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Assessment of bioavailability of engineered nanomaterials by single particle ICP-MS and amphipod haemolymph isolation

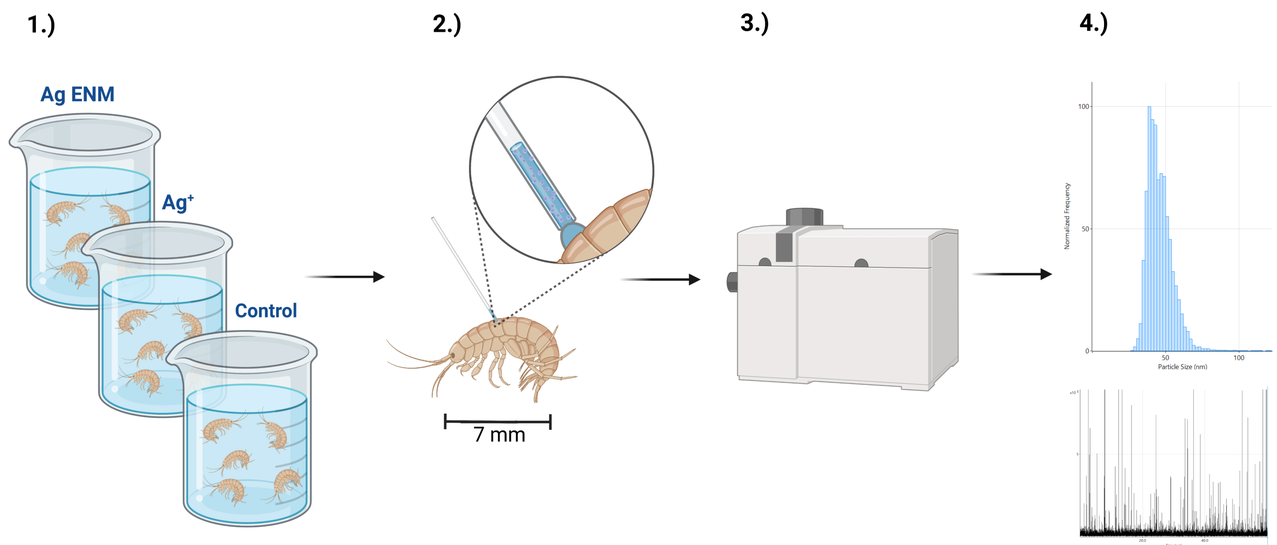
Objective

Bioaccumulation tests with the benthic freshwater amphipod ***Hyalella azteca*** (**HYBIT**) are currently discussed as part of a tiered approach to determine the bioaccumulation potential of **engineered nanomaterials** (**ENMs**) [1 & 2]. This may allow to avoid further vertebrate tests for regulatory bioaccumulation assessment, such as the fish bioaccumulation test (OECD TG 305 [3]). However, the small size of the amphipod, does not allow to distinguish ENMs present in their intestinal content from the bioavailable fraction (ENM in the tissue or body fluids). Even if a concept of a tiered assessment scheme based on **ENM-HYBIT** that takes this ambiguity into account with adjusted endpoints exists [1], methods to gain further data on the **bioavailability of ENMs** are required. Existing methods for the localization of tissue-incorporated ENMs (e.g. correlative microscopy or micro X-ray fluorescence imaging) require sophisticated time- or cost-intensive analytical methods, very high exposure and body burden concentrations. Thus, a microcapillary based simple method that can be used in any laboratory was developed isolating the **haemolymph** of exposed **amphipods** to be analyzed for the presence of ENMs.

Methods

1. Exposure of *H. azteca* for 7 days in culture media spiked with Ag ENM, or Ag^+ and non-spiked culture media. Concentration (7 µg/L) and duration (7 days) required for steady-state conditions are based on previous results [2].

2. Isolation of haemolymph from exposed animals with tapered 5 µl capillary using a microscope. (n = 3, one sample pooled from 20 animals)



3. Measurement of reference material, pristine Ag ENMs, exposure media and diluted isolated haemolymph pooled from 20 animals with single particle inductively coupled plasma mass-spectrometry (spICP-MS)

4. Data processing with single particle software module to calculate the mean particle sizes of detected and measured ENMs.

Fig. 1: Schematic overview of the experiment including exposure of *H. azteca*, extraction of haemolyph and analysis using spICP-MS.

Results and Conclusion

- Ag particles were detected in haemolymph after 7 days of exposure with Ag ENMs and could be measured for size by spICP-MS. (Fig. 2 A - D)

- The detected Ag particles (33.7 ± 5.5 nm) showed a strong comparability regarding mean particle size and size distribution with the exposed Ag ENMs (36 nm). (Fig. 2 B & C) The decreased size may be the result of dissolution effects.

- Strong signals were gained during measurement of haemolymph from Ag^+ exposure resulting in a calculated mean particle size of 57.7 ± 5.5 nm. (Fig. 2 D)

- The measured particles from the Ag^+ exposure group can be secondary particles formed by precipitation of Ag from Ag^+ in the media or within the organisms as observed before [4 - 6].

- The coupled method represents an easy, inexpensive and valuable method for ENM bioavailability and tissue translocation assessment that can support data interpretation from ENM-HYBIT for regulatory bioaccumulation assessment.

