Investigations on the uptake pathway and accumulation of silver from manufactured silver nanoparticles in the freshwater amphipod *Hyalella azteca*

Sebastian Kühr[1], Jessica Klehm[2], Christian Schlechtriem[1]

[1]Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Schmallenberg, Germany

[2]Fraunhofer Institute for Microstructure of Materials and Systems IMWS, Halle (Saale), Germany

Contact: sebastian.kuehr@ime.fraunhofer.de

Introduction

Testing nanomaterials (NMs) under environmentally relevant conditions is an important aspect regarding the risk assessment of nanomaterials that enter the water cycle. Due to the fact, that sewage treatment plants (STPs) are the main pathway of NMs into the aquatic environment we developed a coupled test system using the effluents of model STPs in a chronic exposure test with the epibenthic amphipod *Hyalella azteca*, which is commonly used for ecotoxicity studies.
Previous studies with this test system (Kühr et al., 2018) showed that silver (Ag) from silver NMs (NM 300K) is accumulated by *H. azteca* exposed to model STP effluents. However, the pathway of Ag accumulation, via ingestion of particulate Ag and/or bioconcentration of dissolved ionic silver, is still unknown. To further elucidate the uptake pathway of silver from model STP effluent and sludge the following investigations were carried out.

Testsystem & Methods

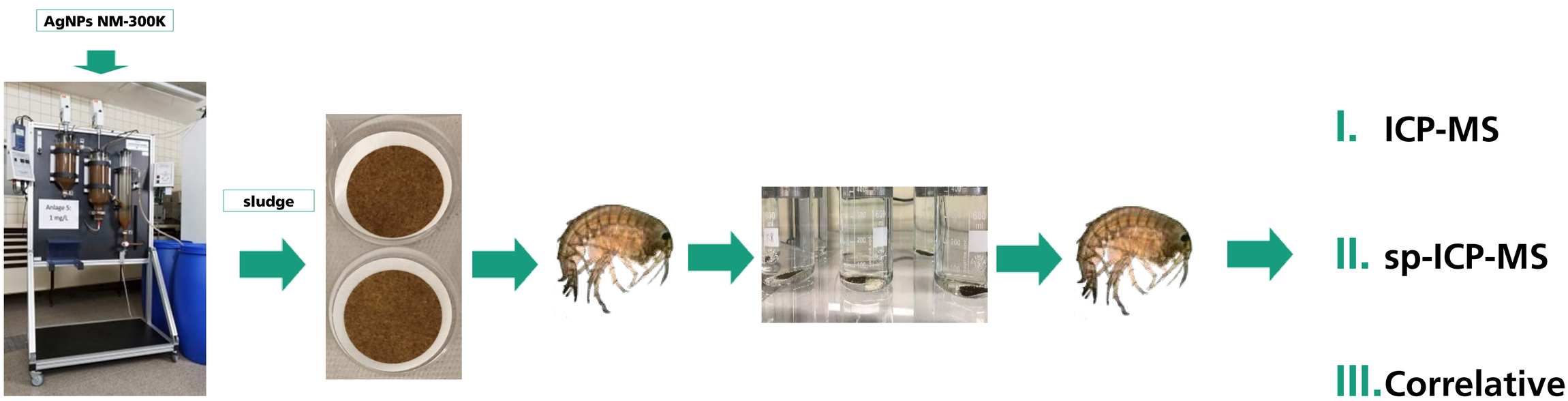

Fig. 1: Testsystem to investigate the uptake pathway and accumulation of Ag from AgNPs (NM 300K) in the freshwater amphipod *H. azteca*.

- Sludge from AgNP spiked STP was collected and loaded to glass fiber filters
- Five amphipods were kept in 500 ml glass beakers (n=5) filled with copper reduced tap water and exposed for a 7 (21) days period
- Animals were fed with AgNP contaminated sludge from model STP
- After the exposure period investigations on total Ag content of test media and animals (ICP-MS), were carried out. Sp- content in animal tissue was determined
- *H. azteca* individuals were also investigated by techniques of the correlative microscopy to get an additional overview of the particle amount and there localization in the animals tissue

Results

I. ICP-MS

	concentration	concentration factor	magnification factor
Contaminated water [mg/L]	0,03		
Ag contaminated sludge [mg/kg]	7245,18		
Treated animals [mg/kg]	201,36	6542	0,03

Tab. 1: ICP-MS measurement results from animals of both test groups and calculated concentration factors and magnfication factor (based on test media and sludge Ag concentration)

