

Combined Effects of Test Media and Dietary Algae on the Toxicity of CuO and ZnO Nanoparticles to Freshwater Microcrustaceans *Daphnia magna* and *Heterocypris incongruens*: Food for Thought

Marge Muna ^{1,2,*}, Irina Blinova ¹, Anne Kahru ^{1,3}, Ivana Vinković Vrček ⁴, Barbara Pem ⁴, Kaja Orupõld ⁵ and Margit Heinlaan ^{1,*}

¹ Laboratory of Environmental Toxicology, National Institute of Chemical Physics and Biophysics, Akadeemia tee 23, Tallinn 12618, Estonia; irina.blinova@kbfi.ee (I.B.); anne.kahru@kbfi.ee (A.K.)

² Department of Materials and Environmental Technology, Tallinn University of Technology, Ehitajate tee 5, Tallinn 19086, Estonia

³ Estonian Academy of Sciences, Kohtu 6, Tallinn 10130, Estonia

⁴ Institute for Medical Research and Occupational Health, Ksaverska cesta 2, Zagreb 10001, Croatia; ivinkovic@imi.hr (I.V.V.); bpem@imi.hr (B.P.)

⁵ Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Fr.R. Kreutzwaldi 5, Tartu 51006, Estonia; kaja.orupold@emu.ee

* Correspondence: marge.muna@kbfi.ee (M.M.); margit.heinlaan@kbfi.ee (M.H.); Tel.: +372-639-8361 (M.M. & M.H.)

Table S1. Characterisation of CuO and ZnO NP suspensions at 0 h, 48 h, and 6 days in five different test media. Nominal concentration was 10 mg metal/L. The mean (SD) of 3 technical replicates of one experiment is given. NP suspension may be characterised as highly unstable if ζ values are in range of ± 0 –10 mV; relatively stable for ± 10 –20 mV; moderately stable for ± 20 –30 mV, and highly stable for values $> \pm 30$ mV [40].

| Time (h) | | MQ | | | AFW | | | MHW | | | Lake Raku | | | Lake Ülemiste | | |
|----------|---------------------|-----------------|-----------------|------------------|-----------------|-----------------|----------------|-----------------|----------------|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|------------------|
| | | 0 | 48 | 144 | 0 | 48 | 144 | 0 | 48 | 144 | 0 | 48 | 144 | 0 | 48 | 144 |
| CuO | D _h (nm) | 207 (14) | 236 (8.9) | 198 (3.9) | 573 (41) | 1826 * | 2142 * | 565 (41) | 1638 * | 2645 * | 197 | 393 | 449 | 335 | 440 | 452 (6.5) |
| | ζ (mV) | -15 (0.15) | 12 (1.3) | 23 (0.46) | -7.3 (2.0) | -4.6 | -3.5 | -13 (0.7) | -8.4 | -13 | -19 | -18 | -17 | -18 | -17 | -17 (0.55) |
| | pdi | 0.17 (0.062) | 0.21 (0.015) | 0.24 (0.0040) | 0.22 (0.045) | 0.78 (0.023) | 0.81 (0.12) | 0.24 (0.015) | 0.77 (0.23) | 1 (0.0) | 0.19 (0.010) | 0.28 (0.016) | 0.35 (0.017) | 0.25 (0.016) | 0.26 (0.018) | 0.35 (0.0065) |
| ZnO | D _h (nm) | 116 (2.5) | 337 (121) | NS | 639 (94) | 3855 * | 3871 * | 1225 (27) | 2846 * | 3385 * | 283 | 1135 | 1052 * | 177 | 609 | 445 (17) |
| | ζ (mV) | 18 (0.42) | -10 (1.5) | NS | 3.4 (0.40) | -4.1 | -5.5 | -5.3 | -2.0 | -5.0 | -17 | -15 | -15 | -16 | -16 | -16 (0.21) |
| | pdi | 0.20 (0.019) | 0.53 (0.14) | NS | 0.34 (0.017) | 0.86 (0.25) | 0.84 (0.28) | 0.45 (0.049) | 0.60 (0.14) | 0.30 (0.13) | 0.40 (0.016) | 0.56 (0.11) | 0.46 (0.036) | 0.34 (0.015) | 0.38 (0.023) | 0.37 (0.029) |

AFW: OECD202 artificial freshwater; MHRW: US EPA moderately hard reconstituted water; D_h: Hydrodynamic diameter; ζ : Zeta potential; pdi: Polydispersity index; NS: DLS analysis found the sample unsuitable for particle analysis; *: Data did not meet the DLS analysis quality criteria (particle concentration was low etc.).

