

Toxicity and Accumulation of Cu and ZnO Nanoparticles in *Daphnia magna*

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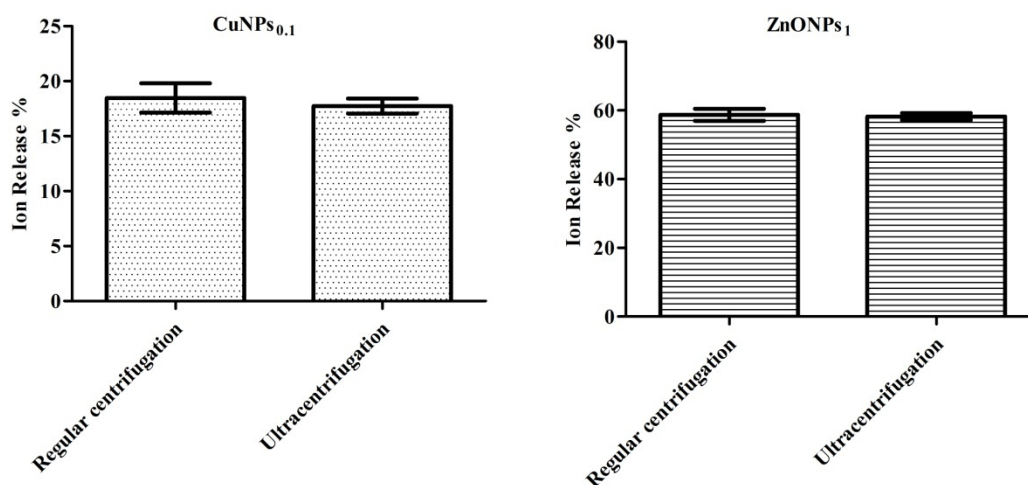
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SUPPORTING INFORMATION

Supporting information 1

To analyze whether there were still NP_(particle) remaining in the supernatants after centrifugation, we firstly conducted an ultracentrifugation experiment. Specifically, ultracentrifugation of nanoparticle suspensions was performed at 192700 g for 30 min at 4 °C (Kontron Centrikon T-2070, TFT 50.38 rotor). Subsequently, the dissolved ion concentration in the supernatants prepared by the two methods of centrifugation was compared. If there was no significant difference of dissolved ion concentration in the supernatants obtained by regular centrifugation and ultracentrifugation, it was to be concluded that regular centrifugation can be applied in this study and NP_(particle) may be completely removed in the supernatant after centrifugation, on the other hand if there was a significant difference, indeed NP_(particle) would be remaining in the supernatants after regular centrifugation and ultracentrifugation should replace regular centrifugation to be employed in this study. After comparing, it was found that there was no significant difference between the ion concentration in the supernatants disposed by the two different centrifugation methods (Figure S1), which means that the supernatants obtained by centrifugation at around 30000 g and subsequent filtration through a syringe filter with 0.02 µm pore diameter,

may only contain $\text{NP}_{(\text{ion})}$. Furthermore, besides comparing the ion concentration by means of ICP-OES in the supernatants after regular centrifugation and ultracentrifugation, DLS was used to confirm $\text{NP}_{(\text{particle})}$ were removed in the supernatants after filtration. From Figure S2, it is clear that the particle profile of the cultural media (STM) was similar to the profiles of the supernatants of CuNPs and ZnONPs. Moreover, the polydispersity indexes (PDI) of the supernatants of the STM, CuNPs and ZnONPs were very high (higher than the recommended PDI values for the DLS measurement: 0-0.7) and the count rates of them were very low (lower than the recommended count rates for the DLS measurement: 100-500 kcps), which did not meet the quality criteria of the DLS test. These results indicate that the particle concentration in the supernatants obtained by centrifugation at around 30000 g and subsequent filtration through a syringe filter with 0.02 μm pore diameter was less than the combined detection limit of the ICP-OES and DLS of 0.01 mg/L.



