Park K, Chung KH, Ryu D et al (2009) Ecotoxicity of silver nanoparticles on the soil nematode *Caenorhabditis elegans* using functional ecotoxicogenomics. Environ Sci Technol 43:3933–3940

Siegel S, Castellan J (1988) Non parametric statistics for the behavioural sciences, 2nd edn. McGraw-Hill International, New York

Unrine JM, Colman BP, Bone AJ, Gondikas AP, Matson CW (2012) Biotic and abiotic interactions in aquatic microcosms determine fate and toxicity of Ag nanoparticles. Part 1: aggregation and dissolution. Environ Sci Technol 46:6915–6924

van Aerle R, Lange A, Moorhouse A, Paszkiewicz K, Ball K, Johnston BD et al (2013) Molecular mechanisms of toxicity of silver nanoparticles in zebrafish embryos. Environ Sci Technol 47:8005–8014

Vance ME, Kuiken T, Vejerano EP, McGinnis SP, Hochella MF Jr, Rejeski D et al (2015) Nanotechnology in the real world: redeveloping the nanomaterial consumer products inventory 6:1769–1780

Wang Z, Liu S, Ma J, Qu G, Wang X, Yu S et al (2013) Silver nanoparticles induced RNA polymerase-silver binding and RNA transcription inhibition in erythroid progenitor cells 7:4171–4186

Wingert-Runge B, Andren AW (1993) Adsorptive behavior of silver to synthetic and natural sediments in aqueous systems. The 1st international conference proceedings: transport, fate and effects of silver in the environment, session B, pp 19–22

II

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

by

Juho Elias Rajala, Eeva-Riikka Vehniäinen, Ari Väisänen & Jussi Vilho Kalevi Kukkonen 2017

Environmental Toxicology and Chemistry. In press.

Environmental Toxicology and Chemistry, Vol. 9999, No. 9999, pp. 1–9, 2017

Environmental Toxicology

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

JUHO E. RAJALA,[a],* EEVA-RIIKKA VEHNIÄINEN,[a] ARI VÄISÄNEN,[b] and JUSSI V.K. KUKKONEN[a]
[a]Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland
[b]Department of Chemistry, University of Jyväskylä, Jyväskylä, Finland

(*Submitted 19 January 2017; Returned for Revision 26 February 2017; Accepted 14 March 2017*)

Abstract: Sediments are believed to be a major sink for silver nanoparticles (AgNPs) in the aquatic environment, but there is a lack of knowledge about the environmental effects and behavior of AgNPs in sediments. The release of highly toxic Ag^+ through dissolution of AgNPs is one mechanism leading to toxic effects in sediments. We applied an ultrasound-assisted sequential extraction method to evaluate the dissolution of AgNPs and to study the partitioning of dissolved Ag in sediments. Silver was spiked into artificial and 2 natural sediments (Lake Höytiäinen sediment and Lake Kuorinka sediment) as silver nitrate ($AgNO_3$), uncoated AgNPs, or polyvinylpyrrolidone-coated AgNPs (PVP-AgNPs). In addition, the total body burdens of Ag in the sediment-dwelling oligochaete *Lumbriculus variegatus* were assessed over a 28-d exposure period. The dissolution rate was found to be similar between the uncoated AgNP and PVP-AgNP groups. 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 Lake Kuorinka sediment, dissolved Ag that originated from PVP-AgNPs was relatively more bioaccessible, also resulting in higher total body burden in *L. variegatus* than that from uncoated AgNPs or $AgNO_3$. In artificial sediment and Lake Höytiäinen sediment, $AgNO_3$ was significantly more bioaccessible than AgNPs. Our results highlight the importance of sediment properties and AgNP surface chemistry when evaluating the environmental exposure of AgNPs. *Environ Toxicol Chem* 2017;9999:1–9. © 2017 SETAC

Keywords: Sediment chemistry Nanoparticles Sequential extraction Nanoecotoxicology Silver

INTRODUCTION

Silver nanoparticles (AgNPs) 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]. Thus, AgNPs have been released into surface waters, mainly through untreated wastewaters and effluents from wastewater treatment plants [2].

Residence time of AgNPs in the surface waters is typically short as a result of 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 such aggregation, AgNPs 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 AgNPs [3]. Under environmental conditions where electrolytes and suspended solids are present, aggregation and sedimentation of AgNPs are likely to occur despite the coating agents [4,5]. The effects and behavior of AgNPs 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 AgNPs to dissolve makes quantification challenging, because it is difficult to separate dissolved Ag ions (Ag^+) from AgNPs in complex media. Dissolution rate depends on such particle properties 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 AgNPs can be explained solely by dissolved Ag^+ [12]. Recent studies indicate that AgNPs are also toxic in the nanoparticle form and that the toxicity of AgNPs can be higher than that of 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 AgNPs in soil [17,18]. The partitioning of Ag spiked as uncoated AgNPs was found to be strikingly different compared with Ag spiked as silver nitrate ($AgNO_3$) or citrate-coated AgNPs. Both AgNPs were quickly immobilized, but the bioaccessible fraction of Ag spiked as uncoated AgNPs increased slowly over time, resulting in values many times higher than those in soil spiked with $AgNO_3$. The differences among Ag compounds were more pronounced in the soil containing more organic carbon, and both the surface chemistry of the AgNPs and the matrix properties were important to the partitioning of Ag [18]. Similar research has not been conducted in sediment previous to the present study. Because sediments are a major sink for AgNPs, it is crucial to study the

---

This article includes online-only Supplemental Data.

* Address correspondence to juho.e.rajala@jyu.fi

DOI: 10.1002/etc.3798

fractionation of AgNP-derived Ag to better evaluate possible environmental effects.

The aim of the present study was to determine the partitioning of dissolved Ag in different sediments spiked with 2 types of AgNPs, and to compare the results with $AgNO_3$-spiked sediments. Our hypotheses, based on the soil study conducted by Coutris et al. [18], were that aging of AgNPs 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-d period in Ag-spiked artificial sediment and 2 natural sediments collected from lakes with different sediment characteristics. The effect of PVP as a capping agent of AgNPs was assessed by spiking the sediment with uncoated AgNPs and PVP-AgNPs of roughly similar size. As a reference material for the partitioning of dissolved Ag, $AgNO_3$ was used. In addition, the dissolution of AgNPs was estimated using the sequential extraction data and the bioaccessible fraction determined by the sequential extraction was compared with the total body burden of Ag in the benthic oligochaeta *Lumbriculus variegatus*.