Table S2. Speciation of metals in different test media predicted by Visual MINTEQ simulation.

| Test medium | | pH ¹ | Dissolved fraction (%) | | | Precipitated fraction (%) | |
|-------------------|----------|-----------------|------------------------|-----------|--------------|---------------------------|--|
| | | | Inorganic | | Bound to DOM | Fraction | Type of mineral |
| | | | Free ion | Bound ion | | | |
| CuSO ₄ | MQ | 4.9 | 97 | 2.7 | 0 | 0 | |
| | AFW | 7.5 | 0.080 | 0.12 | 0 | 100 | Tenorite (CuO) |
| | MHW | 7.7 | 0.021 | 0.093 | 0 | 100 | Tenorite (CuO) |
| | Raku | 7.9 | 0.0077 | 0.082 | 1.0 | 99 | Tenorite (CuO) |
| | Ülemiste | 8.3 | 0.0016 | 0.077 | 2.0 | 98 | Tenorite (CuO), Covellite (CuS) |
| ZnSO ₄ | MQ | 6.4 | 97 | 2.6 | 0 | 0 | Hydrozincite |
| | AFW | 7.4 | 52 | 4.5 | 0 | 43 | Hydrozincite |
| | MHW | 7.5 | 52 | 8.3 | 0 | 40 | Hydrozincite |
| | Raku | 8.1 | 3.3 | 1.3 | 0.071 | 95 | Hydrozincite, Smithsonite, Zn-Al layered double hydroxide |
| | Ülemiste | 8.4 | 0.47 | 1.3 | 0.016 | 99 | Hydrozincite |

¹ recorded after 6 days of incubation at 10 mg metal/L at 20 °C; AFW: OECD202 artificial freshwater; MHW: US EPA moderately hard reconstituted water; Ülemiste: water from lake Ülemiste; Raku: water from lake Raku.

Tabel S3. Change in *H. incongruens* growth (%) at the end of the 6-day experiment at sublethal concentrations. Negative values show growth inhibition and positive values growth enhancement in comparison to control organisms. Each value is based on measurements of 5 to 12 organisms from one exposure concentration. The mean (SD) of 2 experiments is given when possible.

| | Test concentration (mg metal/L) | MHW | Raku | Ülemiste |
|-------------------|---------------------------------|-------------|------|-----------|
| CuO NP | 0.1 | -9.2 | -5.6 | 1.2 |
| | 1 | -4.2 | 6.3 | -10 |
| CuSO ₄ | 0.05 | | 27 | |
| | 0.1 | 41 | -23 | 13 |
| | 0.2 | 4.1 | | |
| | 0.25 | | | 9.8 (7.4) |
| | 0.38 | | | -1.9 |
| ZnO NP | 0.1 | -9.8 (0.35) | | |
| | 0.25 | | -39 | -20 |
| ZnSO ₄ | 0.5 | | -47 | -40 |
| | 0.01 | | 3.9 | -8.6 |
| | 0.05 | | 0.7 | -17 |
| | 0.1 | 17 | -1.7 | -21 |
| | 0.25 | | -53 | -39 |
| | 0.5 | -41 | | |

MHW: US EPA moderately hard reconstituted water; Ülemiste: water from lake Ülemiste; Raku: water from lake Raku; NP: nanoparticles.

