Effects of wastewater borne silver nanoparticles on the reproduction and biochemical markers of *Daphnia magna*

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Introduction

The production of silver nanoparticles (AgNPs) has been strongly growing in the last decades. Their extensive usage as antimicrobial agents in several products leads to rising entries of AgNPs into wastewater treatment plants (WWTPs) via industrial and urban sewage. Although most of the nanoparticles adsorb to the sludge, a small amount of mostly transformed AgNPs passes the WWTP and is finally released into the environment. The aim of this study was to investigate and compare the effects of pristine and transformed AgNPs on the reproduction and biochemical markers of the freshwater cladoceran *Daphnia magna*.

Materials & Methods

Four model WWTPs were conducted according to OECD Guideline 303A. The collected effluents were used to perform a chronic reproduction test with *Daphnia magna* according to OECD guideline 211. Animals were exposed to AgNPs in three different media (Figure 1). In a further approach, 96 h old daphnids were exposed to the same treatments during four days and analyzed for changes in the levels of several biochemical markers [lipid peroxidation; activities of acetylcholinesterase (AChE), lactate dehydrogenase (LDH), superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST)].

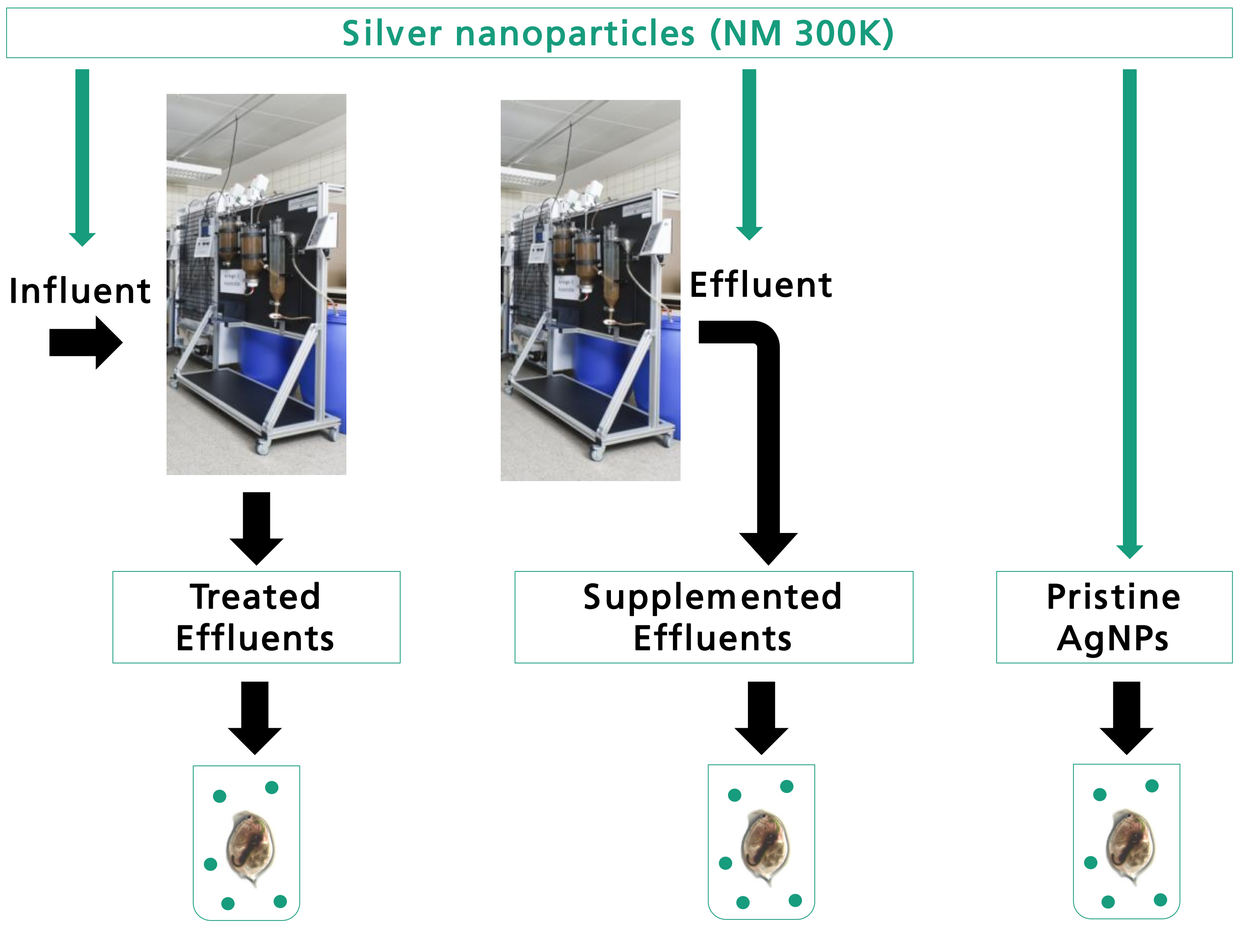

Figure 1: Experimental scenarios for the assessment of the environmental effect of nanosilver. Test animals were exposed to (i) WWTP effluent from AgNP-contaminated plants, (ii) uncontaminated effluent, manually spiked with AgNPs and (iii) pristine AgNPs.

