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# Toxicity of zinc oxide nanoparticle suspensions to aquatic biota

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## INTRODUCTION

By common consent nanomaterials are chemical structures with at least one dimension between 1-100 nm, while for ecotoxicology it is suggested to apply a broader definition by including materials of a few hundreds nm [*Handy et al., 2008*]. Due to pushing development of nanotechnology in various fields such as chemical industry, electronics, (bio)medicine, cosmetics, etc. there is a threat of nanoparticle appearance in hydrosphere, e.g. through municipal treatment plant effluents. Data concerning nanoparticle toxicity and possible pathways of entering into the organism/cell are mainly obtained on humans [*Geiser et al., 2006; Oberdoerster et al., 2005*], while scarce ecotoxicological knowledge on the effects to biota is available to date [Blaise et al, 2008]. It is supposed that the main route to pass the cell interior consists of endocytosis [*Moore, 2006*], however, metal oxide nanoformulations such as CuO and ZnO evoke bactericide effect [*Heinlaan et al., 2008*], despite the endocytosis is not an inherent mechanism to prokaryotic cells.

One of the conventional approaches used to stabilize nanomaterial suspensions and therefore increase bioavailability of nanoparticles is their sonication [*Handy et al., 2008*]. However, during the sonication ions can be released from metallic nanoparticle suspensions, e.g. it was revealed that 1000 mg/l suspension of Cu nanoparticles sonicated for 1 h releases 0.3 mg/l of cupric ions [*Lee et al., 2008*]. Under similar magnitude of concentrations of cupric ions toxic effects were observed on unicellular algae *Selenastrum capricornutum* (growth inhibition, 72-h EC50 = 0.04 mg/l, [*Persoone, 1998*]), macrophytic algae *Nitellopsis obtusa* (96-h LC50 = 0.13 mg/l, [*Manusadžianas et al., 2002*]), rotifers *B.calyciflorus* (24-h LC50 = 0.023 mg/l, [*Clemedson et al., 1996*]) or shrimps *T.platyurus* (24-h LC50 = 0.04 mg/l, [*Heinlaan et al., 2008*]). When assessing ecotoxicological effects of nanosuspensions obtained by means of sonication, it has to be taken into account that a possible contribution of the toxicity due to metal ions may occur.

The aim of this study was to compare lethality response (LC50s) of aquatic organisms (macrophytic algae cells of *N.obtusa*, shrimps *T.platyurus* and rotifer *B.calyciflorus*) induced by sonicated and non-sonicated nano-ZnO suspensions with various particle size (10 and 20-30nm). To distinguish toxicities of different zinc chemical forms, ionic- ($Zn^{2+}$) and nanoform, the presence of $Zn^{2+}$ in both sonicated and non-sonicated suspensions were analysed by capillary electrophoresis and AAS. The dynamics of toxicity effects induced by nano-ZnO suspensions were investigated by evaluating the time-course curves of lethality and electrophysiological reactions.

## METHODS

### Algal material

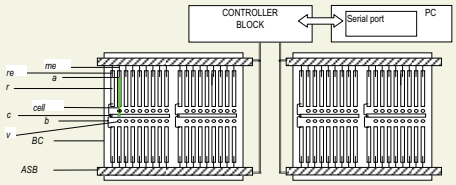
The freshwater charophyte *Nitellopsis obtusa* (Desv.) was harvested in Lake Švenčius (Lithuania) in 2008. Separated from neighbouring cells, internodes (2nd or 3rd ones below the tip) were kept at room temperature (20±2°C) in glass aquariums filled with equal parts of non-chlorinated tap water, lake water and artificial pond water (mM): 0.1 $KH_2PO_4$, 1.0 $NaHCO_3$, 0.4 $CaCl_2$, 0.1 m, $Mg(NO_3)_2$, and 0.1 $MgSO_4$ (pH 7-7.4, unbuffered).

