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Platinum nanoparticle toxicity in freshwater algae and crustaceans: A physical or chemical effect?

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1. Introduction

Although the aquatic fate and toxicity of various carbon and metal nanoparticles has been studied intensively in recent years, only very few studies have focused on the effects of platinum nanoparticles (PtNPs) to aquatic organisms. From an environmental exposure perspective, PtNPs are highly relevant due to their extensive use in automobile catalytic converters and the findings of particulate matter containing platinum (Pt) alongside roadways [1]. The possible emissions of PtNPs to natural waters, e.g. by transport with storm-water runoff, raises the question of the potential environmental effects associated herewith.

The aims of this study was to: 1) Determine the toxicity of PtNPs towards freshwater algae and crustaceans, 2) Investigate if the observed responses are results of physical effects such as obstruction of light in algal tests and mechanical fixation of organisms in daphnia tests, rather than a chemically induced toxicity of PtNPs and 3) Propose alternative test setups to distinguish the pure physical effects from chemical toxicity.

The hypothesis is that part of the observed response in aquatic toxicity testing of NPs arises from physical NP effects that are not related to their chemical composition or nanosize. Ultimately, we aim at distinguishing between the two types of effects in order to better understand the toxicity related to PtNPs.

2. Materials and methods

Test materials were starch stabilized PtNPs of nominal size 2 ± 0.2 nm and PtCl4 as a dissolved Pt reference. Characterization of PtNPs included ICP-OES, DLS and TEM.

Toxicity towards *D. magna* was assessed in a standard 48h acute immobilisation tests according to OECD 202. In addition, 48h mobility and lethality was assessed by use of a double-beaker test setup, where a net separated the daphnia neonates from direct contact with any larger, agglomerated, aggregated and sedimented PtNPs, see figure 1. Concentration-response curves and EC/LC-values were established using TOXCALC®.

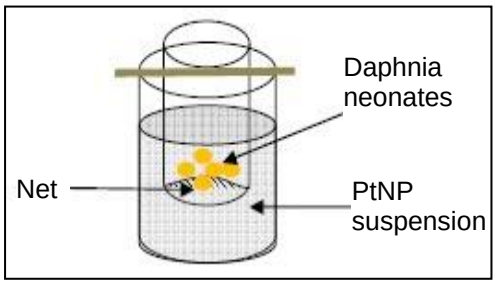

Figure 1: Illustration of double-beaker test setup.

The freshwater green algae *P. subcapitata* were used as test organism in a standard growth inhibition test (ISO 8692:2004) with 48h incubation and a short-term (2h) test. In the standard test, growth rates were determined from fluorometric quantification of extracted algal pigments after 0, 24 and 48 hours, whereas the toxic endpoint of the 2h test is assimilation of ^{14}C during photosynthesis. Briefly, ^{14}C-labelled bicarbonate was added at test start and after 2h incubation the ^{14}C-assimilation into algal biomass was determined by liquid scintillation counting. Concentration-response curves and corresponding EC-values were established using the LOG457 statistical program, assuming logarithmic-normal distribution of data and fitting curves by nonlinear regression analysis [2].

3. Results and discussion

The EC_{50}-values established for PtNPs in algal and daphnia standard tests were in the range 10-100 mg Pt/L and as Pt is a non-degradable element, this leads to a classification of PtNPs as “Harmful to aquatic life with long lasting effects” in accordance with CLP [3]. In comparison, $PtCl_4$ was more toxic with EC_{50}-values, roughly 10 times lover. Considering the great exposure release potential of PtNPs, additional hazard identification and assessment is crucial.

In the standard algal test, the algal pigments are quantified by fluorometry. The presence of NPs may possibly disturb this process [4], especially PtNPs as they are very darkly colored. Thus, the observed response may be due to physical interference of the PtNPs with the fluorometry, rather than a toxic response in the algae.

The 2h algal ^{14}C-asisimliation test employed in this study relies on scintillation counting and is not influenced by the presence of NPs. From this test, an EC_{50} of 28 mg Pt/L were obtained, supporting the result of the 48h test and indicating that a toxic response is caused by PtNPs (see figure 2). The NPs may also obstruct light during exposure and thereby influence the observed response in the two tests. This should be investigated further, although the 48h algal growth rate inhibition test has proved applicable even for the testing of very darkly colored suspensions [5].

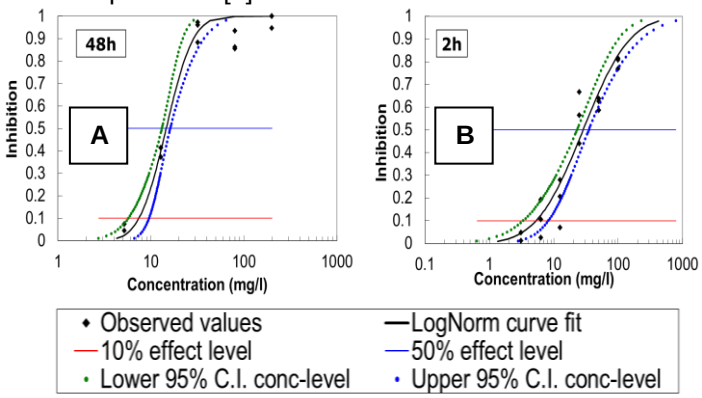

Figure 2: Concentration-response curves for algae exposed to PtNPs in a) 48h growth rate inhibition test and b) 2h ^{14}C-assimilation inhibition test. Responses are given as relative inhibition to control and the concentration as mg Pt/L.

In the daphnia immobilization test, substantial adhesion of PtNPs to the exterior of the organisms was observed. Apparently, this resulted in immobilization of the organisms due to a physical "fixation" rather than a toxic action of the PtNPs. Consequently, both immobilization and lethality was registered, leading to EC_{50} and LC_{50} values of 17 and 26 mg Pt/L respectively. When exposing the daphnia neonates in the double-beaker setup (figure 1), the resulting EC_{50} and LC_{50} values were 31 and 34 mg Pt/L respectively. While the effective concentration based on immobilization increased two-fold when the physical response is overlooked, interestingly, the lethal concentration roughly remained the same in the two test setups. This indicates that lethality is a more robust and accurate endpoint for NP toxicity testing in *D. magna*. The use of a double-beaker system enables observations of both immobilization and lethality in the daphnia tests, as the animals can be difficult to identify and observe in very dark NP suspensions such as PtNPs.

4. Conclusions

The tested PtNPs were found to be harmful to aquatic life, with 48h EC_{50} values from standard tests with algae and daphnia of 14 and 17 mg Pt/L respectively. Considering this toxicity along with the extensive exposure potential of PtNPs, additional hazard and risk assessment is crucial.

The inhibition of growth rate and carbon assimilation in the algal tests with PtNPs is likely due to a toxic response and not a mere result of physical shading effects during exposure and analysis. In the daphnia test, PtNPs adhere to the animals exterior, thus increasing immobilization, but not lethality.

A 2h algal ^{14}C-assimilation test is proposed to avoid shading effects of NPs in the spectrophotometry step. For daphnia toxicity testing of NPs, a double-beaker setup enables the distinction between mere physical immobilization and a real toxic response. Lethality was found to be a more robust and appropriate endpoint, than immobilization in *D. magna*.

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