- Au particles could also be isolated from the hemolymph after exposure to Au ENMs. The isolated particles must therefore be the exposed ENMs and cannot be secondary particles formed from an ionic fraction. (Fig. 2 E & F; exposure only with Au ENMs - no ions)

- Comparison of spICP-MS measurement results from exposure media and heamolymph can be used to identify secondary particles. (Fig. 2 B, C & D and Fig. 2 E & F)

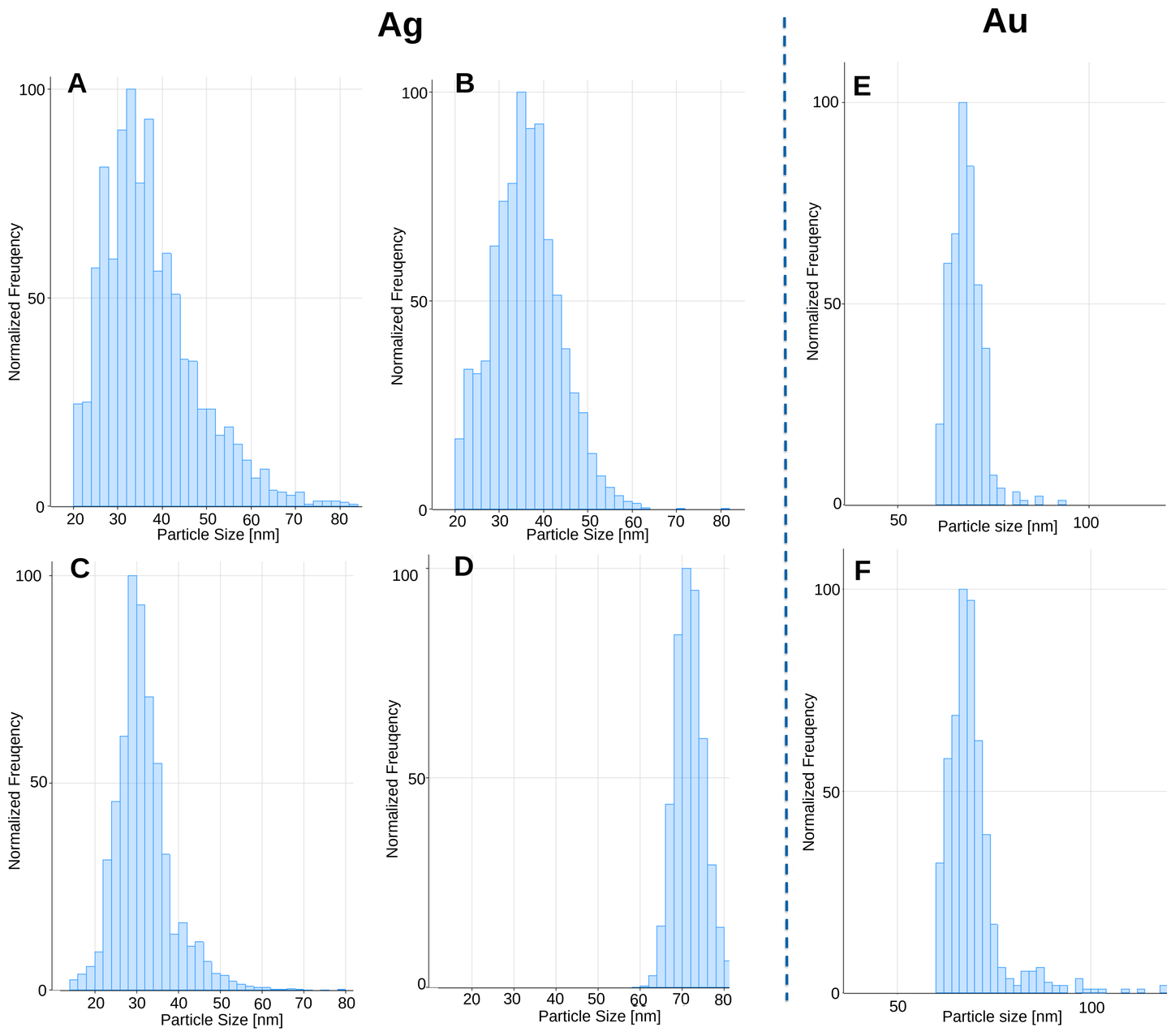


Fig. 2: Results of the spICP-MS measurement: Relative particle size distribution in nm. A: Ag ENMs in MQ water; B: Ag ENMs in exposure media; C: Haemolymph after Ag ENMs exposure; D: Haemolymph after Ag^+ exposure; E: Au ENMs in MQ water; F: Haemolymph after Au ENMs exposure

References

[1] Kuehr et al. 2021, Environmental Science Europe, 33, 9; [2] Kuehr et al. 2021, Chemosphere, 263, 127961; [3] OECD 2012, Guidelines for the Testing of Chemicals, Section 3; [4] Georgantzopoulou et al. 2020, Environmental Science & Technology, 54, 19, 12316-12325; [5] Clark et al. 2021, Environmental Science Nano, 8, 1642; [6] Baccaro et al. 2018, Environmental Science Nano, 5, 1107-1116

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