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Ecotoxicity of nanoparticles of CuO and ZnO in natural water

I. Blinova [a,*], A. Ivask [a], M. Heinlaan [a,b], M. Mortimer [a,c], A. Kahru [a]

[a] Laboratory of Molecular Genetics, National Institute of Chemical Physics and Biophysics, Akadeemia tee 23, Tallinn 12618, Estonia
[b] Estonian University of Life Sciences, Kreutzwaldi 5, Tartu 51014, Estonia
[c] Tallinn University of Technology, Akadeemia tee 15, Tallinn 12618, Estonia

Natural waters remarkably reduced the toxicity of nanoCuO but not nanoZnO.

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ABSTRACT

The acute toxicity of CuO and ZnO nanoparticles in artificial freshwater (AFW) and in natural waters to crustaceans *Daphnia magna* and *Thamnocephalus platyurus* and protozoan *Tetrahymena thermophila* was compared. The L(E)C_{50} values of nanoCuO for both crustaceans in natural water ranged from 90 to 224 mg Cu/l and were about 10-fold lower than L(E)C_{50} values of bulk CuO. In all test media, the L(E)C_{50} values for both bulk and nanoZnO (1.1–16 mg Zn/l) were considerably lower than those of nanoCuO. The natural waters remarkably (up to 140-fold) decreased the toxicity of nanoCuO (but not that of nanoZnO) to crustaceans depending mainly on the concentration of dissolved organic carbon (DOC). The toxicity of both nanoCuO and nanoZnO was mostly due to the solubilised ions as determined by specific metal-sensing bacteria.

1. Introduction

The last decade is distinguished by the drastic growth of production and use of manufactured nanoparticles (NPs). NPs of metal oxides such as ZnO and TiO_2 are already widely used in personal care products (e.g., sunscreens), coatings and paints; CuO is used in gas sensors, photovoltaic cells, in catalyst applications and in heat transfer nanofluids. Subsequently, the risk of natural water contamination by synthetic NPs continuously increases (Klaine et al., 2008).

It should be stressed that natural NPs, including nano-sized particles of metal oxides, exist in all ecosystems and play important role in biogeochemical processes (Wigginton et al., 2007). During the evolution living organisms have adapted to the presence of natural NPs in the environment. For synthetic NPs, however, it is recognized that their potential harmful properties on ecosystems have to be evaluated (Handy et al., 2008; Nowack, 2009). Despite the rapidly increasing amount of nanotoxicological peer-reviewed papers (Medina et al., 2007) data on ecotoxicity of synthetic NPs (Baun et al., 2008; Handy et al., 2008) and especially on metal oxide NPs, except nanoTiO_2, are rare (Kahru et al., 2008). As water is an essential compartment in ecosystems and natural vehicle for pollutant migration, the data on fate and behavior of synthetic NPs in different types of natural waters as well as their potential ecotoxic effects are essential for evaluation of the environmental risks of nanotechnologies (Nowack and Bucheli, 2007).

The main goal of evaluation of ecotoxicological properties of chemicals is to prevent the hazard to the ecosystems via establishing respective environmental standards, which guarantee the absence of negative effects of those compounds on living organisms. At the same time, it is widely accepted and also shown in our earlier works (Aruoja et al., 2004; Blinova, 2004; Kahru et al., 2005) that due to the environmentally non-relevant conditions used in regulatory testing, most of the standardized bioassays do not appropriately characterize the potential impacts of hazardous substances on the environment, in particular, on water ecosystems (Allen and Hansen, 1996; Hyung et al., 2007; Lewis, 1995). Most of the ecotoxicity data on chemicals available for standard freshwater test organisms such as crustaceans, algae and fish have been generated using so-called artificial freshwater (AFW), which composition differs from natural waters. However, as bioavailability and toxic effect of a chemical depend on its speciation and hence, on water composition (Witters, 1998), the hydrochemical parameters of water used as test medium are very important.

Till now, the impact of the composition of natural water on fate and biological effects of chemicals in the aquatic ecosystems has not been adequately explored. For example, in spite of the intensive investigation of the effects of natural water composition on bioavailability of heavy metals during the last decades leading even to the elaboration of several models, which are used for the prediction of metal toxicity

* Corresponding author. Tel.: +372 6 398 361.
E-mail address: irina.blinova@kbfi.ee (I. Blinova).

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in natural water (Jager et al., 2006; Kim et al., 1999; Long et al., 2004; McGeer et al., 2002; Niyogi and Wood, 2004; Pagenkopf, 1983, etc.), understanding of the behavior and biological effects of trace metals in natural waters is still limited (Borgmann, 2000; Handy et al., 2008; Town and Filella, 2002; Van Leeuwen et al., 2005). For synthetic NPs, it is known that the type and amount of natural organic matter in the water affects their stability and bioavailability (Giasuddin et al., 2007; Hyung et al., 2007; Wigginton et al., 2007), and may strongly influence their behavior in surface waters (Klaine et al., 2008; Lead and Wilkinson, 2006). However, the effect of organic ligands as well as of other hydrochemical parameters (pH, hardness, ionic strength, etc.) on the bioavailability of NPs to aquatic organisms is still inadequately investigated. The latter decreases the practical value of ecotoxicity test results obtained with AFW (Handy et al., 2008; Nanotechnology, 2006; Velzeboer et al., 2008).