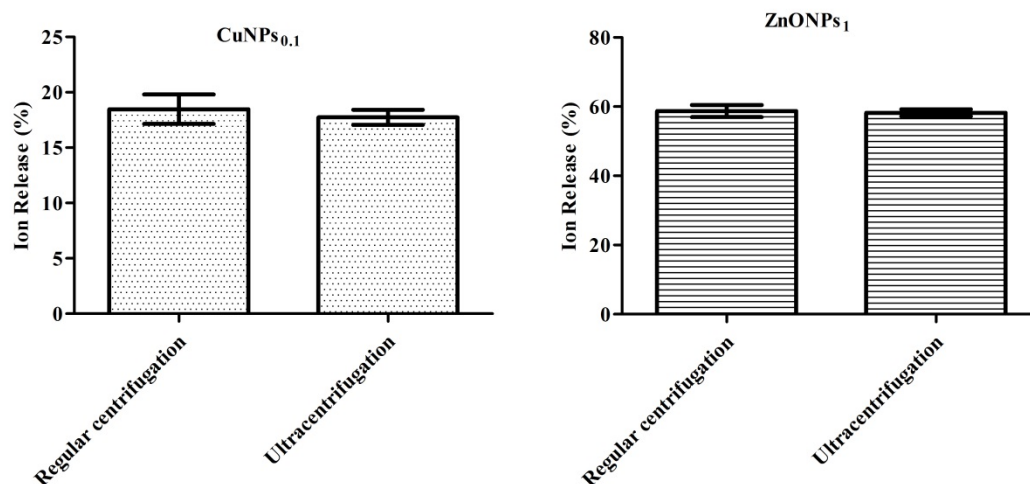


Figure S1. The comparison of the ion release profiles in the supernatants obtained by regular centrifugation for 30 min at 4 °C with 30392 g and ultracentrifugation for 30 min at 4 °C with 192700 g, respectively. The left graph exhibits the relative percentage of dissolved Cu released from the CuNPs at the concentration of 0.1 mg/L and the right graph displays the relative percentage of dissolved Zn released from the ZnONPs at the concentration of 1 mg/L. Results are expressed as mean $\pm$ SD ($n = 3$).

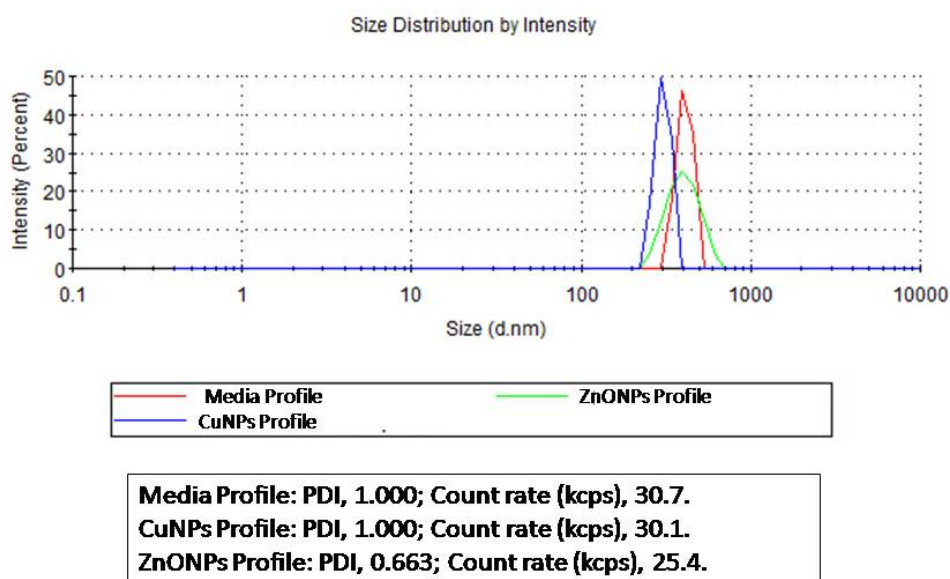


Figure S2. Dynamic light scattering data for particle profiles of the supernatants of STM, CuNPs and ZnONPs. As control, the particle profile of the supernatant of the cultural media (STM) obtained by centrifugation at 30392 g and subsequent filtration through a syringe filter with 0.02 μm pore diameter has been measured first, which was shown in the figure with red line. Then the particle profiles of the supernatants of CuNPs and ZnONPs also obtained by centrifugation at 30392 g and subsequent filtration through a syringe filter with 0.02 μm pore diameter were represented by blue and green lines, respectively.

