Toxicity of zinc oxide nanoparticle suspensions to aquatic biota

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 [König 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 are mainly obtained on humans [Gieser et al., 2006; Oberdörster et al., 2003], 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 (Moor, 2009); however, metal oxide nanof ormulations such as CuO and ZnO evoke bactericide effect [Kheirani et al., 2009], despite the endocytosis is not an inherent mechanism to prokaryotic cells.

One of the conventional approaches used to stabilize nanoparticle suspensions and therefore increase bioavailability of nanoparticles is their sorption [Handy et al., 2008]. However, during the sorption ions can be released from metallic nanoparticle suspensions, e.g. it was revealed that 1000 mg/L suspension of Cu nanoparticles sonicated for 1 hour released 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) [Pires et al., 1998]. Magnetic alumina Nitellopsis obtusa (96-h LC50 = 0.13 mg/L) [Manusadžianas et al., 2002], rotifers Brachionus calyciflorus (24-h LC50 = 0.022 mg/L) [Orgaz-Diez et al., 1998] or shrimps Thamnocephalus platyurus (24-h LC50 = 0.04 mg/L) [Kheirani et al., 2009]. When assessing ecotoxicological effects of nanoparticle suspensions obtained by 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 lethal response (LC50) of aquatic organisms (magnetic alumina magnetic algae cells of N. obtusa, shrimps T. platyurus and rotifers B. calyciflorus) induced by sonicated and non-sonicated nano-ZnO suspensions with various particle sizes (10 and 20-30 nm). To distinguish toxicities of different zinc chemical forms, ionic (ZnSO4) and nanoparticles (ZnO), the presence of ZnO 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 Sventoji, Lithuania in 2009. Separated from neighbouring cells, internodes (2-3 cm 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 KH2PO4, 1.0 NaHCO3, 0.4 CaCl2, 0.1 MgSO4, pH 7.4 (unbuffered).

Preparation of nano-ZnO suspensions

Powder of ultratine zinc(II)oxide nanoparticles (NP), average size 10 and 20-30 nm, were purchased from AlfaAesar. A stock solution of 10 gl ZnO was prepared by dispersing the NPs in deionized H2O with sonication for 30 min in a bath-type sonicator (46-52, 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 (s), non-sonicated (n), sonicated-centrifuged (s) and non-sonicated-centrifuged (n) suspension toxicity to algae and crustaceans were carried out. The suspensions for the s suspensions were prepared just prior testing; for the n suspensions were prepared for 30 min & then centrifuged for 10 min (Jenlab, 3000 rpm), and for the c-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 30-60s exposure period to an initial concentration of CuO nanoparticles.

Observation of Cell Lethality

Lethality response of algae was investigated during 8-day exposure period. Single intermolecular cells (each 4-10 cm in length) were placed in Petri dishes (10 cells per dish, 3 replicates), preincubated for 1 day in artificial pond water (APW) containing 0.1 mM Na2CO3, 1.0 mM NaHCO3, 2.4 mM CaCl2, 0.1 mM MgSO4, and 0.1 mM MgSO4, pH 7.4 (unbuffered), and then were kept at room temperature (18–24°C) in the dark. Survivability 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 spatala and loses its cylindrical shape.

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 10 nm 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 (CuSO4 and Cu(NO3)2) solutions) on charophyte algae cells of Nitellopsis obtusa, shrimps Thamnocephalus platyurus and rotifers Brachionus calyciflorus expressed as LC50s (mg/L, copper, measured).

Charophyte cell membrane response

Figure 2. The kinetics of lethal potential (P(E)) of Nitellopsis obtusa exposed to 8 concentrations of non-sonicated (s) and sonicated (n) ZnO suspensions (10 nm and 20-30 nm). Each curve represents averages of 14-16 cells. Treatments were started at 0 time.

CONCLUDING REMARKS

Among the three test organisms, magnetic alumina cells of M. obtusa was substantially less susceptible to nano-ZnO toxicity (effect concentrations ranging 500-1000 mg/L) than shrimps Thamnocephalus platyurus (0.09-0.21 mg/L) and rotifiers 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 electrophysiologic al response). Generally, both unsonicated and 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.