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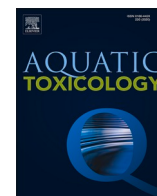
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Long-term effects of sediment-associated silver nanoparticles and silver nitrate on the deposit-feeding polychaete *Capitella teleta*

Maria Bille Nielsen^{a,*}, Janna Vavra^b, Annemette Palmqvist^c, Valery E. Forbes^d

^a Department of Environmental Engineering, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark

^b School of Biological Sciences, University of Nebraska-Lincoln, United States

^c Department of Science and Environment, Roskilde University, Denmark

^d Department of Ecology, Evolution and Behavior, University of Minnesota, United States

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ABSTRACT

Aquatic sediments are predicted to be an important sink for released silver nanoparticles (AgNPs). Knowing the long-term effects of AgNPs on benthic deposit-feeders is therefore an important step towards assessing their potential environmental risks. The aim of this study was to examine the effects on survival, growth and reproduction of the deposit-feeding polychaete *Capitella teleta* exposed for ten weeks to sediment-associated un-coated AgNPs or silver nitrate (AgNO₃). *C. teleta* exhibited tolerance towards exposure to both AgNPs and AgNO₃. Significant effects were observed for percentage of pairs that reproduced as well as worm growth after eight weeks, but the effects did not show a clear concentration- or Ag type-dependent pattern. Further investigations of long-term effects of un-coated AgNPs in additional sediment-dwelling organisms are needed and should involve comparisons to coated AgNPs.

Silver is a widely used metal in many consumer products due to its antimicrobial properties (Hernández et al., 2021), and the emergence of nanotechnology has further expanded its uses as silver nanoparticles (AgNPs). Applications of AgNPs include e.g. clothing, detergents, food, paints and disinfection products (Hansen et al., 2020; Hansen et al., 2016; Gottschalk et al., 2015). Environmental concerns about the use of AgNPs have been raised due to release of the particles to the environment. Toxic effects have been reported in a range of organisms, such as fish (Zhao et al., 2019; Rajkumar et al., 2016), algae (Johari et al., 2018) and invertebrates (Luo et al., 2016; Ribeiro et al., 2015). Luo et al. (2016) observed negative effects on reproduction and survival of the invertebrate *Caenorhabditis elegans* when exposed to polyvinylpyrrolidone-coated AgNPs, and in Ribeiro et al. (2015), exposure to AgNPs (AgNM300K standard particles) was found to induce oxidative stress in the invertebrate *Enchytraeus crypticus*. Aquatic sediments have been identified as an important sink for AgNPs (Ramskov et al., 2015; Baun et al., 2008). Concentrations of AgNPs in sediments have been estimated to be in the range of 18.3–43.4 µg/kg in Europe (Sun et al., 2016) with yearly increases between 1.8 and 3.3 µg/kg (Sun et al., 2014). Gottschalk et al. (2015) reported AgNP concentrations in Danish marine sediments of 0–0.7 µg/kg.

The marine polychaete *Capitella teleta* is an opportunistic sediment-

dwelling deposit feeder, often inhabiting polluted and organically enriched environments (Blake et al., 2009; Ramskov et al., 2009; Selck et al., 1998). It lives buried in the upper few centimeters of the sediment in which it feeds on living and dead organic matter (Lopez and Levinton, 1987). In order to meet its nutritional needs, *C. teleta* ingests daily sediment quantities of up to 12 times its own body weight (Selck et al., 1998; Dai et al., 2012) and is therefore likely to be exposed to sediment-associated contaminants, such as AgNPs. The aim of this study was to investigate the effects on survival, growth and reproduction of long-term exposure (ten weeks) to sediment-associated AgNPs in *C. teleta*. To assess if potential effects were related to the nanoparticles (NPs) themselves or to release of Ag ions (Ag⁺), exposure to Ag⁺ was included as a treatment.