In order to be relevant for the use in the risk assessment and establishment of environmental quality standards, ecotoxicity tests should give information on a chemical's bioavailability and toxicity in the given environment (McGeer et al., 2002; Van Assche et al., 2002). Additional knowledge required to extrapolate laboratory test results to field populations may be received by extending the standard protocols of ecotoxicological testing (Jager et al., 2006), for example, by replacing the AFW with a natural one.

The aim of the present study was to compare the acute toxicity of CuO and ZnO NPs towards particle-ingesting aquatic species (two crustaceans and one protozoan) in AFW and in six natural waters with different hydrochemical characteristics. Bulk CuO and ZnO and the respective soluble salts ($CuSO_4$ and $ZnSO_4 \cdot 7H_2O$) were used as controls for size-dependent and solubility effects. Bioavailability of Cu and Zn as well as the solubilisation of metal oxides in natural waters was studied by recombinant sensor bacteria.

2. Materials and methods

2.1. Natural water

Natural water samples were taken during November–December 2007 from six Estonian rivers with different hydrochemical characteristics. Sampling places were chosen according to the data of the national monitoring program. The chemical analysis of water samples (Table 1) was performed in a certified laboratory using the following standard analytical methods: EN 1899-1:1998 for biochemical oxygen demand (BOD_7), ISO 9963-1:1994 for alkalinity, ISO 11905-1:1997 for total nitrogen (N_{tot}), ISO 6878:1998 for total phosphorus (P_{tot}), ISO 8245:1999 for dissolved organic carbon (DOC), ISO 17294-2:2003 for Zn and Cu, ISO 10304-1:1992 for sulphate, ISO 10304-1:1992 for chloride, ISO 6058:1984 for calcium, SFS 3032 (1976) for ammonium. Before the biotesting, suspended solids and plankton were separated from the water samples by filtration through a 0.45 μm pore size standard filter (Millipore).

Table 1
Characterization of natural waters used as test media.

Parameter	Unit	River 1	River 2	River 3	River 4	River 5	River 6
pH		8.2	8.2	7.9	8.1	7.5	8.1
DOC[a]	mg C/l	13.3	13.2	25.9	29.2	34.5	31.5
BOD_7[b]	mg O_2/l	1.1	1.5	1.5	1.3	1.1	1.9
N_{tot}[c]	mg/l	7.6	3.2	9	10.1	4.7	5.2
P_{tot}[d]	mg/l	0.039	0.045	0.037	0.028	0.052	0.015
Alkalinity	meq/l	4.4	5.6	4.1	3.9	3.8	2.7
Ca^{2+}	mg/l	122	124	106	111	82.0	58.0
Cl^-	mg/l	15.4	17	4.6	13.5	9.2	7.7
SO_4^{2-}	mg/l	96.1	69.1	55.6	76.1	20.9	14.9
Zn_{tot}[e]	μg/l	2.6	2.6	1.4	2.2	1.5	3.1
Cu_{tot}[f]	μg/l	2.0	3.0	4.6	21.3	2.0	11
NH_4^+	mg N/l	0.06	0.15	0.11	0.09	0.53	0.19

[a] DOC – dissolved organic carbon.
[b] BOD_7 – biochemical oxygen demand.
[c] N_{tot} – total nitrogen.
[d] P_{tot} – total phosphorus.
[e] Zn_{tot} – total zinc.
[f] Cu_{tot} – total copper.

2.2. Chemicals

NanoCuO (advertised particle size 30 nm), nanoZnO (advertised particle size 70 nm) and $ZnSO_4 \cdot 7H_2O$ were purchased from Sigma–Aldrich, the bulk form of ZnO from Fluka, the bulk CuO and $CuSO_4$ from Alfa Aesar. The stock solutions of metal salts and suspensions of metal oxides were prepared in MilliQ water. The suspensions of metal oxides (40 g/l) were sonicated for 30 min and stored in the dark at +4 °C.

2.3. Electron microscopy imaging

The aqueous suspensions of the studied metal oxides (both nano- and bulk formulations) have been previously characterized by scanning electron microscopy (SEM): despite of agglomeration, individual nanoscale particles were present in nanoZnO and nanoCuO suspensions (Kahru et al., 2008). For the current study, size distribution of CuO NPs (40 mg CuO/l) was analysed by transmission electron microscopy in *Daphnia magna* test medium (AFW) using a JEOL 1230 TEM at 120 kV. For the characterization of NPs after ingestion by *D. magna*, organisms were exposed to nanoCuO (4 mg/l) and live crustaceans were fixed for TEM observations using a JEOL 1011 TEM at 100 kV. TEM photographs were taken from the gut after thin-sectioning. Individual particles were sized (based on 200 measurements) from TEM photographs using the freeware ImageJ (NIH, USA).