MATERIALS AND METHODS

Silver nanoparticles

The PVP-AgNPs (purity, 99.9%; diameter, 30–50 nm; PVP content = 0.2 wt %) and uncoated AgNPs (purity, 99.5%; nominal size, 35 nm) were obtained as dry powder from Nanostructured and Amorphous Materials (Nanoamor). Analytical grade silver nitrate ($AgNO_3$, supplied by J.T. Baker) was used as an Ag reference. The size distribution of AgNPs was estimated by measuring the diameter of 344 uncoated AgNPs and 205 PVP-AgNPs from 3 grids using the 25 000× 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 Supplemental Data.

Sediments

Natural sediments collected from Lake Höytiäinen (North Karelia, Finland) and Lake Kuorinka (North Karelia, Finland) and artificial sediment prepared in the laboratory were used in the experiments. Both Lake Höytiäinen sediment and Lake Kuorinka sediment have been used as clean reference sediments in toxicity tests and contain minimal amounts of organic contaminants [20]. The artificial sediment was prepared following Organisation for Economic Co-operation and Development (OECD) guideline 225 [21] and consisted of 75% combusted quartz sand (grain size, 100–200 μm), 5% finely ground (<500 μm) sphagnum peat (Kekkilä), 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 artificial sediment was adjusted to 7.0 with calcium carbonate. Sediments were characterized for dry weight percentage; loss of ignition; organic carbon content; 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. The details of the characterization and reagents used are given in the Supplemental Data.

Test organisms

Lumbriculus variegatus oligochaetes were cultured at the University of Jyväskylä in large aquaria filled with artificial freshwater (hardness, 1.0 mM L^{-1} as Ca + Mg) at a constant temperature of 20 °C and a 16:8-h light:dark regime. Cellulose paper tissues were used as a substrate, and worms were fed with ground tetra fish food once a week (Tetrawerke). The *L. variegatus* were synchronized by cutting them in half 14 d before the experiment, and only the posterior parts 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}) was mixed into 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 AgNPs was 300 mg kg^{-1} (dry wt), and the concentration of $AgNO_3$ was 20 mg kg^{-1} (dry wt). The concentration of AgNPs used was higher than estimated to be found in the environment [22]. The concentration was selected according to previous studies done in soil, where dissolution of AgNPs and association of dissolved Ag with certain soil fractions was low [17,18]. To ensure analytical precision, a higher concentration of AgNPs than $AgNO_3$ was used in the present study. Despite the elevated AgNP concentration, the processes explored in the present study 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 Lake Höytiäinen sediment, 6.8 g of Lake Kuorinka sediment, and 15 g of artificial sediment as dry wt) was transferred to the bottom of each test unit, and the beakers were filled with artificial freshwater (hardness, 1.0 mM L^{-1}). Test units were left 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 dry weight of test organisms to the amount of NOM in the sediment [21]. Temperature in the test room was kept constant at 20 °C, and a 16:8-h light:dark regime was 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 burden of Ag in the test organisms were measured after 2, 5, 9, 14, and 28 d of exposure. After the exposure period, the worms were sieved out from the sediment and transferred into clean artificial freshwater for 6 h to empty their gut, dried at 105 °C overnight, weighed, and stored at –20 °C for further metal extractions. The total body burden of Ag in worm tissue was normalized for the total dry biomass (μg g^{-1} of Ag in dry tissue). When the total body burdens 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 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 analysis of Ag in the overlying water (discussed in the Supplemental Data). All metal concentrations were analyzed by ICP–OES. Details of the sample treatment and analysis can be found in the Supplemental Data.

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 porewater, 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 porewater 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 Supplemental Data.

Dissolution of AgNPs

Total extracted concentration of Ag in each sampling point ($exC_{\mathrm{Agx(t)}}$) was calculated

$$exC_{\mathrm{Agx(t)}} = F1_{\mathrm{Agx}} + F2_{\mathrm{Agx}} + F3_{\mathrm{Agx}} + F4_{\mathrm{Agx}} + F5_{\mathrm{Agx}} \quad (1)$$

where $F1_{\mathrm{Agx}}$, $F2_{\mathrm{Agx}}$, $F3_{\mathrm{Agx}}$, $F4_{\mathrm{Agx}}$, and $F5_{\mathrm{Agx}}$ represent the concentration of Ag (mg kg^{-1} dry wt) in the extracted fractions of Ag-spiked test sediment. The $exC_{\mathrm{Agx(t)}}$ was used to estimate the dissolution rate, $d\%_{\mathrm{AgNP(t)}}$, of AgNPs in each test sediment at every exposure time

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

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

Data treatment and statistical tests

The Ag measurements that fell under the method level of detection of ICP–OES (1.9 $\mu g\,L^{-1}$) were considered as zero. The nonparametric pairwise Wilcoxon rank sum test was used for comparisons of the dissolution rates of the AgNPs among the sediments, and the exact Wilcoxon rank sum test was used for comparison of the average bioaccessible Ag (at 9,14, and 28 d) 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 analysis of variance with Tukey's honest significant difference 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 in terms of robustness of the test, this does not preclude the use of parametric tests [25]. Analyses were performed using R Ver 3.0.1 (R Foundation for Statistical Computing).

RESULTS AND DISCUSSION

Characterization of AgNPs

The diameter of uncoated AgNPs measured from the pictures taken by transmission electron microscope varied from 11.9 to 48.8 nm, resulting in a mean (±standard deviation [SD]) diameter of 39.4 nm (± 12.6 nm), and from 7.5 to 74.8 nm for PVP-AgNPs, resulting in a mean (±SD) diameter of 28.6 nm (± 5.8 nm; Supplemental Data, Figure S1). Both AgNPs formed large agglomerates when suspended in water (Supplemental Data, Figure S1). Further characterization of AgNPs in water suspension was not considered relevant, because AgNPs 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-AgNPs and 0.30% (± 0.01) for uncoated AgNPs.