C. teleta worms were exposed to un-coated silver nanoparticles (AgNPs) or silver nitrate (AgNO₃) added to marine sediment collected from Munkholm, Isefjorden, Denmark. Un-coated AgNPs are environmentally relevant to test as nanoparticle coatings may be degraded or substituted with natural organic matter upon environmental exposure (Diegoli et al., 2008; Ellis et al., 2016). Three nominal sediment Ag concentrations were prepared for both AgNPs and AgNO₃: 1 (low), 10 (medium) and 100 (high) µg Ag/g dry weight sediment (dw sed), providing 7 treatment groups: AgNP-low, AgNP-medium, AgNP-high,

* Corresponding author.

E-mail address: mabini@env.dtu.dk (M.B. Nielsen).

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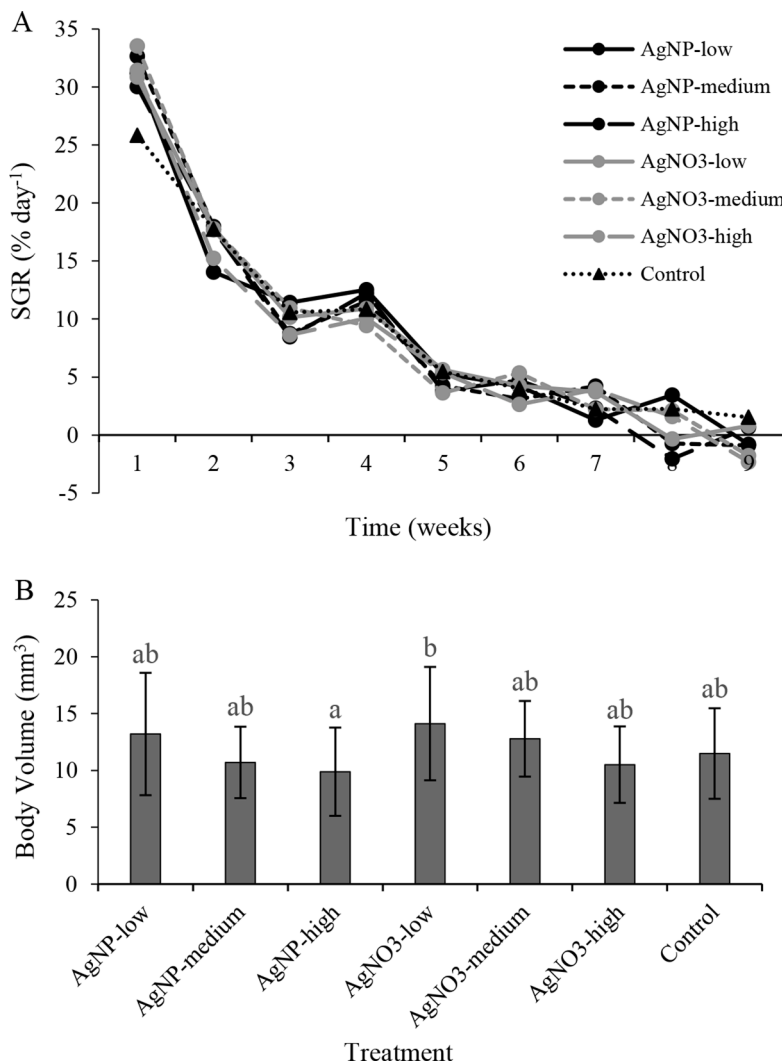


Fig. 1. A, Mean specific growth rates (SGR) of *C. teleta* during nine weeks of exposure to either un-coated silver nanoparticles (AgNPs) or silver nitrate (AgNO₃) added to sediment in nominal concentrations of 1 (low), 10 (medium) and 100 (high) µg Ag/g dry weight sediment or to clean sediment (control), calculated as % change in body volume relative to time (% day⁻¹), and B, *C. teleta* final body volume (mm³) after nine weeks of exposure (mean ± SD). Different letters on bars indicate significant ($p \leq 0.05$) difference between treatments.