Results

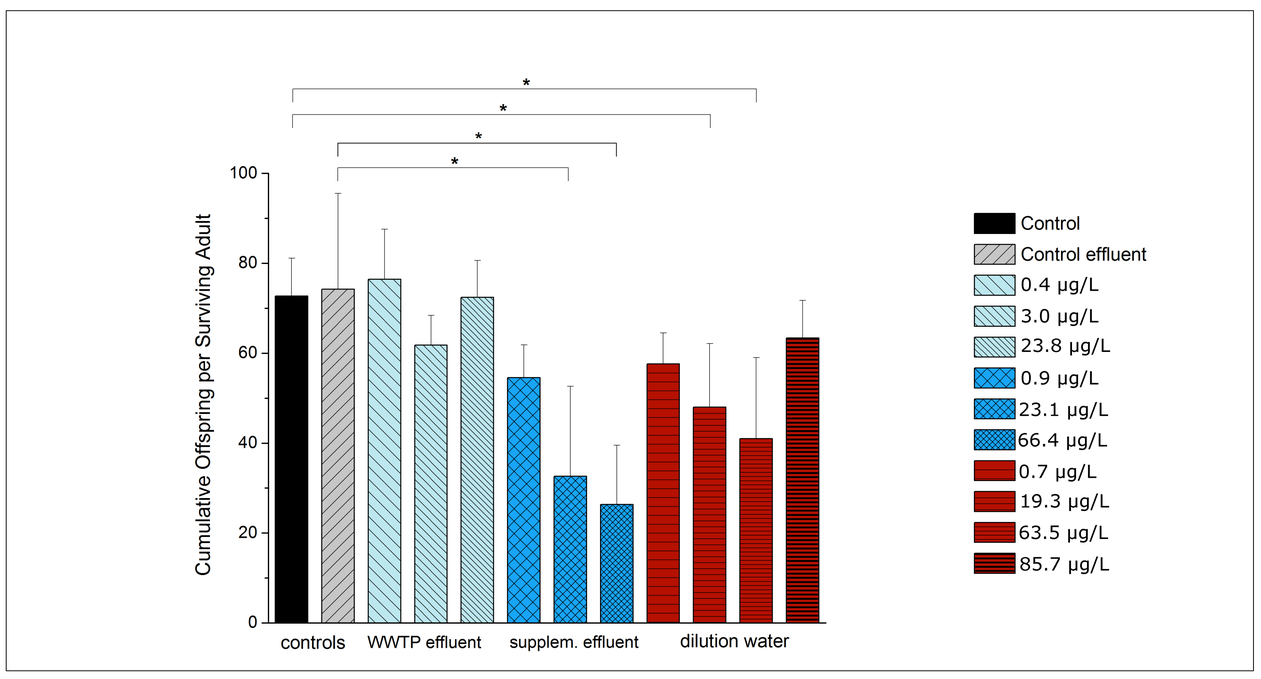

Figure 2: Effects of NM 300K AgNPs on the reproduction of *D. magna* after 21 d exposure. Concentrations of total silver were measured by ICP-MS. Asterisks indicate significant differences from the respective controls (Dunnett's test).