Sediment characterization

In the Lake Höytiäinen sediment, the pH stayed relatively constant (5.8–6.2) throughout the experiment, decreased in the Lake Kuorinka sediment from 6.2 to 4.7, and increased from 7.0 to 8.3 in artificial sediment during the 28-d 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 Lake Kuorinka 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-d exposure period [26]. The concentration of Fe_{ox}, Mn_{ox}, Cd, Cr, Cu, Ni, Pb, Zn, and organic carbon content was highest in the Lake Höytiäinen sediment, followed by Lake Kuorinka sediment, and artificial sediment (Table 1). The variation of the total Ag concentrations in the spiked sediments was highest

Table 1. Characteristics of the test sediments

	Höytiäinen	Kuorinka	Artificial
Dry weight (%)[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 ($\mu mol\,g^{-1}$)[b]	ND	2.83 (0.68)	ND
SEM ($\mu mol\,g^{-1}$)[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]	19 700 (730)	9840 (340)	153 (2)
Mn_{ox}[c]	20 400 (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)
Uncoated AgNPs[d]	178 (112)	171 (137)	315 (122)
PVP-AgNPs[d]	196 (45)	262 (33)	278 (42)

[a] At the end of exposure, $n = 3$ (± standard deviation).

[b] Before the exposure, $n = 2$ (± standard deviation).

[c] At the end of exposure, $n = 3$, mg kg^{-1} dry weight (± standard deviation).

[d] At the end of the exposure, $n = 6$, mg kg^{-1} dry weight (± standard deviation).

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; PVP-AgNPs = polyvinylpyrrolidone-coated silver nanoparticles; ND = not detected.

with the uncoated AgNPs, indicating possible heterogeneous distribution of uncoated AgNPs in the sediments (Table 1).

Dissolution rate of AgNPs

Aquatic toxicity of AgNPs is typically found to be several times lower than toxicity of Ag^+ [27]. Toxic effects often derive from Ag^+ released from the AgNPs by dissolution. It is difficult to measure the dissolution rate of AgNPs in sediment, because 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 AgNPs to be similar. A sequential extraction method accompanied by 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 AgNPs is minimal and Ag is mostly present as dissolved compounds (see discussion in the Supplemental Data). Using Equations 1 and 2, the dissolution rate was estimated individually at each sampling point (Table 2). The association of Ag released from AgNPs may, however, be slower than Ag spiked as $AgNO_3$, because AgNPs need to first dissolve and $AgNO_3$ was spiked already dissolved. Thus, we considered the average dissolution among the sampling points to be the best estimate of the dissolution rate of AgNPs in the test sediments. In Lake Höytiäinen sediment, the extractable concentration of Ag spiked as $AgNO_3$ at 14 d was unexpectedly low (Figure 1), leading to relatively high estimates of dissolution (Table 2). This estimate was excluded from the average, because the estimates of dissolution rates among the other sampling points were close to each other, and a similar decrease was not observed in the AgNP-spiked sediments at 14 d. In Lake Kuorinka sediment and artificial sediment, the dissolution rate is given as an average of all sampling points (Table 2).

The PVP coating did not have an effect on the average dissolution of AgNPs, because no significant difference was observed in the dissolution rates between the uncoated AgNPs and PVP-AgNPs in the Lake Höytiäinen sediment, Lake Kuorinka sediment, or artificial sediment ($p = 1.000$; Table 2). Surface chemistry is reported to be one of the major factors influencing the dissolution rate of AgNPs in addition to size and morphology of the particles [7,28]. Sterically stabilized particles such as PVP-AgNPs have typically reduced dissolution rates compared with uncoated or electrostatically stabilized particles because of the physical protection provided by the coating [29]. However, the PVP coating does not totally eliminate the dissolution of AgNPs [30]. The similar dissolution rate between PVP-AgNPs and uncoated AgNPs in the present study can be explained by the interaction of AgNPs with NOM in the sediments. The presence of NOM is known to increase the stability of AgNPs by coating the particles both sterically and electrostatically and thus protecting initially uncoated particles [31–33]. Also PVP-AgNPs are influenced and coated by the NOM by displacing the PVP coating or associating with the coating, which adds an additional electrostatic barrier [34]. Natural organic matter that is rich in sulfur and nitrogen complex has a great affinity to Ag and a high potential to decrease the dissolution rate of AgNPs 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 uncoated AgNPs and PVP-AgNPs.

The quality of NOM was not assessed in the present study but may be one factor explaining the differences in dissolution rates among the sediments, because the dissolution rate of AgNPs was significantly higher for PVP-AgNPs in Lake Höytiäinen sediment than in Lake Kuorinka sediment or artificial sediment ($p < 0.001$), but was similar between Lake Kuorinka sediment and artificial sediment (Table 2). A significant difference was also found for uncoated AgNPs between Lake Höytiäinen sediment and artificial sediment ($p < 0.001$) but not between Lake Kuorinka sediment and artificial sediment ($p = 0.747$). The high organic carbon content was not observed to be a significant dissolution reducing factor, because dissolution increased with sediment organic carbon content (Lake Höytiäinen sediment > Lake Kuorinka sediment > artificial sediment). Low pH has also been found to promote the dissolution of AgNPs in water, but in soils, dissolution was independent of pH, possibly because of the increased coagulation of AgNPs [8,10]. However, dissolution in the soil suspension was reported to be fast, and it was concluded that unmodified AgNPs were not 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, AgNPs seemed to be persistent in sediment, probably because of the fast interaction with NOM in the sediments protected AgNPs from dissolution.

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 AgNPs. This was performed to add comparability between $AgNO_3$ and AgNP treatments. The association of AgNPs with different sediment fractions was not assessed in the

Table 2. Estimated dissolution of silver nanoparticles in the test sediments

	Höytiäinen		Kuorinka		Artificial	
	Uncoated AgNPs (%)	PVP-AgNPs (%)	Uncoated AgNPs (%)	PVP-AgNPs (%)	Uncoated AgNPs (%)	PVP-AgNPs (%)
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 ± standard deviation.

[b] Excluded from the average.

AgNPs = silver nanoparticles; PVP-AgNPs = polyvinylpyrrolidone-coated silver nanoparticles.

