Behaviour of silver and silver sulfide nanoparticles in the environment: Effects on wastewater treatment processes and soil organisms

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Abstract

Manufactured silver nanoparticles (AgNPs) are the most commonly used manufactured nanomaterial in consumer products. They are incorporated into a vast array of products due to their strong, broad-spectrum anti-microbial activity. However, the anti-bacterial properties that render AgNPs desirable may also lead to increased environmental risks. This thesis examines that impact in wastewater treatment plants (WWTP) and terrestrial environments – the key risk pathways for AgNPs.

This thesis studied the life-cycle of released AgNPs, from their release to potential plant uptake, with a focus on their effects and fate in the environment. Four main experiments were undertaken to investigate 1) the effects of AgNPs on WWT processes, 2) the bioavailability of AgNPs and transformed AgNPs (Ag_2S-NPs) in soils to lettuce, 3) the effect of fertiliser addition on the bioavailability of AgNPs and Ag_2S-NP and 4) the effects of AgNPs and Ag_2S-NPs on soil microbial communities. The experiments were carried out to model realistic exposure concentrations and pathways (e.g. wastewater and soil cf. synthetic wastewater and hydroponic conditions, and Ag_2S-NPs cf. 'pristine' AgNPs).

The results from this thesis demonstrate that sub-dominant wastewater microbial communities can be affected by AgNPs. However, dominant microbial communities and key WWT processes, such as nitrification and methanogenesis, are unlikely to be affected by AgNPs at realistic exposure concentrations. During wastewater treatment it was found that AgNPs were almost completely transformed (> 95%) to sulfidised Ag species, predominantly as Ag-sulfide (Ag_2S-NPs).

The bioavailability of sludge-borne Ag_2S-NPs in soil was found to be very low. However, when thiosulfate fertiliser was added to soil, significantly more Ag was taken up by plants. Despite this increased uptake, the overall plant concentrations of Ag remained low; the Ag concentrations in edible plant parts (shoots) increased from 0.02% to 0.06% of the total amount of added Ag.

Finally, to assess the degree of risk that AgNPs and Ag_2S-NPs pose to soil microorganisms, a new molecular-based approach was developed to determine the effect on whole soil microbial communities. This new approach was used to calculate toxicity values for individual soil microbial populations following their exposure to Ag^+, AgNPs and Ag_2S-NPs. A combination of quantitative PCR (qPCR) and pyrosequencing-based analysis of the 16S rRNA gene region was used to develop dose-response curves for sensitive microbial populations. Based on pyrosequencing results, similar sequences were assigned to operational taxonomic units (OTUs); the abundances of which were then converted to absolute values. Toxicity values (EC_{20}) for sensitive soil OTUs were then plotted on a sensitivity distribution in order to calculate the Ag concentration that would theoretically protect a specified percentage of soil microorganism gene sequences (HC_x values). At the HC5 and HC10 values (95% and 90% of soil OTUs protected, respectively), there were no significant differences between Ag treatments, while at the HC20 (80% of OTUs protected), Ag_2S-NPs were significantly less toxic than AgNPs and Ag^+. The most sensitive OTUs (EC_{20} < HC5) were predominantly from the *Bacillaceae* family, with lower abundances of other families including *Frankiaceae*, *Comamonadaceae* and *Pseudonocardiaceae*.

In all experiments described in this thesis, the negative impacts of AgNPs and Ag_2S-NPs were less than or equal to the effects observed in ionic Ag (Ag^+) treatments. Overall, results from this thesis show that the risks associated with AgNPs and Ag_2S-NPs are overestimated (and conservatively covered) by the risk of ionic Ag^+ in terrestrial environments.

Declaration

I, Casey Doolette, certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution, and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide.