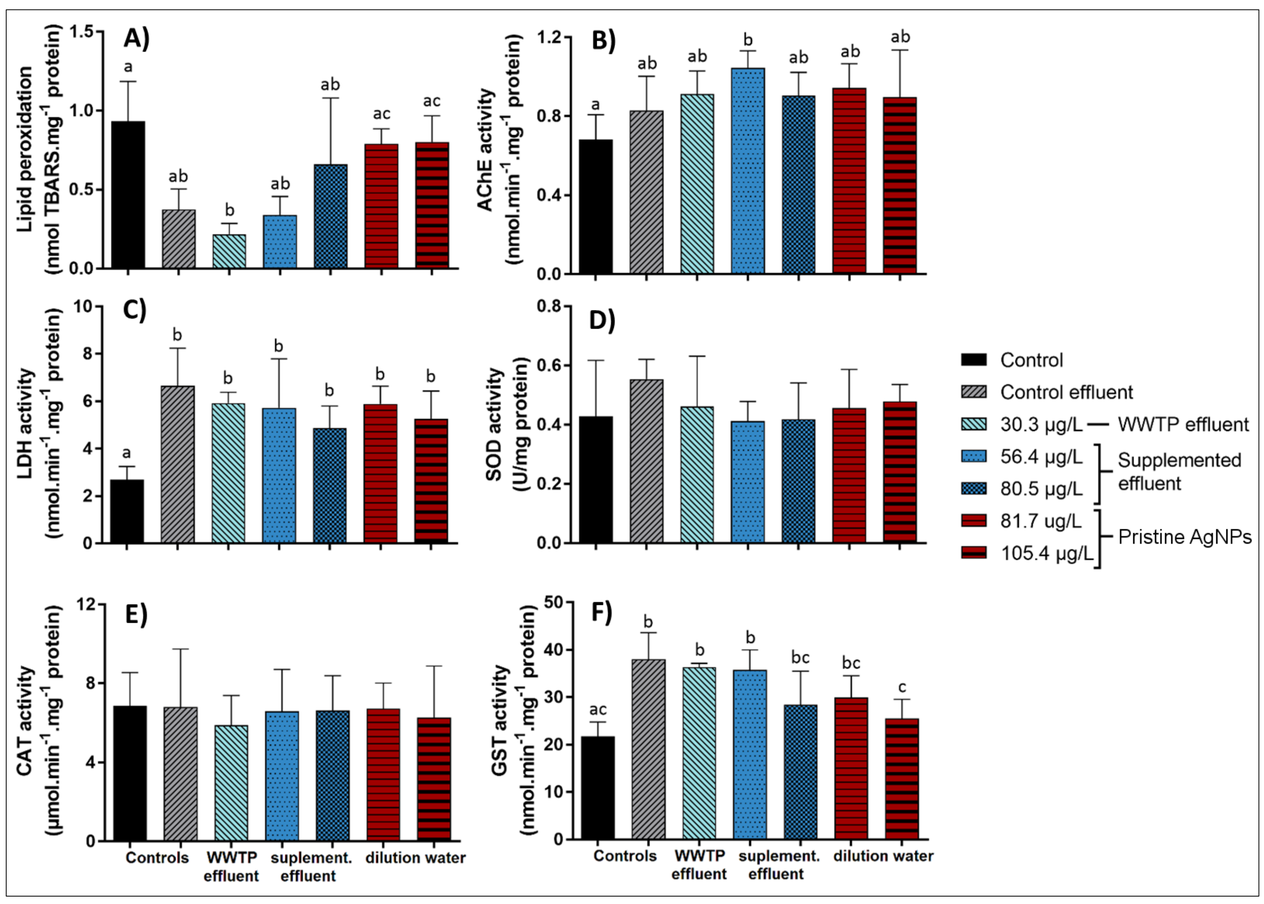

Figure 3: Effect of NM 300K AgNPs on biochemical markers of *D. magna* after 96 h. Bars are means of five independent experiments ± SD and those with different letters are statistically different ($p \leq 0.05$) according to Tukey's **(B, C, E, F)** and Dunn's **(A, D)** *post-hoc* tests.

Conclusions

- Exposition with contaminated WWTP effluents had no effect on *D. magna* reproduction;
- Dilution water and control effluent supplemented with AgNPs, induced a decrease of offspring per adult daphnia with increasing AgNP concentration;
- The highest concentration tested (85.7 µg/L) showed no significant effect on daphnia reproduction. A possible explanation for this might be a potential agglomeration of AgNPs at high concentrations and/or an acclimation of daphnia in the tested media;
- Results suggest no induction of oxidative stress by AgNPs in none of the conditions;
- The significant increase of LDH activity in daphnids exposed to pristine AgNPs suggests an increased rate of anaerobic metabolism (conversion of lactate to pyruvate and then to glucose);
- The significant increase of both LDH and GST activities in the control effluent in comparison to the negative control suggests the presence of additional substances in the effluent, *e.g.* organic compounds that can trigger anaerobic metabolism and detoxification mechanisms.

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- Implications of the environment as a global reactor for nanomaterials along their life-cycle (P)
Poster
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Silver nanoparticles (AgNPs) are widely used in a variety of products, e.g. textiles, bandages, and building facades, mainly due to their antimicrobial properties. Their increasing use leads to the transfer of AgNPs into wastewater treatment plants (WWTP) via industrial and urban sewage. During water treatment, silver nanomaterials remain in the sewage sludge to a great extent. Only a small amount of mostly transformed AgNPs is finally released into the environment. In this study we investigated the toxic effect of pristine and transformed AgNPs on the reproduction and biochemical markers of the freshwater cladoceran *Daphnia magna*.

Four model WWTPs were conducted according to OECD Guideline 303A. The collected effluents were used to perform a chronic reproduction test with *Daphnia magna* according to OECD guideline 211. Animals were exposed to (i) effluent from model WWTPs previously contaminated with AgNPs, (ii) uncontaminated effluent, manually spiked with AgNPs and (iii) dilution water enriched with pristine AgNPs. In a further approach, 96 h old daphnids were exposed to the same treatments for 4 days, collected and analysed for changes regarding the biochemical markers superoxide dismutase, glutathione S-transferase, thiobarbituric acid reactive substances to assess lipid peroxidation, lactate dehydrogenase, catalase and acetylcholinesterase.

No chronic effects were found in *D. magna* after exposure to effluents with transformed AgNPs. However, when supplemented into uncontaminated effluents or dilution water, the amount of offspring per adult daphnid decreased with increasing AgNP concentration. Interestingly, the highest test concentration of 100 µg/L showed no significant effect on the reproduction of the daphnids. These results can be explained by the transformation of AgNPs during their passage through the WWTP and potential agglomeration of AgNPs at high concentrations in the test media. Regarding the responses at the biochemical level, though significant changes were observed for daphnids exposed to the AgNPsfree effluent, in general, the presence of pristine and transformed AgNPs in the effluent did not significantly alter the measured parameters comparatively to the control effluent.