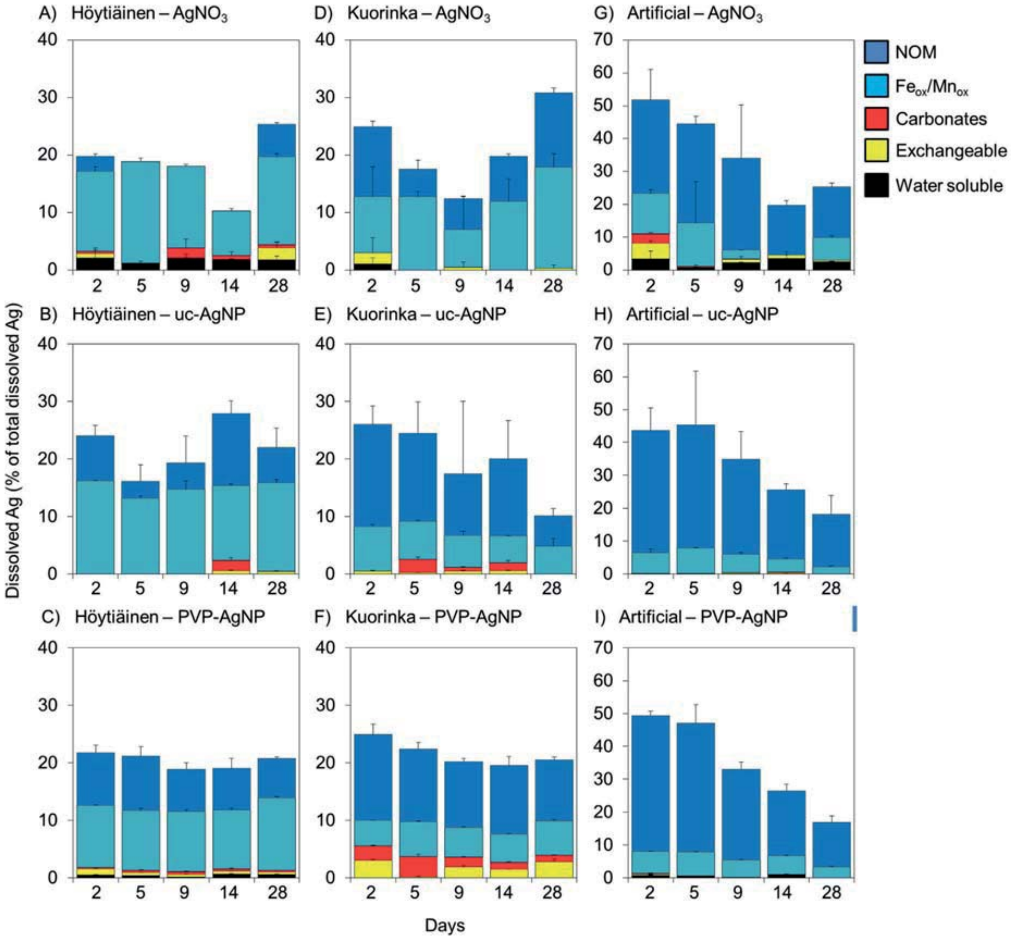


Figure 1. Relative distributions of dissolved Ag (normalized to estimated total dissolved concentration of Ag in sediments) associated with the different sediment fractions, water-soluble, exchangeable fraction, carbonates, Fe and Mn oxides, and natural organic matter. The sum of water-soluble and exchangeable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

present study, because they were removed from the samples by the centrifugation.

The residual fraction. The Ag in the residual fraction is considered to be highly stable and is 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). The results compare well with the soil studies, where the majority of Ag is also reported to lie in the residual fraction [38,39]. In artificial sediment, the concentration of residual Ag was seen to increase during the experiment with all Ag compounds, indicating slower association of Ag in the artificial sediment compared with natural Lake Höytiäinen and Lake Kuorinka sediments (Figure 1). The coarse grain size of artificial sediment can explain this observation. The artificial sediment consisted mainly of 100- to 200-μm quartz sand, whereas the natural sediments of Lake Höytiäinen and Lake Kuorinka are reported to consist mainly of material under 65 μm: 79.0% in Lake Höytiäinen sediment and 77.9% in Lake Kuorinka sediment [40].

NOM and sulfides and the Fe_{ox}/Mn_{ox} fractions. Affinity of Ag toward NOM, sulfides, Fe_{ox}, and Mn_{ox} is high [41]. The role of sulfides in the present study can be considered negligible, because the test sediments were treated under oxidized conditions in which 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 uncoated AgNPs, and 10–13% of PVP-AgNPs) than NOM (0.0–5.6% of $AgNO_3$, 2.9–13% of uncoated AgNPs, and 7.3–9.5% of PVP-AgNPs) with all Ag compounds in Lake Höytiäinen sediment (Figure 1A–C). The high concentration of Fe_{ox} and especially Mn_{ox} in Lake Höytiäinen sediment may explain the relatively higher association (Table 1). The NOM fraction seemed to be more important in binding Ag spiked as uncoated AgNPs (5.3–18%)

and PVP-AgNPs (10–15%) also in Lake Kuorinka sediment, because it was dominant over the Fe_{ox}/Mn_{ox} fraction (4.6–7.7% with uncoated AgNPs and 4.4–6.0% with PVP-AgNPs; Figure 1D–F). 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 Lake Kuorinka sediment (Figure 1D–F).

As discussed in *Dissolution rate of AgNPs*, NOM is likely to coat initially uncoated AgNPs and partially replace the PVP coating on the particles [31–34]. Interactions between AgNPs and NOM can increase the total surface area of NOM, because the molecules become well arranged on the surface of AgNPs, opening more specific binding sites for Ag. The coating process is reported to be fast and is therefore expected to take place simultaneously with dissolution of AgNPs [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 the surface of AgNPs also susceptible. This can lead to overestimation of the NOM-bound dissolved Ag in the samples.

In artificial sediment, NOM-associated concentration of Ag decreased during the experiment from 28 to 15% with $AgNO_3$, from 37 to 16% with uncoated AgNPs, and from 41 to 14% with PVP-AgNPs. In addition, 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 $AgNO_3$-spiked, uncoated AgNP-spiked, and PVP-AgNP–spiked sediment, respectively (Figure 1G–I). The coarse grain size of artificial sediment compared with natural sediments can explain the slower association kinetics, as discussed in the section *The residual fraction*.

