Development of Microscopic and Spectroscopic Techniques for the Detection and Characterization of Manufactured Nanomaterials in Aquatic Samples

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Due to their increased application in various fields, the environment is at least indirectly exposed to manufactured nanomaterials (MNMs). Among those, silver (Ag) and titanium dioxide (TiO_2) nanoparticles (NPs) represent two examples, which are used for commercial purposes in high volume outputs and are hence very widespread.[1] However, their end-of-life impact on the environment remains, as for many other materials, unclear. MNMs are typically not found in their pristine state, but are subject to physico-chemical changes like oxidation or the adsorption of biomolecules, especially during wastewater treatment.[2] This alteration in NP properties may lead to an increase in bioavailability and toxicity for water organisms.[3] In the framework of FENOMENO we investigate the fate of pristine as well as wastewater-borne Ag (NM-300K) and TiO_2 (NM-105) NPs after exposure to relevant aquatic species (algae, *Daphnia*, fish eggs, fish) in order to validate and optimize microscopic methods for the detection and characterization of MNMs. Among those methods, Darkfield Optical Microscopy has proven to be a valuable technique for rapid overview scans of thin tissue sections due to intense light scattering effects of small particles. This first and crucial step is followed by more detailed investigations including Scanning Electron Microscopy (SEM) in combination with Energy-dispersive X-ray Spectroscopy (EDX) to determine the elemental composition. Laboratory studies are compared with field samples from Lake Mondsee, Austria. Additionally, artificial wastewater effluents containing Ag NPs are under investigation to study the chemical transformation during wastewater treatment. The major challenge here is to separate and concentrate nanoparticles from their corresponding matrix. A commonly used method in this context is the so-called Cloud Point Extraction (CPE) in which Ag NPs are encapsulated in micelles and hence removed from their aqueous environment via phase separation.[4]