II. sp-ICP-MS

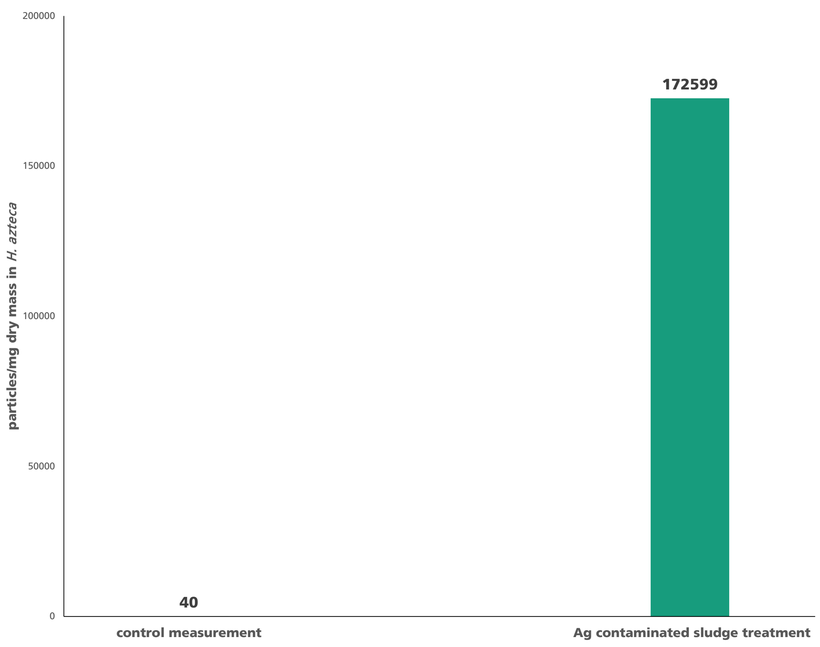

Fig. 2: sp-ICP-MS measurement results from animals (n= 15) of both test groups and uncontaminated control animals after enzymatic digestion

III. Correlative microscopy

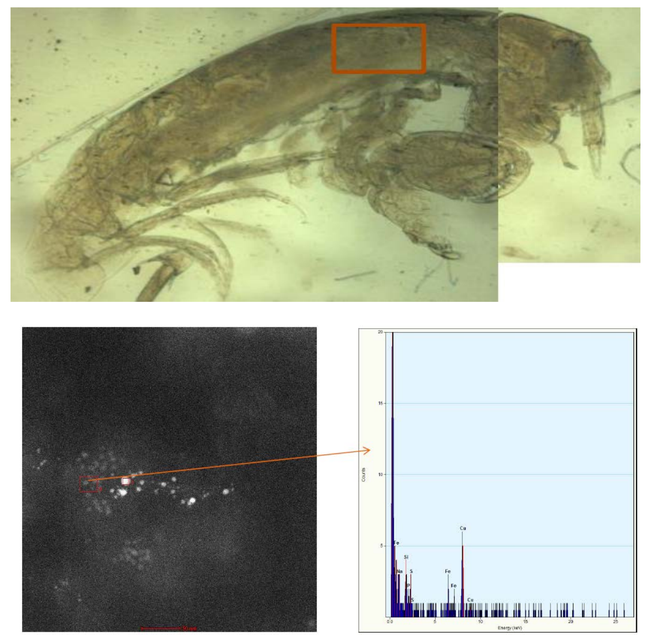

Fig. 3: First step light microscopic and final STEM / EDX image (example pictures with typical sample area)

Conclusions

- Animals with direct contact to the AgNP contaminated sludge show high tissue concentrations of silver.
- Calculated concentrations and magnification factors indicate a limited uptake of particulate silver.
- However examination of the animal tissue by sp-ICP-MS measurement show the presence of particulate Ag in the animals.
- Correlative microscopy showed that there was a limited amount of sulfidized AgNPs in the gastrointestinal tract.
- These results indicates that the main uptake path of Silver, that leads to the observed accumulation, are dissolved Ag-cations from the media.

References: S. Kühr, S. Schneider, B. Meisterjahn, K. Schlich, K. Hund-Rinke, and C. Schlechtriem, "Silver nanoparticles in sewage treatment plant effluents: chronic effects and accumulation of silver in the freshwater amphipod Hyalella azteca," Environ. Sci. Eur., vol. 30, no. 1, p. 7, Feb. 2018.

Session: The environment as a reactor determining fate and toxicity of nanomaterials (P)
Poster / Exhibition Hall, Monday May 14th, 2018, 08.30 a.m., ID: MO411

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[1]Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Auf dem Aberg 1, 57392 Schmallenberg, Germany

[2]Business Unit Biological and Macromolecular Materials, Fraunhofer Institute for Microstructure of Materials and Systems IMWS, Germany

E-mail contact: sebastian.kuehr@ime.fraunhofer.de

Keywords: correlative microscopy, nanomaterials, sewage treatment plant effluent, bioaccumulation

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Previous studies with this test system showed that silver (Ag) from silver NMs is accumulated by *H. azteca* exposed to model STP effluents. However, the pathway of Ag accumulation, via ingestion of particulate Ag and/or bioconcentration of dissolved ionic silver, is still unknown. To further elucidate the uptake pathway of silver from model STP effluent, two groups of *H. azteca* with five animals each were placed in a single test vessel. The two groups were separated by a stainless-steel strainer. One group was fed contaminated sludge from model STPs, loaded on glass fibre filters. The second group, located in the stainless-steel strainer, was fed uncontaminated control STP sludge and had no direct contact to the test sludge containing Ag NMs. The study was carried out with five replicated test vials with two groups of amphipods each. Water samples were taken within the strainers to measure the silver content in the media and to prove that the animals fed control sludge were not in contact with Ag NMs potentially released from the contaminated sludge.

After an exposure period of 7 (21) days Ag content of the water and animal samples collected at the end of the exposure period was measured by ICP-MS or ICP-OES to determine the accumulation of Ag in both groups. The presence of NMs in the animals was examined by high-resolution transmission electron microscopy (TEM) and methods of correlative microscopy. The derived accumulation factors and the results of the TEM investigations allow to evaluate the contribution of particulate and dissolved ionic Ag to the accumulation of Ag from STP effluent.