The carbonate fraction. The association of Ag on carbonates is reported to be low [17,18,38,39]. Our results compare well with previous studies. In Lake Höytiäinen sediment, the recoveries of dissolved Ag were 0.0–1.7%, 0.0–1.8%, and 0.3–0.5% spiked as $AgNO_3$, uncoated AgNPs, and PVP-AgNPs, respectively (Figure 1A–C). In Lake Kuorinka sediment, no Ag spiked as $AgNO_3$ was extracted from the carbonate fraction, but a small proportion was recovered with uncoated AgNPs (0.0–2.3%) and PVP-AgNPs (1.2–3.4%; Figure 1D–F). In artificial sediment, 2.9% of Ag spiked as $AgNO_3$ at 2 d was associated with the carbonate fraction, but only marginal proportions at later sampling points or when spiked as AgNPs (Figure 1G–I). 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, because bioaccessible Ag can potentially be bioavailable to benthic organisms.

In Lake Höytiäinen sediment, 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). Uncoated AgNP was not bioaccessible up to 9 d, but small amounts of bioaccessible Ag were extracted at 14 d (0.6%) and at 28 d (0.4%; Figure 1B). In contrast to uncoated AgNPs, PVP-AgNPs were more bioaccessible through the experiment: 1.5% at the beginning and 0.9% at the end of the experiment (Figure 1C).

In Lake Kuorinka sediment, at the 2-d 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 uncoated AgNPs, 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 the Lake Höytiäinen sediment, the concentration of bioaccessible Ag originating from PVP-AgNPs remained constant through the experiment: 3.0% at the start and 2.7% at the end of the experiment (Figure 1F).

In artificial sediment, 8.1% of the $AgNO_3$-spiked Ag was bioaccessible 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 uncoated AgNPs and from 0.1 to 1.0% with PVP-AgNPs between 2 and 14 d, but no bioaccessible Ag was recovered at the end of the exposure from the AgNP-spiked artificial sediment (Figure 1H and I).

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

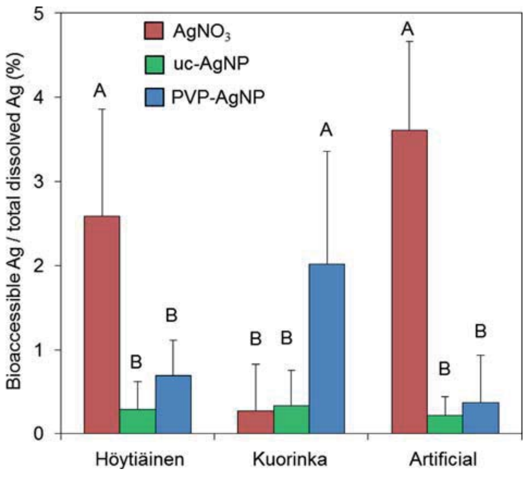


Figure 2. The percentage of bioaccessible Ag of the total dissolved Ag in Höytiäinen, Kuorinka, and artificial sediment spiked as silver nitrate ($AgNO_3$), uncoated silver nanoparticles (un-AgNPs), and polyvinylpyrrolidone-coated silver nanoparticles (PVP-AgNPs). Each bar represents an average % (+standard deviation) of bioaccessible Ag between 9 and 28 d 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.

Our results indicate that dissolved Ag released from PVP-AgNPs is more bioaccessible in the Lake Kuorinka 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-AgNPs were found to be toxic only in the Lake Kuorinka sediment, but the toxicity of $AgNO_3$ was highest in artificial sediment followed by Lake Höytiäinen sediment and Lake Kuorinka sediment [42]. Increased bioaccessibility of dissolved Ag spiked as PVP-AgNPs was unexpected, because released Ag from AgNPs was predicted to be bound by the sediment in a similar manner as Ag in the $AgNO_3$ treatment. Coutris et al. [18] observed similar increased bioaccessibility of Ag spiked as uncoated AgNPs compared with citrate-coated AgNPs or $AgNO_3$ in organic and mineral soil. They proposed that larger and more easily aggregated uncoated AgNPs 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 toward bioaccessible fractions during the 70-d exposure time. This might not be the case in the present study, because the dissolution of AgNPs was found to be immediate, and bioaccessibility of Ag increased only with PVP-AgNPs. In contrast, the bioaccessibility of Ag spiked as uncoated AgNPs decreased to a negligible amount at 28 d in artificial sediment and Lake Kuorinka sediment (Figure 1E and H).

The binding of Ag^+ on the surface of PVP-AgNPs could explain the elevated bioaccessibility of dissolved Ag [7,8,43]. The affinity of pristine PVPs to Ag^+ has been experimentally proved 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 the 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 Lake Kuorinka sediment.

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 total body burden of Ag measured from *L. variegatus* in natural sediments, even though the digested worm samples also contained AgNPs attached onto or taken up by the test organisms. The results suggest that most of the Ag is taken up as dissolved Ag rather than AgNPs in natural sediments. No conclusions on whether the differences in total body burden are statistically significant could be made because of the small sample size resulting from the unexpected outliers and mortality in the Lake Höytiäinen sediment samples (see Supplemental Data for details).

In the Lake Höytiäinen sediment, the total body burdens of Ag for *L. variegatus* were very similar between $AgNO_3$ and PVP-AgNPs (Figure 3A). The total body burden increased linearly ($r^2 = 0.91–0.93$) as a function of time in both treatments. The sediment uptake coefficients determined from the slope were: $AgNO_3 = 3.04\ \mu g\ g^{-1}\ d^{-1}$ and $PVP = 2.61\ \mu g\ g^{-1}\ d^{-1}$. In uncoated AgNP-spiked Lake Höytiäinen sediment, the total body burdens were smaller compared with other Ag compounds. No statistically significant difference in the absolute extracted bioaccessible concentration of Ag among the treatments was found (Figure 3AB).

In Lake Kuorinka sediment, Ag spiked as PVP-AgNPs produced a higher total body burden than other Ag compounds, as also predicted by the higher amount of Ag in the bioaccessible fraction (Figure 3C and D). Significantly more bioaccessible Ag was extracted with PVP-AgNPs at the 2-d sampling point compared with uncoated AgNPs ($p = 0.020$), and at the 28-d sampling point compared with $AgNO_3$ ($p = 0.016$) and uncoated AgNPs ($p = 0.010$). The total body burden of Ag increased linearly as a function of time in the PVP-AgNP ($r^2 = 0.87$) and $AgNO_3$ treatments ($r^2 = 0.93$). The sediment uptake coefficients determined from the slope were: $AgNO_3 = 1.31\ \mu g\ g^{-1}\ d^{-1}$ and $PVP\text{-}AgNP = 3.41\ \mu g\ g^{-1}\ d^{-1}$. No Ag was detected in the worms exposed to uncoated AgNPs (Figure 3C).