Darkfield Optical Microscopy of … …*Daphnia magna*

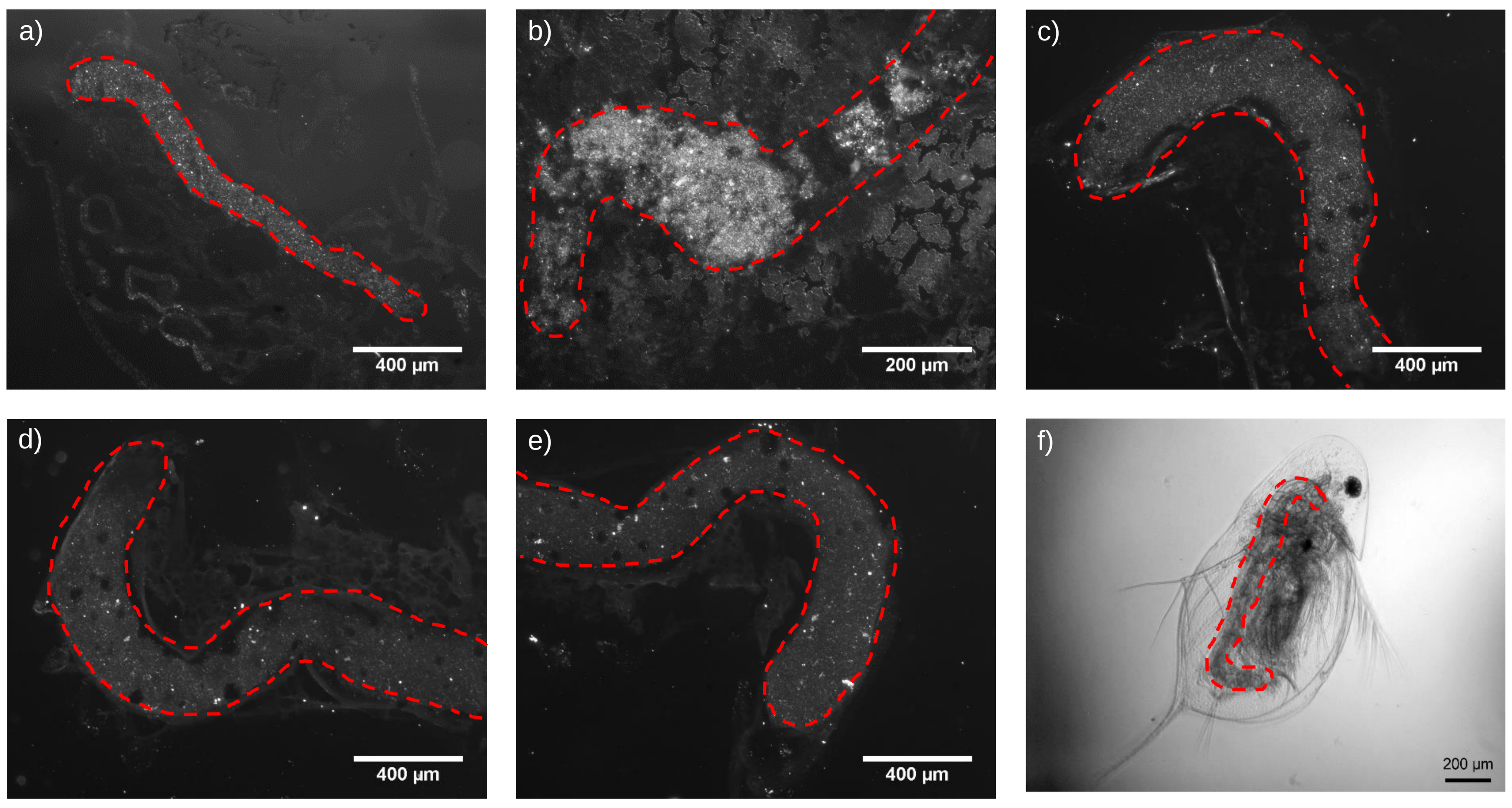

Figure 1. Daphnia exposed to MNMs: (a) 75 µg/L Ag NPs (96 h), (b) 200 µg/L TiO_2 (21d). Daphnia exposed to Ag-contaminated algae: (c) dispersant control, (d) 1.25 µg/L, (e) 5 µg/L. (f) Brightfield image of whole daphnia for orientation. Dashed lines represent gut region.

SEM-EDX Analysis of daphnia gut

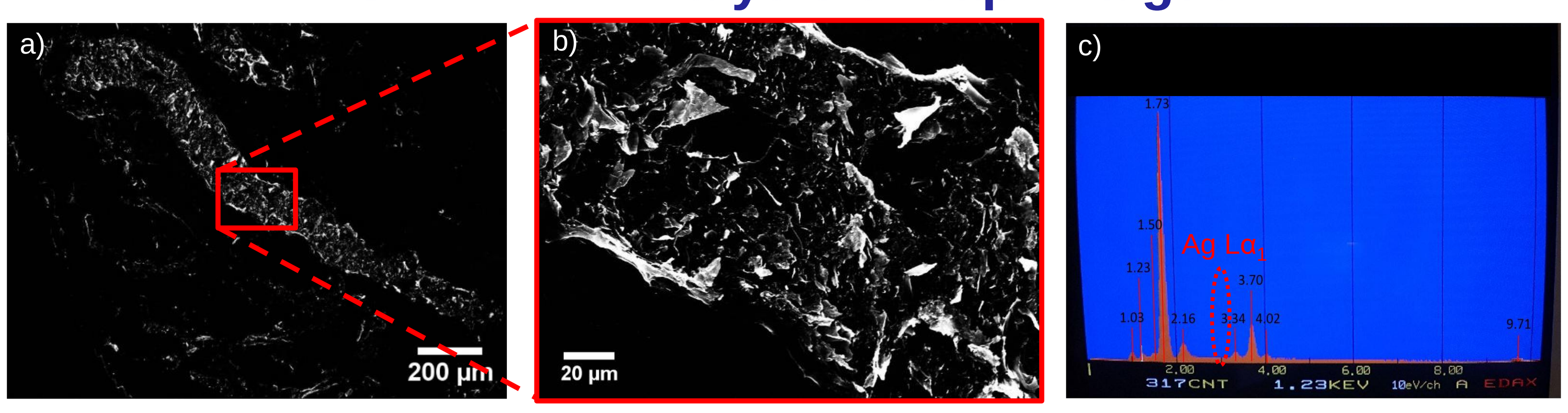

Figure 4. (a) SEM image of the daphnia section shown in Figure 1a. (b) Close-up of daphnia gut and region of interest for EDX analysis with corresponding EDX spectrum (c). The strongest and most characteristic silver peak at 2.98 keV is missing, indicating either the absence of Ag NPs or a signal below the limit of detection. Due to the high interaction volume, a very weak silver signal is to be expected but parameters such as acceleration voltage and acquisition time have to be adjusted.