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Publications arising from this thesis

Journal articles

Doolette, C.L, McLaughlin, M.J., Kirby, J.K., & Navarro, D.A. (2015) Bioavailability of silver and silver sulfide nanoparticles to lettuce (*Lactuca sativa*): Effect of agricultural amendments on plant uptake. *Journal of Hazardous Materials* (accepted pending revision)

Doolette, C.L, McLaughlin, M.J., Kirby, J.K., Batstone, D.J., Harris, H.H., Ge, H., & Cornelis, G. (2013) Transformation of PVP coated silver nanoparticles in a simulated wastewater treatment process and the effect on microbial communities. *Chemistry Central Journal* 7(1), 46-64.

Conference abstracts

Doolette, C.L., McLaughlin, M., Kirby, J., Navarro, D.G., & Gupta, V.G. (2014) The influence of sulfidised silver nanoparticles on soil nitrification processes and soil microbial communities. *Proceedings of: Society of Environmental Toxicology and Chemistry (SETAC) North America 35th Annual Meeting*, Vancouver, Canada.

Doolette, C.L., McLaughlin, M.J, Kirby, J., Navarro, D.G., Harris, H.H., & Cornelis, G. (2014) The influence of agricultural amendments on the bioavailability of silver sulfide nanoparticles in soil. *Proceedings of: 9th Society of Environmental Toxicology and Chemistry (SETAC) Asia Pacific 2014 Conference*, Adelaide, Australia.

Doolette, C.L., McLaughlin, M., Kirby, J., Harris, H.H., Navarro, D.G., & Cornelis, G. (2013) Will the environmental impacts of manufactured silver nanoparticles be more than a small problem? *Proceedings of: 3rd Society of Environmental Toxicology and Chemistry (SETAC) Australasia Conference,* Melbourne, Australia.

McLaughlin, M.J., Navarro, D.A., Langdon, K., Kirby, J.K., Kookana, R.S., **Doolette, C.L.**, Settimio, L., & Kumar, A (2013) Comparing the fate of ionic silver and nanometallic silver in soils - implications for ecotoxicity tests and risk assessment. *Proceedings of*: *SETAC North America 34th Annual Meeting*, Nashville, United States.

Doolette, C.L., McLaughlin, M.J., Kirby, J.K., Harris, H.H., Navarro, D., Cornelis, G., Batstone, D., & Ge, H. (2013) Will silver nanoparticles affect the efficient functioning of wastewater treatment plants? *Proceedings of*: *CSIRO Nanosafety Workshop for staff involved in nanosafety projects within the Advanced Materials Transformational Capability Platform,* Sydney, Australia

Doolette, C.L., McLaughlin, M.J., Kirby, J.K., Cornelis, G., Batstone, D.J., & Ge, H. (2012) Soil amendment with biosolids exposed to silver nanoparticles: is silver bioavailable? *Proceedings of: Joint SSA and NZSSS Soil Science Conference,* Hobart, Australia.

McLaughlin, M., Kirby, J., Batstone, D.J., **Doolette, C**., & Ge, H. (2012) Manufactured nanomaterials - An emerging issue for wastewater treatment and biosolids reuse. *Proceedings of: AWA Biosolids and Source Management National Conference*, Gold Coast, Australia.

Additional journal articles of related work not described in this thesis

Cornelis, G., Pang, L., **Doolette, C.**, Kirby, J.K., & McLaughlin, M.J. (2013) Transport of silver nanoparticles in saturated columns of natural soils. *Science of the Total Environment* 463–464, 120-130.

Cornelis, G., **Doolette, C.**, Thomas, M., McLaughlin, M.J., Kirby, J.K., Beak, D.G., & Chittleborough, D. (2012) Retention and dissolution of engineered silver nanoparticles in natural soils. *Soil Science Society of America Journal* 76(3), 891-902.

Statement of authorship

Components of the research described in this thesis have been published, or have been submitted for publication and are in review, or, are currently being prepared for publication. The contribution of each author to these works is described below.

Chapter 2: *Chemistry Central Journal;* 2013, **7**, 46 – 64.