In artificial sediment, the total body burden in PVP-AgNP–treated sediment peaked during early exposure but then decreased and reached a plateau at 9 d (Figure 3E). A similar peak was seen after a 9-d contact time in $AgNO_3$-spiked sediment (Figure 3E), but the total body burdens decreased after that, possibly because of the growth of the test organisms (Supplemental Data, Figure S5). The concentration of bioaccessible Ag was significantly higher at the beginning of the experiment with $AgNO_3$ compared with uncoated AgNPs ($p < 0.001$) and PVP-AgNPs ($p = 0.017$) or 5- to 9-d sampling points with $AgNO_3$ ($p = 0.001–0.017$; Figure 3F).

Selection of equilibrium time is essential in laboratory dosed sediment studies. For AgNPs, no information is available on the proper contact time with the sediment before adding the organisms. Short equilibrium times may overestimate the bioavailability, because the association with sediment may be slow and high amounts of Ag can stay bioavailable during early exposure. On the other hand, dissolution of AgNPs can lead to increasing bioavailability of Ag during 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 artificial sediment, the bioaccessibility of $AgNO_3$ was highest at the beginning of the experiment. The slower equilibrium in artificial sediment can be explained by the coarser grain size of the artificial sediment: the association of dissolved Ag and AgNPs is slower compared with the relatively finer grain natural sediments [44]. Most likely PVP-AgNPs were also bioavailable at early exposure in artificial sediment, because the highest total body burden was observed at the beginning of the experiment despite the low bioaccessibility of dissolved Ag. The decreasing total body burden at the later sampling points can be explained by the growth of the test organisms and the stronger complexation of PVP-AgNPs with the sediment (also supported by the increasing amount of Ag in the residual fraction). In the uncoated AgNP-spiked sediments, this effect was not seen, probably because of a lack of stabilizing PVP coating, resulting in more rapid association with the sediment components. Coleman et al. [45] concluded in their study that neither the coating nor the size of AgNPs influenced the bioaccumulation factors of the Ag from the sediment to *L. variegatus*. For the marine benthic polychaete *Nereis virens*, bioaccumulation was influenced by the AgNP coating agent [46]. In the present study, the PVP-coating on AgNPs 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 uncoated AgNPs and PVP-AgNPs. Sufficient equilibrium time was also dependent on

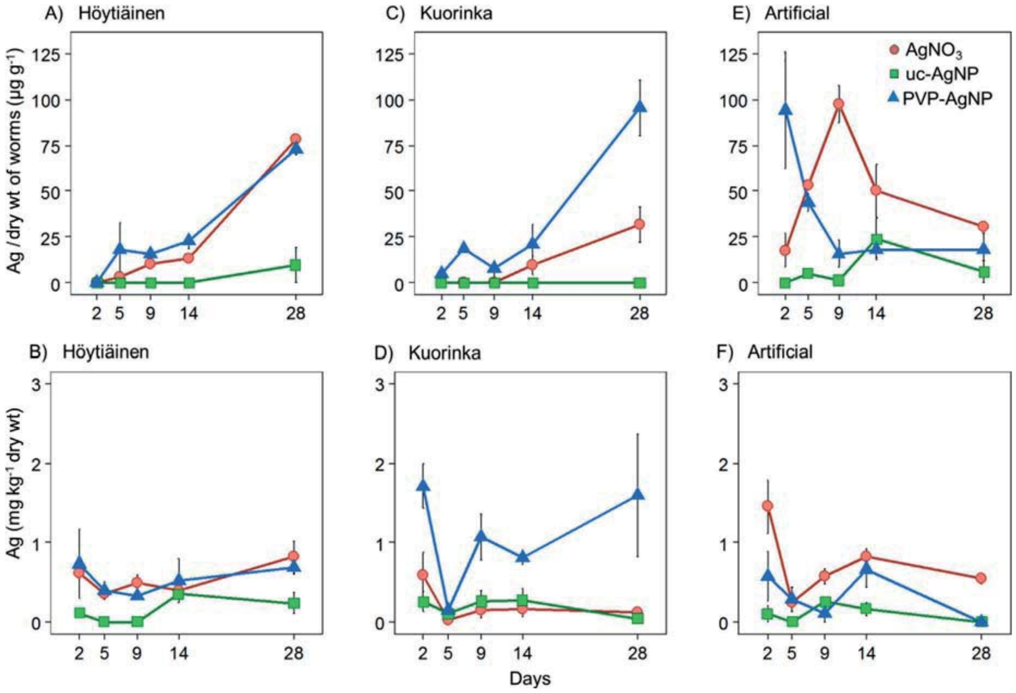


Figure 3. The total body burdens of Ag in dry *Lumbriculus variegatus* tissue (μg g^{-1} dry wt; **A,C,E**) and the concentration of Ag (mg kg^{-1} dry wt) associated with the bioaccessible fraction (sum of fractions 1 and 2) of the sediment (**B,D,F**). The Ag was spiked to Höytäinen (**A,B**), Kuorinka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

the media and AgNP properties. According to our results, an equilibrium time of 24 h was sufficient for AgNPs in natural sediments; but in artificial sediment, equilibrium times up to 9 d 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 to 169.8 μg g^{-1} for AgNPs with varying capping agents [47]. In our experiments, the total body burdens of Ag exceeded these values in all tested sediments for $AgNO_3$, but mortality (as missing worms) was only observed in the Lake Höytiäinen sediment after 28 d of exposure. Ingestion of Ag is minimal in waterborne exposures, and comparing the values is complicated by the different uptake route. However, the total body burdens increased linearly in the natural sediments spiked with $AgNO_3$ and PVP-AgNPs. Thus, it is possible that in the natural Lake Kuorinka and Lake Höytiäinen sediments, these compounds have toxic effects for chronic exposures longer than 28 d.