NP characterization via TEM

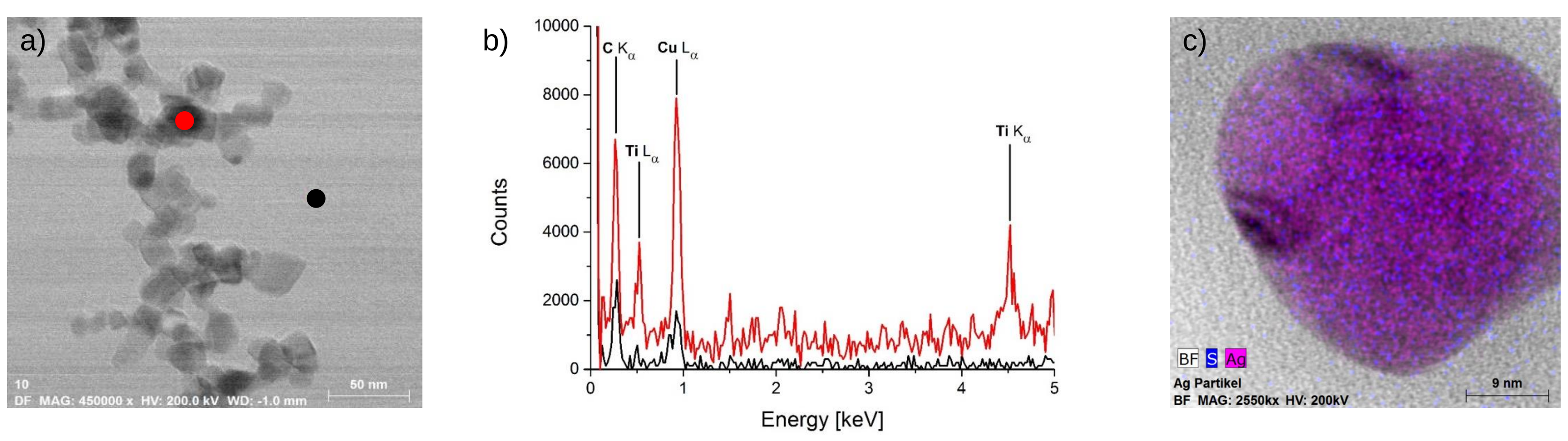

Figure 6. (a) TEM image of TiO_2 NPs with corresponding (b) EDX spectrum. (c) TEM image merged with EDX mapping of Ag NPs.

…fish tissue from lab studies

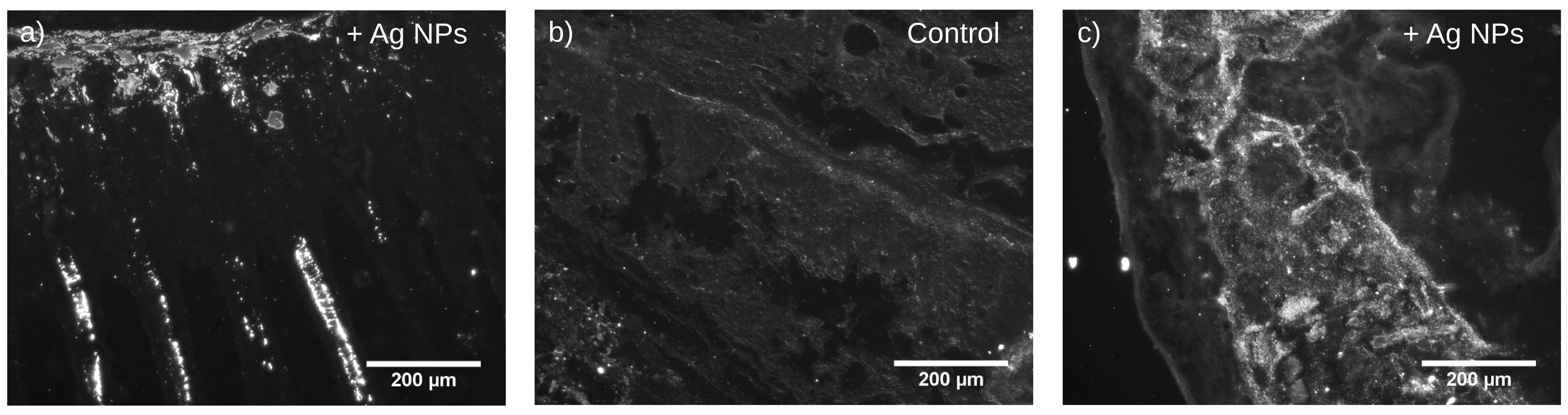

Figure 2. (a) Sections of gill tissue and (b+c) intestine tissue of *Coregonus lavaretus* test specimen exposed to Ag NPs (45 µg/L, 7 days). Bright scattering signals along the gill filaments as well as an increased signal for intestine tissue compared to its control indicate the accumulation of Ag NPs in those organs. Other investigated organs (liver, stomach, kidney, brain and muscle) did not show comparable results.