AgNO₃-low, AgNO₃-medium, AgNO₃-high and Control. The concentration range was selected as it covers both the lower end of the analytical detection limit as well as exceeds the estimated environmental sediment concentrations. Sediment was spiked according to the USEPA method for Slurry Spiking (US Environmental Protection Agency, 2001) with slight modifications. Ag concentrations were measured with inductively coupled plasma mass spectrometry (ICP-MS) and AgNP characterization included measurement of hydrodynamic diameter, size distribution, zeta potential, particle size and morphology. Dynamic light scattering (DLS) measurements and images taken using transmission electron microscopy (TEM) revealed roughly spherical and relatively unstable AgNPs with a mean primary particle size of 74.1 nm (± 25.4 , $n = 66$). *C. teleta* juveniles were 8,9 days old when the experiment was initiated, and for ten weeks worms were monitored for survival, growth and reproductive output. In Supplementary Material more details on experimental setup, sediment characteristics (Table S1), Ag analysis (Table S2) and AgNP characterization (Table S3 and Fig. S1) are provided.

Measured sediment Ag start concentrations were lower than nominal (approximately 3.5 to 11 times), and Ag concentrations were higher in sediments spiked with AgNP than AgNO₃. The highest Ag concentrations were 28.8 and 16.2 µg Ag/g dw sed for sediment spiked with AgNPs and AgNO₃, respectively. One explanation of the lower Ag concentrations may be that Ag was lost to the extracted water upon sediment spiking, however, as Ag concentrations were not measured in overlying water or

porewater, this is uncertain. In Ramskov et al. (2015), the concentration of AgNPs and AgNO₃ was measured in both the sediment and the overlying water in a test system with *C. teleta*, and the results showed higher Ag concentrations in the overlying water in AgNO₃ treatments (25 ± 19 µg Ag/L) compared to AgNP treatments (< 3 µg Ag/L). Zeta potential of the AgNP suspension in this study prior to sediment spiking was between -30 and 30 mV, indicating unstable particles that likely settled out of suspension (by adsorption to sediment particles or forming aggregates/agglomerates) to a higher extent than AgNO₃. Despite the instability, TEM images revealed that at least some AgNPs were dispersed as single particles and therefore were probably available for uptake in *C. teleta* if adsorbed to sediment particles ingested by the worms.

After ten weeks of exposure to AgNPs and AgNO₃, there was no effect of any treatment on survival, and almost all worms survived to the end of the experiment. Only minor effects on growth were observed (Fig. 1A). After eight weeks, growth rates of worms from the AgNP-high treatment were significantly lower than worms in the AgNP-low, AgNO₃-medium and control treatments (Dwass-Steel-Christlow-Fligner, $p \leq 0.008$). After nine weeks, worm growth rates were significantly lower for worms in the AgNO₃-high treatment compared to worms in the control (Dwass-Steel-Christlow-Fligner, $p = 0.032$). Comparison of final body volumes of *C. teleta* after nine weeks of exposure (Fig. 1B) showed that body volumes of *C. teleta* were significantly lower in the AgNP-high treatment than the AgNO₃-low treatment

Table 1

Number of *C. teleta* pairs in each treatment, number and percentage of pairs that reproduced at least once during the experimental period (breeding pairs), time to first reproduction (TTFR), total number of brood tubes produced per pair, number of eggs in each brood tube and total number of eggs produced per pair for *C. teleta* pairs exposed to silver nanoparticles (AgNPs) and silver nitrate (AgNO₃) added to sediment in nominal concentrations of 1 (low), 10 (medium) and 100 (high) µg Ag/g dry weight sediment or to clean sediment (control) for ten weeks. TTFR, brood tubes per pair, eggs per brood tube and eggs per pair are given as mean ± SD.