CONCLUSIONS

The hypothesis that aging of AgNPs would lead to a higher bioaccessible concentration of Ag in sediment was rejected, because the concentration of bioaccessible Ag did not increase in any treatment during the 28-d 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 originated from PVP-AgNPs was more bioaccessible than that from uncoated AgNPs or $AgNO_3$ in the natural Lake Kuorinka sediment. The dissolution rates of uncoated AgNPs and PVP-AgNPs were estimated to be similar, thus varying among the sediments. The measured sediment characteristics, such as organic carbon content, Fe_{ox}/Mn_{ox}, or pH, could not explain the observed differences. More research on NOM(–PVP) –AgNP interactions should be conducted to reveal the mechanisms behind our observations. Higher bioaccessibility of dissolved Ag also resulted in a higher total body burden in *L. variegatus*, indicating that the uptake of Ag happens mainly as dissolved Ag species rather than AgNPs. Overall, our results suggest that the possibility of hazardous effects in the environment is lower for uncoated AgNPs than for $AgNO_3$ or PVP-AgNPs.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3798.

Acknowledgment—J.E. Rajala acknowledges support from the University of Jyväskylä Graduate School for Doctoral Studies. E.-R. Vehniäinen was supported by the Academy of Finland (285296).

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

REFERENCES

1. Project on Emerging Nanotechnologies. 2016. Consumer Products Inventory. [cited 2017 January 19]. Available from: http://www.nanotechproject.org/cpi/.
2. Gottschalk F, Sonderer T, Scholz RW, Nowack B. 2009. Modeled environmental concentrations of engineered nanomaterials (TiO_2, ZnO, Ag, CNT, fullerenes) for different regions. *Environ Sci Technol* 43:9216–9222.
3. Wang H, Burgess RM, Cantwell MG, Portis LM, Perron MM, Wu F, Ho KT. 2014. Stability and aggregation of silver and titanium dioxide

nanoparticles in seawater: Role of salinity and dissolved organic carbon. *Environ Toxicol Chem* 33:1023–1029.