### Preparation of nano-ZnO suspensions

Powder of ultrafine zinc(II)oxide nanoparticles (NP), average size 10 and 20-30nm, were purchased from Alfa/Aesar. A stock solution of 10 g/L ZnO was prepared by dispersing the NPs in deionized $H_2O$ with sonication for 30 min in a bath-type sonicator (IS-2, Poland). The concentrations of 1, 10, 30, 100, 300, 500 and 1000 mg/l were prepared immediately prior to the experimentation. The trials with sonicated (1), non-sonicated (2), sonicated+centrifuged (3) and non-sonicated+centrifuged (4) suspension toxicity to algae cells and crustaceans were carried out. The suspensions for the (1) were sonicated for 30 min just prior testing; for the (3) – sonicated for 30 min & then centrifuged for 10 min (Janitzki, 3000 rpm); and for the (4) – centrifuged for 10 min. The supernatant was removed from pellet and kept in +4°C.

### Measurement of Electrophysiological Reaction

The observation of the kinetics of electrophysiological response of up to 32x2 single cells was conducted within 30-h exposure to a given concentration of CuO nanosuspensions.

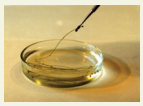


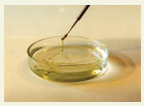

a - pool where the main part of cell and chlorinated silver wire measuring electrode are placed; b - П-shaped glass tube bridge filled with 3% (w/v) agar solidified in a 3 M KCl; c - common central pool for the small part of each of the 16 cells filled with 100 mM KCl; r - reference pool with reference electrode (re); v - vaseline insulation; me - measuring electrode; BC – biotesting chamber; ASB – analogue signal block.

### Observation of Cell Lethality

Lethality response of algal cells was investigated during 8-day exposure period. Single internodal cells (each 4-10 cm in length) were placed in Petri dishes (10 cells per dish, 3 replicates), preadapted for 1-2 days in artificial pond water (APW) containing 0.1 mM $KH_2PO_4$, 1.0 mM $NaHCO_3$, 0.4 mM $CaCl_2$, 0.1 mM $Mg(NO_3)_2$ and 0.1 mM $MgSO_4$ (pH 7-7.4, unbuffered), and then were kept at room temperature (18–24°C) in the dark. Survival of the cells was checked daily or when required by gently picking up each cell with a spatula. A cell was judged to be dead when picked up if there was disappearance of turgor pressure, a state in which a cell bends on the spatula and looses its cylindrical shape.

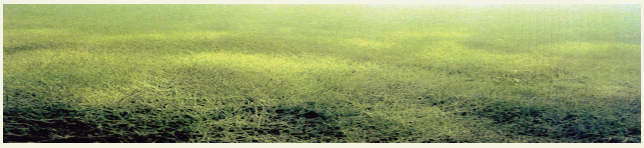
Cell with turgor pressure





Dead turgor-less cell

Underwater habitat of *Nitellopsis obtusa* (Desv). J. Groves



### Toxkit bioassays

The 24 h mortality of shrimps *Thamnocephalus platyurus* (Thamnotoxkit F™) and rotifers *Brachionus calyciflorus* (Rotoxkit F™, 2003) bioassays were performed following the SOP of the respective toxkits

## RESULTS

### Charophyte cell lethality kinetics

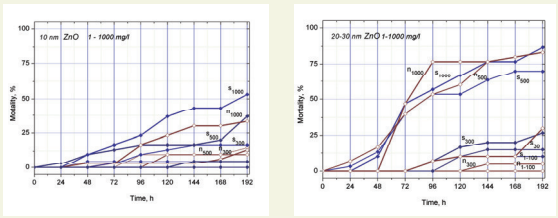



Figure 1. Mortality time-courses of charophyte algae cells of *Nitellopsis obtusa* during the 192 h exposure period to non-sonicated (n) and sonicated (s) suspensions of 10nm and 20-30 nm ZnO (mg/l).