…fish tissue from field studies

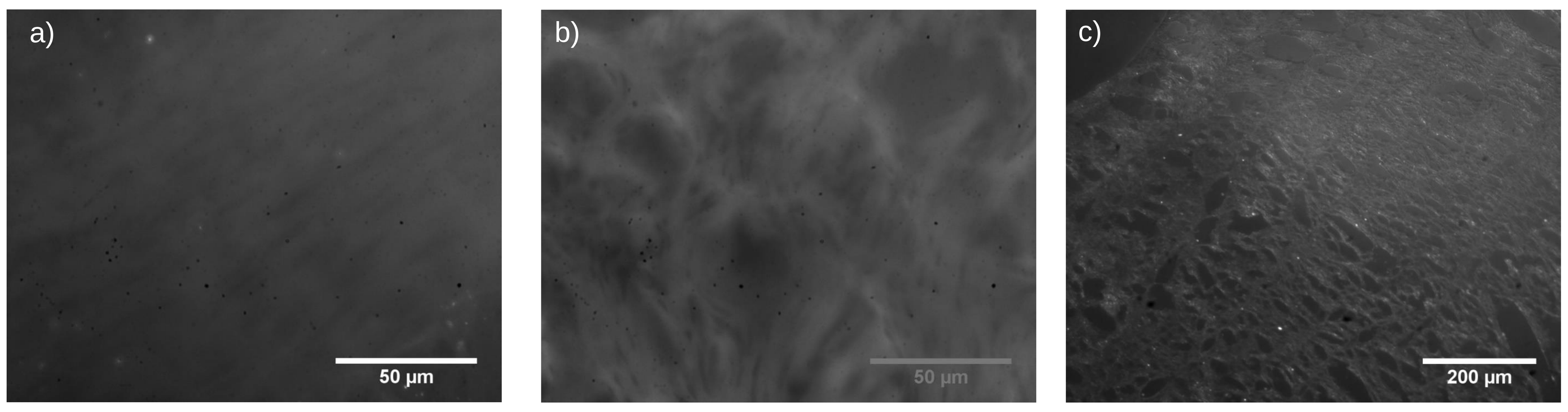

Figure 3. *Coregonus lavaretus* tissue sections from Lake Mondsee ((a) intestine, (b) stomach) and a reference lake ((c) intestine). There is no visible evidence for the presence of nanoparticles.