Chapter 3: *Journal of Hazardous Materials*; 2015 (submitted).

Chapter 4: *The ISME Journal* (in preparation).

DOOLETTE, C.L. (Candidate)

Chapters 2 – 5: Experimental development; performed experiments; data analysis and critical interpretation; prepared the manuscripts; created all figures.

I hereby certify that the statement of contribution is accurate.

Signed Date

MCLAUGHLIN, M.J., KIRBY, J., NAVARRO, D.A. (Supervisors)

Chapters 2 – 5: Project design; experimental development; data analysis and critical interpretation of results; manuscript review.

I hereby certify that the statement of contribution is accurate.

Signed Date

Signed Date

Signed Date

HARRIS, H.H. (Supervisor)

Chapter 2: Assisted and supervised XAS experiments; data interpretation; manuscript review.

I hereby certify that the statement of contribution is accurate.

Signed Date

CORNELIS, G. (Supervisor)

Chapter 2: Assisted with experimental design; data interpretation; manuscript review.

I hereby certify that the statement of contribution is accurate.

Signed Date 24 June 2015

BATSTONE, D.

Chapters 2 and 4: Assisted with project design and set-up; data analysis and interpretation; manuscript review.

I hereby certify that the statement of contribution is accurate.

Signed Date 25/6/15

GE, H.

Chapter 2: Set-up sequencing batch reactors; collected and analysed anaerobic sludge samples; manuscript review.

I hereby certify that the statement of contribution is accurate.

Signed Date 25/6/15

PAYNE, J.L.

Chapter 4: Co-wrote the R code; data analysis and critical interpretation of results; manuscript review.

I hereby certify that the statement of contribution is accurate.

Signed Date

GUPTA, V.V.S.R.

Chapter 4: Project design; data analysis and interpretation; manuscript review.

I hereby certify that the statement of contribution is accurate.

Signed Date

LU, Y.

Chapter 4: Assisted with project design; prepared the supplied DNA samples for pyrosequencing analysis; performed qPCR analysis; processed raw data from pyrosequencing and qPCR analysis; manuscript review.

I hereby certify that the statement of contribution is accurate.

Signed Date 25/6/15

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This thesis is dedicated to the memory of my Dad, Kevern Doolette, who encouraged me to take every opportunity to keep learning. More importantly, he taught me that it's the people you meet and friendships you make along the way that are the most rewarding.

Motivations and aims of the thesis

The past two decades have seen a dramatic increase in the use of silver nanoparticles (AgNPs) in consumer products. The widespread use of AgNPs can be attributed to their unique and enhanced physicochemical properties compared to the bulk material (> 100 nm). However, in the majority of AgNP-enabled consumer products, AgNPs are incorporated due to their broad-spectrum antimicrobial activity. It is this antimicrobial property that may pose a potential risk to the environment.

The main environmental exposure pathway of AgNPs is *via* soil application of AgNP-containing biosolids. When AgNP-containing products are washed, a portion of AgNPs are released into wastewater streams. Silver NPs – and ionic Ag (Ag^+) – can be released readily from AgNP-containing textiles, food containers and paints, and through the use of AgNP-coated washing machines. During wastewater treatment (WWT), the majority of AgNPs (or Ag^+) will adsorb to the biosolids (stabilised sludge) and depending on location, biosolids may then be applied to soil as an agricultural amendment to improve soil fertility and act as a soil conditioner. For example, in Australia, approximately 60% of biosolids are used in this way. Therefore, when considering the lifecycle of AgNPs and the potential environmental risks, the two main compartments that are susceptible to deleterious effects are WWT processes and soil environments.

In both WWT plants and terrestrial systems, microorganisms are responsible for a number of essential processes. Therefore, given the strong antimicrobial properties of AgNPs it is important to understand the fate and behaviour of AgNPs in soils and WWT plants: it is arguably more important to understand if the potential risks are over/under-estimated by the risks of Ag^+ in these two systems. A number of studies that have investigated the toxicity and behaviour of AgNPs have not included Ag^+ treatments; hence, it remains unclear whether the observed effects are nano- specific.