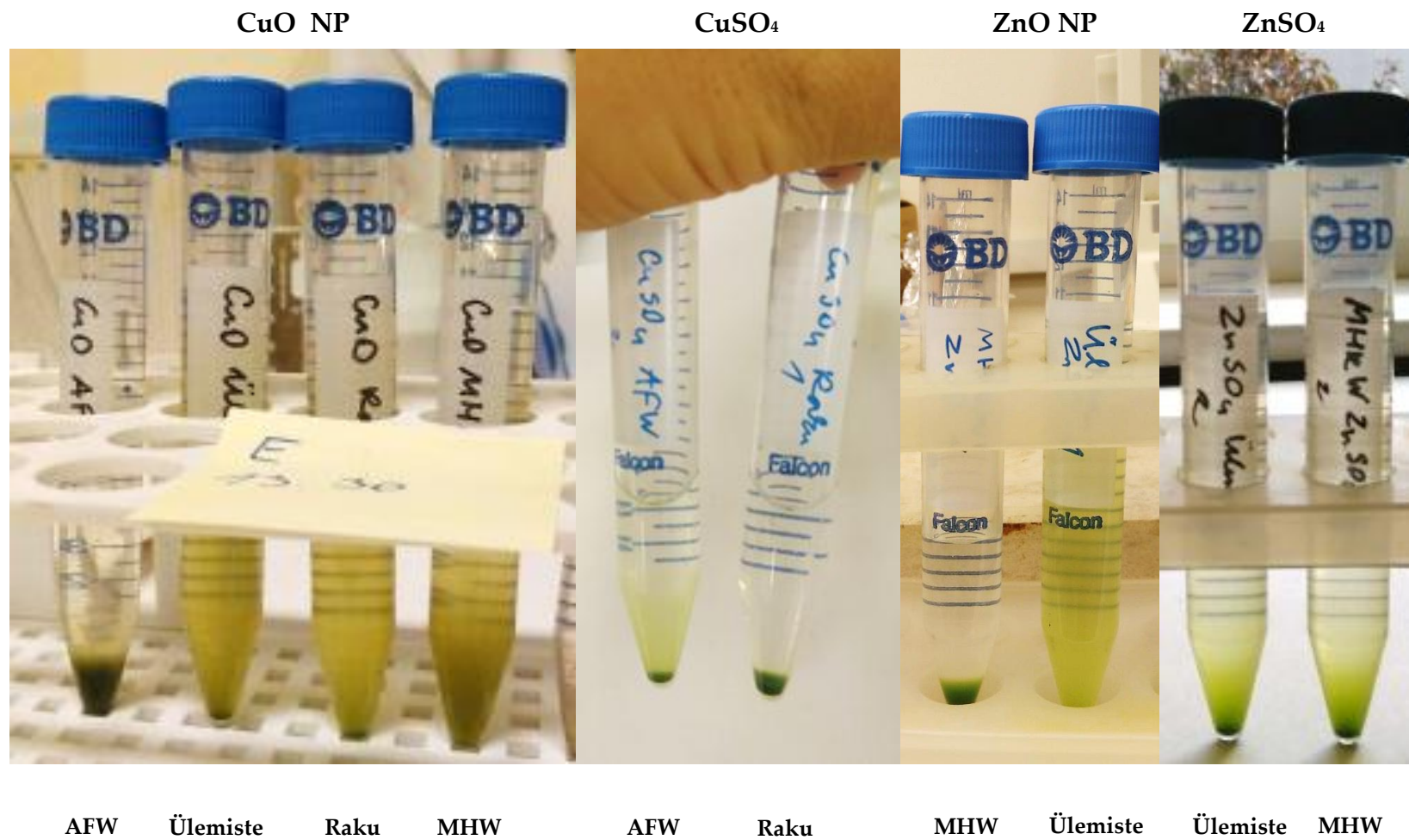


Figure S1. Examples of typical sedimentation of algae after 6 days of incubation with CuO and ZnO NP and respective soluble salts at 10 mg metal/L. MHW: US EPA moderately hard water; AFW: OECD 202 artificial freshwater; Ülemiste: water from lake Ülemiste; Raku: water from lake Raku.