2.4. Aquatic bioassays

The crustacean *D. magna* acute immobilisation assay (Daphtoxkit F™) adhering to OECD 202 guidelines, crustacean *Thamnocephalus platyurus* acute mortality test (Thamnotoxkit F™) and ciliate protozoan *Tetrahymena thermophila* growth inhibition test (Protoxkit F™) were used. The Toxkits were purchased from MicroBioTests, Inc. (Nazareth, Belgium). In both crustacean assays viable and dead organisms were counted under dissection microscope after 24 h (*T. platyurus*) or 48 h (*D. magna*) of exposure. For protozoan growth inhibition test, the investigated compound and *T. thermophila* culture were added to the food substrate suspension in test medium. While normal proliferating protozoan culture clears the substrate suspension in 24 h, inhibition of the growth of protozoa is reflected by residual turbidity of the food substrate measured by optical density (OD) of the tests samples at 440 nm. All the tests were performed in the dark at constant temperature (20 °C for *D. magna*, 25 °C for *T. platyurus* and 30 °C for *T. thermophila*) according to the respective guidelines of the Toxkits.

The test organisms were exposed to different concentrations of CuO and ZnO (both, nano- and bulk forms), $CuSO_4$ and $ZnSO_4 \cdot 7H_2O$. The filtered river waters (Table 1) were used as basic test medium in the tests, i.e. for the dilution of studied compounds and as a control. Evaluation of the toxicity was performed in two steps: i) determination of 0–100% tolerance range of the test species to the respective compound and ii) determination of the 50% effect values $L(E)C_{50}$. The toxicity was evaluated from 2 to 3 independent experiments, each in several replicates (four for *D. magna*, three for *T. platyurus* and two for *T. thermophila*).

The AFW (test medium used in the standard test procedure) for crustaceans has following composition (mg/l): for *D. magna* – $CaCl_2 \cdot 2H_2O$ – 294, $MgSO_4 \cdot 7H_2O$ – 123.25, $NaHCO_3$ – 64.75, KCl – 5.75, pH −7.8 ± 0.2 and for *T. platyurus* – $CaSO_4 \cdot 2H_2O$ – 60, $MgSO_4 \cdot 7H_2O$ – 123, $NaHCO_3$ – 96, KCl – 4 mg/l; pH – 7.8 ± 0.2 dissolved in MilliQ water, i.e. AFW does not contain organic compounds. MilliQ water was used as standard test medium for *T. thermophila*.

2.5. Bacterial metal-specific biosensors

In parallel to the aquatic biotests, dissolved bioavailable Zn^{2+} and Cu^{2+} in the solutions/suspensions of tested compounds were quantified using recombinant bioluminescent Zn-sensor bacteria *Escherichia coli* MC1061(pSLzntR/pDNPzntAlux) and Cu-sensor bacteria *E. coli* MC1061(pSLcueR/pDNPcopAlux), respectively (Ivask et al., 2009). Bioluminescence of those sensor bacteria increases proportionally with the concentration of bioavailable Cu^{2+} (Cu-sensor) or Zn^{2+} (Zn-sensor) in the test medium (Ivask et al., 2002). A constitutively luminescent control strain *E. coli* MC1061(pDNlux) (Leedjärv et al., 2006) not induced by heavy metals, but otherwise similar to sensor strains, was used to take into account the potential quenching of bacterial bioluminescence by the turbid suspensions of metal oxides. 100 μl of the suspension of Zn- or Cu-sensor bacteria or the constitutively luminescent control bacteria in 9 g/l of NaCl supplemented with 1 g/l of cas-aminoacids (acid hydrolysate of casein, LabM) and 0.9 g/l of glucose was mixed with 100 μl of the solution or suspension of studied metal compound diluted either in MilliQ, AFW for *D. magna* or *T. platyurus* or in the natural river waters and incubated for 2 h at 30 °C as described previously (Heinlaan et al., 2008). The amount of bioavailable Cu and Zn was quantified assuming that $CuSO_4$ and $ZnSO_4 \cdot 7H_2O$ were 100% bioavailable to the sensor bacteria, when compounds were diluted in MilliQ. Detection limits of this method were 2 μg Cu^{2+}/l and 20 μg Zn^{2+}/l.

2.6. Statistical analysis

One-way analysis of variance (ANOVA) followed by *t*-tests was used to determine statistical significance of the differences between toxic effects of the compounds in different test media. The differences were considered significant, when $p < 0.05$.