In the environment, the concentration of AgNPs is expected to be in the ng kg^{-1} to mg kg^{-1} range. Given the limited number of studies that have used realistic exposure concentrations, there is significant motivation to investigate the effects of AgNPs on environmental systems using realistic exposure scenarios. In addition to AgNP concentration, a number of other factors must also be considered to ensure that the risks of AgNPs are not overestimated, including the test matrix (e.g. synthetic wastewater vs. real wastewater), exposure times and AgNP transformation products.

During WWT, AgNPs may be transformed into nano- sized sulfidised Ag aggregates (Ag_2S-NPs) which are adsorbed by, or incorporated into, the biosolids. Therefore, in biosolids, AgNPs will not be present as 'pristine' AgNPs but will exist as Ag_2S-NPs. While studies that use pristine AgNPs are important for understanding the mechanisms of AgNP toxicity, such studies do not accurately predict the potential risks of AgNPs. Hence, to gain a more comprehensive understanding of the effects of AgNPs in WWT plants and soils, it is necessary to undertake experiments that use realistic spiking concentrations, test matrices, time scales and Ag species.

The main objective of this research was to carry out a comprehensive study of the behaviour of AgNPs in the environment; from their release into wastewater to their application to soil as Ag_2S-NPs in biosolids. Specifically, the aims of this thesis were to:

1. Investigate the fate of AgNPs during wastewater treatment and the changes in Ag speciation during this process;
2. Determine the effect of AgNPs on wastewater treatment processes using realistic experimental conditions;
3. Compare the bioavailability of transformed AgNPs to that of AgNPs and ionic Ag^+;
4. Determine the effects of agricultural amendments on the bioavailability of transformed AgNPs and AgNPs;
5. Investigate the effects of transformed AgNPs on soil nitrification processes; and,
6. Use genomic tools to develop a new method to quantify the ecotoxicity, and thus potential risk, of transformed AgNPs to whole soil microbial communities.

Structure of this thesis

This thesis is presented as a combination of papers that have been published, submitted for publication, as well as chapters that have not been submitted for publication.

Chapter 1 provides an overview of the literature on the behaviour of manufactured and naturally occurring nanomaterials in the environment with a focus on silver nanoparticles (AgNPs). Background information is also given for bulk Ag and the potential mechanisms of AgNP toxicity. Additional information that is specific to each study is presented in the introduction of each chapter.

Chapter 2 comprises a paper that is published in the *Chemistry Central Journal*. It describes the effects of AgNPs on wastewater treatment processes and assesses the chemical transformation of AgNPs during wastewater treatment.

Chapter 3 comprises a paper that has been submitted to the *Journal of Hazardous Materials* and has been accepted for publication pending revision. It describes two pot trials that were undertaken to examine the bioavailability of transformed AgNPs to lettuce. In the first pot trial, biosolids that were produced in **Chapter 1** and that contained transformed AgNPs (as Ag_2S-NPs) were applied to soil. In the second experiment, the effects of fertiliser application on the plant uptake of Ag were investigated.

Chapter 4 describes a soil incubation experiment that was carried out to investigate the sensitivity of soil microbial populations to AgNPs and transformed AgNPs. This experiment used molecular-based techniques to develop a new method that could be used to quantify the risks of AgNPs and Ag_2S-NPs to soil microorganisms. These results will be submitted for publication following submission of this thesis.

Chapter 5 provides a summary of the main outcomes of this thesis and includes recommendations for future research in this area.

Appendices 1 and **2** describe the method development for Ag_2S-NPs synthesis and for growing lettuce in a sandy soil.

Appendix 3 describes attempts to investigate the impact of fertiliser application on Ag speciation in soil using synchrotron based X-ray absorption spectroscopy analysis.