4. Velzeboer I, Quik J, van de Meent D, Koelmans A. 2014. Rapid settling of nanoparticles due to heteroaggregation with suspended sediment. *Environ Sci Technol* 33:1766–1773.
5. Quik JT, Stuart MC, Wouterse M, Peijnenburg W, Hendriks AJ, van de Meent D. 2012. Natural colloids are the dominant factor in the sedimentation of nanoparticles. *Environ Sci Technol* 31: 1019–1022.
6. Wu Y, Zhou Q, Li H, Liu W, Wang T, Jiang G. 2010. Effects of silver nanoparticles on the development and histopathology biomarkers of Japanese medaka (*Oryzias latipes*) using the patial-life test. *Aquat Toxicol* 100:160–167.
7. Liu J, Sonshine DA, Shervani S, Hurt RH. 2010. Controlled release of biologically active silver from nanosilver surfaces. *ACS Nano* 4: 6903–6913.
8. Liu J, Hurt RH. 2010. Ion release kinetics and particle persistence in aqueous nano-silver colloids. *Environ Sci Technol* 44:2169–2175.
9. Ho CM, Yau SK, Lok CN, So MH, Che CM. 2010. Oxidative dissolution of silver nanoparticles by biologically relevant oxidants: A kinetic and mechanistic study. *Chem Asian J* 5:285–293.
10. Cornelis G, Doolette M, Thomas C, McLaughlin MJ, Kirby JK, Beak DG, Chittleborough D. 2012. Retention and dissolution of engineered silver nanoparticles in natural soils. *Soil Sci Soc Am J* 76:891–902.
11. Peretyazhko TS, Zhang Q, Colvin VL. 2014. Size-controlled dissolution of silver nanoparticles at neutral and acidic pH conditions: Kinetics and size changes. *Environ Sci Technol* 48: 11954–11961.
12. Yu S, Yin Y, Liu J. 2013. Silver nanoparticles in the environment. *Env Sci Process Impact* 15:78–92.
13. Chan CYS, Chiu JMY. 2015. Chronic effects of coated silver nanoparticles on marine invertebrate larvae: A proof of concept study. *PLoS One* 10: e0132457.
14. García-Alonso J, Rodriguez-Sanchez N, Misra SK, Valsami-Jones E, Croteau M, Luoma SN, Rainbow PS. 2014. Toxicity and accumulation of silver nanoparticles during development of the marine polychaete *Platynereis dumerilii*. *Sci Total Environ* 476:688–695.
15. Cozzari M, Elia AC, Pacini N, Smith BD, Boyle D, Rainbow PS, Khan FR. 2015. Bioaccumulation and oxidative stress responses measured in the estuarine ragworm (*Nereis diversicolor*) exposed to dissolved, nano- and bulk-sized silver. *Environ Pollut* 198:32–40.
16. Semple KT, Doick KJ, Jones KC, Burauel P, Craven A, Harms H. 2004. Defining bioavailability and bioaccessibility of contaminated soil and sediment is complicated. *Environ Sci Technol* 38:228A–231A.
17. Coutris C, Hertel-Aas T, Lapied E, Joner EJ, Oughton DH. 2012. Bioavailability of cobalt and silver nanoparticles to the earthworm *Eisenia fetida*. *Nanotoxicology* 6:186–195.
18. Coutris C, Joner EJ, Oughton DH. 2012. Aging and soil organic matter content affect the fate of silver nanoparticles in soil. *Sci Total Environ* 420:327–333.
19. Tessier A, Campbell PG, Bisson M. 1979. Sequential extraction procedure for the speciation of particulate trace metals. *Anal Chem* 51:844–851.
20. Ristola T, Pellinen J, Van Hoof PL, Leppänen M, Kukkonen J. 1996. Characterization of Lake Ladoga sediments. II. Toxic chemicals. *Chemosphere* 32:1179–1192.
21. Organisation for Economic Co-Operation and Development. 2007. Test no. 225: Sediment-water *Lumbriculus* toxicity test using spiked sediment. *OECD Guidelines for the Testing of Chemicals*. Paris, France.
22. Sun TY, Gottschalk F, Hungerbühler K, Nowack B. 2014. Comprehensive probabilistic modelling of environmental emissions of engineered nanomaterials. *Environ Pollut* 185:69–76.
23. Väisänen A, Kiljunen A. 2005. Ultrasound-assisted sequential extraction method for the evaluation of mobility of toxic elements in contaminated soils. *Int J Environ Anal Chem* 85:1037–1049.
24. Holm S. 1979. A simple sequentially rejective multiple test procedure. *Scand J Stat* 6:65–70.
25. Norman G. 2010. Likert scales, levels of measurement and the "laws" of statistics. *Adv Health Sci Educ Theor Pract* 15:625–632.
26. Van Griethuysen C, De Lange H, Van den Heuij M, De Bies S, Gillissen F, Koelmans A. 2006. Temporal dynamics of AVS and SEM in sediment of shallow freshwater floodplain lakes. *Appl Geochem* 21:632–642.
27. Notter DA, Mitrano DM, Nowack B. 2014. Are nanosized or dissolved metals more toxic in the environment? A meta-analysis. *Environ Sci Technol* 33:2733–2739.
28. Hoheisel SM, Diamond S, Mount D. 2012. Comparison of nanosilver and ionic silver toxicity in *Daphnia magna* and *Pimephales promelas*. *Environ Toxicol Chem* 31:2557–2563.
29. Li X, Lenhart JJ, Walker HW. 2011. Aggregation kinetics and dissolution of coated silver nanoparticles. *Langmuir* 28:1095–1104.
30. Kittler S, Greulich C, Diendorf J, Koller M, Epple M. 2010. Toxicity of silver nanoparticles increases during storage because of slow dissolution under release of silver ions. *Chem Mater* 22: 4548–4554.
31. Seitz F, Rosenfeldt RR, Storm K, Metreveli G, Schaumann GE, Schulz R, Bundschuh M. 2015. Effects of silver nanoparticle properties, media pH and dissolved organic matter on toxicity to *Daphnia magna*. *Ecotoxicol Environ Saf* 111:263–270.
32. Delay M, Dolt T, Woellhaf A, Sembritzki R, Frimmel FH. 2011. Interactions and stability of silver nanoparticles in the aqueous phase: Influence of natural organic matter (NOM) and ionic strength. *J Chromatogr A* 1218:4206–4212.
33. Cumberland SA, Lead JR. 2009. Particle size distributions of silver nanoparticles at environmentally relevant conditions. *J Chromatogr A* 1216:9099–9105.
34. Yang X, Lin S, Wiesner MR. 2014. Influence of natural organic matter on transport and retention of polymer coated silver nanoparticles in porous media. *J Hazard Mater* 264:161–168.
35. Gunsolus IL, Mousavi MP, Hussein K, Bühlmann P, Haynes CL. 2015. Effects of humic and fulvic acids on silver nanoparticle stability, dissolution, and toxicity. *Environ Sci Technol* 49:8078–8086.
36. Yamada H, Michalik J, Sadlo J, Perlinska J, Takenouchi S, Shimomura S, Uchida Y. 2001. Electron spin resonance studies on silver atoms in imogolite fibers. *Appl Clay Sci* 19:173–178.
37. Akaighe N, MacCuspie RI, Navarro DA, Aga DS, Banerjee S, Sohn M, Sharma VK. 2011. Humic acid-induced silver nanoparticle formation under environmentally relevant conditions. *Environ Sci Technol* 45:3895–3901.
38. Hou H, Takamatsu T, Koshikawa M, Hosomi M. 2006. Concentrations of Ag, In, Sn, Sb and Bi, and their chemical fractionation in typical soils in Japan. *Eur J Soil Sci* 57:214–227.
39. Hou H, Takamatsu T, Koshikawa M, Hosomi M. 2005. Migration of silver, indium, tin, antimony, and bismuth and variations in their chemical fractions on addition to uncontaminated soils. *Soil Sci* 170:624–639.
40. Mäenpää KA, Sormunen AJ, Kukkonen JVK. 2003. Bioaccumulation and toxicity of sediment associated herbicides (ioxynil, pendimethalin, and bentazone) in *Lumbriculus variegatus* (Oligochaeta) and *Chironomus riparius* (Insecta). *Ecotoxicol Environ Saf* 56:398–410.
41. Luoma SN, Bryan G. 1981. A statistical assessment of the form of trace metals in oxidized estuarine sediments employing chemical extractants. *Sci Total Environ* 17:165–196.
42. Rajala JE, Mäenpää K, Vehniäinen E, Väisänen A, Scott-Fordsmand JJ, Akkanen J, Kukkonen JVK. 2016. Toxicity testing of silver nanoparticles in artificial and natural sediments using the benthic organism *Lumbriculus variegatus*. *Arch Environ Contam Toxicol* 71:405–414.
43. Klitzke S, Metreveli G, Peters A, Schaumann GE, Lang F. 2014. The fate of silver nanoparticles in soil solution—Sorption of solutes and aggregation. *Sci Total Environ* 535:54–60.
44. Simpson SL, Angel BM, Jolley DF. 2004. Metal equilibration in laboratory-contaminated (spiked) sediments used for the development of whole-sediment toxicity tests. *Chemosphere* 54:597–609.
45. Coleman JG, Kennedy AJ, Bednar AJ, Ranville JF, Laird JG, Harmon AR, Hayes CA, Gray EP, Higgins CP, Lotufo G, Steevens JA. 2013. Comparing the effects of nanosilver size and coating variations on bioavailability, internalization, and elimination, using *Lumbriculus variegatus*. *Environ Toxicol Chem* 32:2069–2077.
46. Wang H, Ho KT, Scheckel KG, Wu F, Cantwell MG, Katz DR, Horowitz DB, Boothman WS, Burgess RM. 2014. Toxicity, bioaccumulation, and biotransformation of silver nanoparticles in marine organisms. *Environ Sci Technol* 48:13711–13717.
47. Khan FR, Paul KB, Dybowska AD, Valsami-Jones E, Lead JR, Stone V, Fernandes TF. 2015. Accumulation dynamics and acute toxicity of silver nanoparticles to *Daphnia magna* and *Lumbriculus variegatus:* Implications for metal modelling approaches. *Environ Sci Technol* 49:4389–4397.

III

TOXICITY OF SILVER NANOPARTICLES TO *LUMBRICULUS VARIEGATUS* IS A FUNCTION OF DISSOLVED SILVER AND PROMOTED BY LOW SEDIMENT PH

by

Juho Elias Rajala, Eeva-Riikka Vehniäinen, Ari Väisänen & Jussi Vilho Kalevi Kukkonen 2017

Submitted manuscript