### Comparison of lethality end points of various test-organisms

Table 1. Lethal effects of different chemical forms of copper, nano- (applied as non-sonicated (n) and sonicated (s) copper oxide nanopowder suspensions) and ionic form ($CuSO_4$ and $Cu(NO_3)_2$ solutions) on charophyte algae cells of *Nitellopsis obtusa*, shrimps *Thamnocephalus platyurus* and rotifers *Brachionus calyciflorus* expressed as LC50s (mg/l of copper, mean±sd).

| | *N.obtusa*, 96-h LC50 | | *T.platyurus*, 24-h LC50 | | *B.calyciflorus*, 24-h LC50 | |
|---|---|---|---|---|---|---|
| | n | s | n | s | n | s |
| *Nano form* | | | | | | |
| **Zn, mg/l** | | | | | | |
| ZnO: 10nm | **>1000** | **>1000** | **0.20±0.01** | **0.09±0.005** | **2.1±0.9** | **0.6±0.1** |
| ZnO: 20–30 nm | **438±35** | **562±100** | **0.21±0.005** | **0.17±0.01** | **0.6±0.001** | **0.34±0.04** |
| *Ionic form* | | | | | | |
| **$Zn^{2+}$, mg/l** | | | | | | |
| $Zn(CH_3OO)_2$ | **9.1±1.3** | | | | | |
| $ZnSO_4$ | **22.8±6.5** | | | | | |
| $Zn(NO_3)_2$ | **35.1±2.6** | | | | | |

### Charophyte cell membrane response

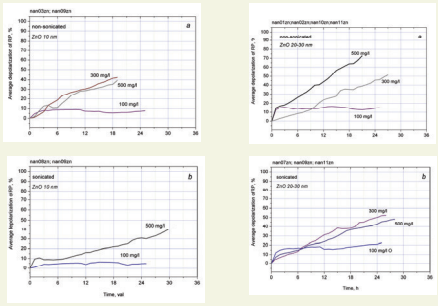



Figure 2. The kinetics of resting potential (RP) of *N.obtusa* cells treated with various concentrations of non-sonicated (a) and sonicated (b) nano-ZnO suspensions (10nm and 20-30nm). Each curve represents average of 14-16 cells. Treatments were started at 0 time.

## CONCLUDING REMARKS

- Among the three test organisms, macrophytic algae cells of *Nitellopsis obtusa* was substantially less susceptible to nano-ZnO particle toxicity (effect concentrations ranging 500-1000 mg/l) than shrimps *Thamnocephalus platyurus* (0.09-0.21 mg/l) and rotifers *Brachionus calyciflorus* (0.34-2.11 mg/l).
- Generally, both unsonicated and sonicated ZnO-formulations of 10 nm (Alfa/Aesar) particle size were less toxic than 20-30 nm (Alfa/Aesar) ones to charophyte cells (96-h and 192-h LC50s, and electrophysiological response expressed as percentage membrane depolarization after 18 h) and rotifers (24-h LC50s), however, no differences were found in the case of shrimps (24-h LC50s).
- No toxic Zn ionic concentrations were measured by capillary electrophoresis and AAS in supernatants of centrifuged zinc oxide suspensions both unsonicated and sonicated, thus confirming toxicity effects to algae cells being induced by nanoforms of suspensions.
- Titanium oxide nanoparticle suspensions (anatase, average size 29 nm, Alfa/Aesar) either sonicated or unsonicated showed no lethal effect to algae within 12 day exposure period in the range 1-1000 mg/L.
- The observed lethal effects and algae cell depolarization were evoked by nanoparticles per se, but not by dissolved zinc since neither chemical nor biological control undertaken after the settlement of nanoparticles by centrifuging were negative. Supernatants of both non-sonicated and sonicated suspensions have been analyzed by capillary electrophoresis and AAS: low (0,6-2,1 mg/l) zinc ion concentrations were detected by this chemical analysis as well as algae survived in the supernatants of suspensions.

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