Supporting Information 2

During the procedures of centrifugation and filtration, NPs and $\text{NP}_{(\text{ion})}$ may be adsorbed to the sidewalls of centrifuge tubes or on the filter membrane. To analyze the losses of NPs and $\text{NP}_{(\text{ion})}$ after centrifugation and filtration, experiments were conducted. Specifically, freshly prepared

nanoparticle suspensions were sampled and the nanoparticle concentration in the samples was detected by ICP-OES after digestion in 65% HNO₃ for at least 1 day. Moreover, to compare the difference of nanoparticle concentration before and after centrifugation, the nanoparticle concentration after centrifugation at 30392 g for 30 min at 4 °C was also tested by ICP-OES after digestion in 65% HNO₃. The difference of NP_(ion) concentration before and after filtration was also detected. After centrifugation at 30392 g for 30 min at 4 °C, Concentration of NP_(ion) in the supernatant was detected by ICP-OES. Furthermore, supernatants obtained by centrifugation at 30392 g for 30 min at 4 °C were filtered through a syringe filter with 0.02 µm pore diameter (Antop 25, Whatman). Subsequently, the concentration of NP_(ion) in the supernatants obtained by centrifugation and subsequent filtration through a syringe filter with 0.02 µm pore diameter was tested. The results were shown in Figure S3. It reported that there indeed exist decrease of nanoparticle concentration after centrifugation and decrease of dissolved ion concentration after filtration. However, the difference of the ZnONPs concentration before and after centrifugation and of the dissolved Zn concentration before and after filtration, as well as of the CuNPs concentration before and after centrifugation and of the dissolved Cu concentration before and after filtration was not significantly different ($p > 0.05$, the p -value between different groups was tested by mean of the t - test).

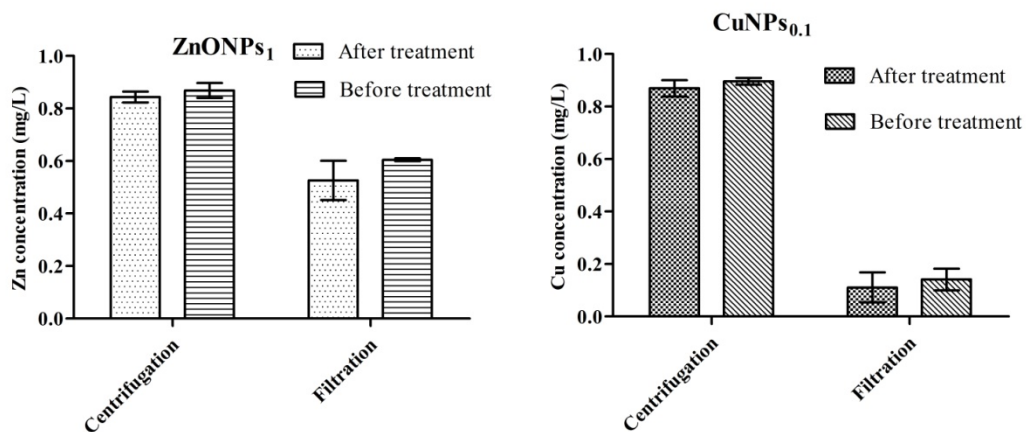


Figure S3. The losses of NPs and NP_(ion) after centrifugation and filtration. The left graph shows the concentration of ZnONPs at 1 mg/L before and after centrifugation and the concentration of dissolved Zn released from ZnONPs at 1 mg/L before and after filtration. The right graph exhibits the concentration of CuNPs at 0.1 mg/L before and after centrifugation and the concentration of dissolved Cu released from CuNPs at 0.1 mg/L before and after filtration. Results are expressed as mean $\pm$ SD ($n = 3$).