Cloud Point Extraction of artificial wastewater effluents

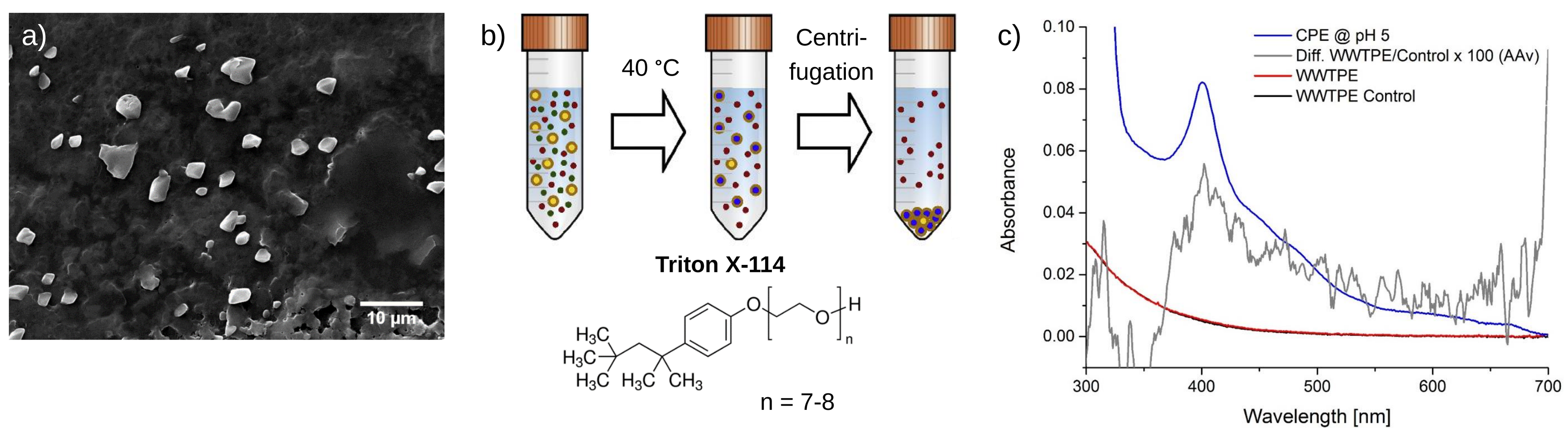

Figure 5. (a) SEM image of inorganic residues from an artificial wastewater effluent containing Ag NPs. (b) Principle of Cloud Point Extraction: Upon heating above their cloud point, TX-114 micelles form around Ag NPs at an acidic pH which are separated from the surrounding matrix via centrifugation. (c) UV-Vis spectra of wastewater effluents (WWTPE) before and after Cloud Point Extraction. The signal at ~ 410 nm corresponds to the SPR peak of Ag NPs.

Sample	Concentration [µg/L]	Extraction efficiency [%]
WWTPE control	15.1 ± 0.1	
WWTPE control (CPE)	17.5 ± 0.3	1 ± 0
WWTPE	224.1 ± 23.7	
WWTPE (CPE)	19089.1 ± 1416.1	106 ± 8
Ag ions	26.0 ± 0.1	
Ag ions (CPE)	21.5 ± 0.9	1 ± 0

Table 1. Extraction efficiencies for Ag NPs containing wastewater effluents as well as Ag ions as determined by ICP-MS. Cloud Point Extraction is an efficient and Ag(0)-selective method.

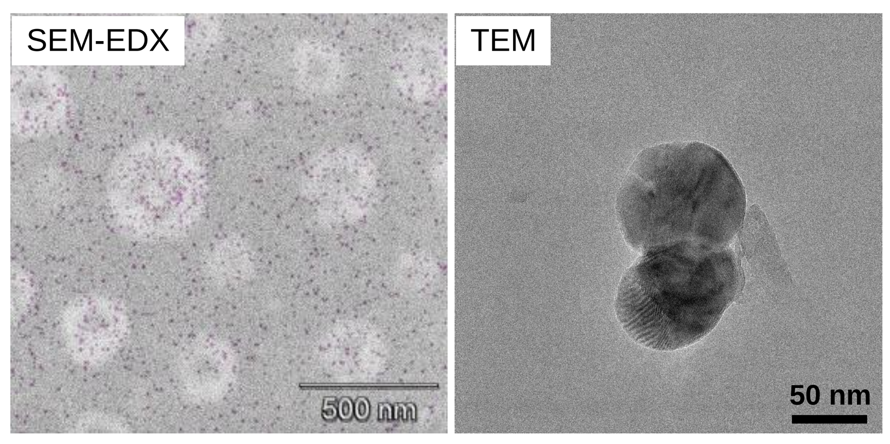

Figure 7. Electron Microscopy images of encapsulated Ag NPs (left, spots represent the Ag EDX signal) and Ag NPs after the removal of micelles using UV/Ozone treatment.

Acknowledgements

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http://www.fenomeno-nano.de/

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Conclusions

Darkfield Optical Microscopy is an essential tool to enable quick overview scans of tissue sections from aquatic specimens concerning the potential presence of nanoparticles. In this context, there is no clear indication for NP uptake in fish tissue from field studies at the Lake Mondsee into which Ag NPs might be discharged via a wastewater treatment plant. This could either mean the absence of NPs throughout the lake or the occurrence of processes that prevent bioaccumulation in living organisms, such as heteroaggregation or premature dissolution. In contrast, there is a strong indication of NP accumulation in the gills or the intestinal tract of test organisms that have been exposed to nanoparticles in the lab. However, the ultimate proof of those light scattering signals as seen by Darkfield Optical Microscopy originating from NPs has yet to be brought forward. In addition, Cloud Point Extraction has been shown to be an effective and specific method for the isolation and preconcentration of silver nanoparticles from complex aqueous matrices such as wastewater effluents.

References

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