	AgNP-low	AgNP-medium	AgNP-high	AgNO ₃ -low	AgNO ₃ -medium	AgNO ₃ -high	Control
Number of pairs	11	11	9	10	7	7	6
Number of breeding pairs (and percentage out of total number of pairs)	5 (45.5%)	6 (54.5%)	5 (55.6%)	5 (50.0%)	1 (14.3%)	4 (57.1%)	4 (66.7%)
TTFR (weeks after start of experiment)	6.4 ± 0.5	6.2 ± 0.4	7.2 ± 1.6	7.4 ± 1.2	8.0 ± 0.0	7.5 ± 1.7	6.8 ± 0.8
Brood tubes per pair	1.5 ± 1.8	2.1 ± 2.0	1.8 ± 1.9	1.2 ± 1.6	0.1 ± 0.3	2.0 ± 2.1	2.2 ± 2.0
Eggs per brood tube	201.4 ± 84.9	186.0 ± 50.4	159.5 ± 51.6	176.3 ± 88.0	455 ± 0.0*	149.5 ± 56.9	164 ± 48.5
Eggs per pair	311.2 ± 407.1	389.0 ± 369.2	283.6 ± 302.2	211.5 ± 249.2	65 ± 159.2	292.3 ± 312.8	355.3 ± 373.5

* Only one pair bred in this treatment and they produced only one brood tube, explaining the lack of variation.

(Tukey, $p = 0.014$).

All worms except for two reached sexual maturity during the third or fourth week of exposure. No significant differences were found in time to reach maturity among treatments (Kruskal-Wallis, $p = 0.742$) and mean maturation time calculated from all worms (in weeks of exposure) was 3.4 weeks (± 0.58 , $n = 158$). In all treatments there were cases of pairs that reproduced (breeding pairs) and pairs that did not. The highest percentage of breeding pairs was found in the control treatment with 66.6% (Table 1). Percentages of breeding pairs in AgNP-low, AgNO₃-low and AgNO₃-medium were found to be significantly lower than the percentage of breeding pairs in the control treatment (Chi-square, $p < 0.05$). There were no effects of AgNPs or AgNO₃ exposure on time to first reproduction, number of brood tubes or total numbers of eggs produced per pair. Visual observations of brood tubes, collected after 9 weeks of exposure, revealed close to 100 percent hatching success of eggs in all treatments. Our study showed a high within-treatment variation in reproductive endpoints, possibly caused by the circumstances under which the worms were paired and pairs were kept. In Ramskov and Forbes (2008) reproduction in *C. teleta* was investigated under different sediment organic matter (OM) concentrations. Under the highest OM concentration (3%), which is lower than the OM concentration in the present study, *C. teleta* started reproducing approximately 2,3 weeks earlier than in this study, and at least 80% of the females reproduced. In Ramskov and Forbes (2008) juveniles were kept in groups of 20 prior to pairing, whereas in the present study, juveniles were kept individually. The lack of presence of other worms during development until sexual maturation may explain the delayed reproductive output. The numbers of eggs in each brood tube in Ramskov and Forbes (2008) were approximately 220 (± 50) on average, which does not differ considerably from this study with an average number of eggs per brood tube of 177.10 (± 72.23).

As is evident from the results, *C. teleta* showed tolerance to both AgNP and AgNO₃ exposure with no effect on survival and only minimal effects on growth and reproduction during ten weeks of exposure. The effects did not show a clear concentration- or Ag type-dependent pattern, rendering the potential impacts of AgNPs and AgNO₃ at the concentrations tested here uncertain. In previous studies by Ramskov et al. (2015) and Dai (2012), *C. teleta* also demonstrated tolerance to sediment-associated Ag exposure in the form of AgNPs and AgNO₃. In both of these earlier studies, no effects were observed on survival or growth at Ag concentrations either higher than or overlapping with the concentrations used here. However, the studies used coated NPs and only considered short-term effects (two weeks of exposure). Since *C. teleta* is considered a pollution tolerant species, adapted to live in organically polluted areas, more studies investigating effects of

long-term exposure of AgNPs in other sediment-dwelling organisms are needed to improve our understanding of the environmental risks of these contaminants.

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CRediT authorship contribution statement

Maria Bille Nielsen: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft, Visualization. **Janna Vavra:** Methodology, Data curation, Writing – review & editing. **Annemette Palmqvist:** Conceptualization, Methodology, Formal analysis, Writing – review & editing. **Valery E. Forbes:** Conceptualization, Methodology, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.aquatox.2021.106046.

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