Zinc bioaccumulation in a terrestrial invertebrate fed a diet treated with particulate ZnO or ZnCl₂ solution

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ABSTRACT

A number of reports on potential toxicity of nanoparticles are available, but there is still a lack of knowledge concerning bioaccumulation. The aim of this work was to investigate how different sources of zinc, such as uncoated and unmodified ZnO nanoparticles, ZnCl₂ in solution, and macropowder ZnO influence the bioaccumulation of this metal in the terrestrial isopod Porcellio scaber. After exposure to different sources of Zn in the diet, the amount of assimilated Zn in whole body, the efficiency of zinc assimilation, and bioaccumulation factors (BAFs) were assessed. The bioaccumulation potential of Zn was found to be the same regardless of Zn source. The amount of assimilated Zn and BAF were dose-dependent, and Zn assimilation efficiency was independent of exposure concentrations. The Zn assimilation capacity was found to be up to 16% of ingested Zn. It is known that as much as approximately 20% of Zn can be accreted from ZnO particles by dissolution. We conclude that bioaccumulation of Zn in isopods exposed to particulate ZnO depends most probably on Zn dissolution from ZnO particles and not on bioaccumulation of particulate ZnO.

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1. Introduction

Concerns of the environmental fate and the effects of increased use of engineered nanoparticles are an important issue. By their nature nanoparticles share properties associated with solutes as well as separate particle phases and an understanding of their environmental behavior and biological effects is consequently very complicated.

An increasing number of research articles have focused on the toxicity of nanoparticles to cells or to standard test organisms. Only very recently however has research focused on their bioavailability and bioaccumulation. Studies published so far point to the bioaccumulation potential of different types of nanoparticles and indicate the need for more knowledge on this matter. For instance, crustacean Ceridaphnia dubia accumulated fluorescent semiconductor nanocrystals in the region of abdominal appendages (Ingle et al., 2008), and gold nanoparticles were found in digestive tract of crustacean Daphnia magna, but they were not taken up and distributed in the body (Lovern et al., 2008). Similarly, the majority of carbon nanotubes were found in the gut of earthworms Eisenia fetida and Lumbricus variegatus, and minimal absorption into the tissue was found (Petersen et al., 2008a,b). On the other hand, Cu nanoparticles were found in the cells of mung bean Phaseolus radiates and wheat Triticum aestivum (Lee et al., 2008), and iron oxide (Fe₂O₃) nanoparticles were accumulated in the roots of pumpkin plants Cucurbita maxima (Zhu et al., 2008). Thorough comparisons of the accumulation of different metal-particles and dissolved metal are still however very uncommon.

A common approach to study the bioaccumulation of metals is the use of different metal accumulating invertebrate species (Hopkin, 1989; Heikens et al., 2001). Among terrestrial invertebrates, isopods appear to be the most efficient accumulators of metals. They accumulate the highest concentrations of metals such as zinc, cadmium, lead and copper so far recorded in any soft tissue (Hopkin, 1989; Hopkin et al., 1993; Vijver et al., 2006; Witzel, 1998). Digestive system of isopods is the main route for metal intake and their accumulation of metals is attributed solely to dietary exposure. For these reasons, terrestrial isopods are a good choice in which to compare bioaccumulation of metals from disparate origins.

Extensive studies exist on toxicity and physiology of Zn assimilation in isopods (Drobne, 1997; Zidar et al., 2003; Odendaal and Reinecke, 2004, 2007). In laboratory studies, the adverse effects of Zn accumulation were studied after dosing food with Zn²⁺ ions from ZnCl₂, Zn(NO₃)₂·6H₂O or with ZnO. Recently, nanosized zinc oxide, a new form containing the element, has received considerable attention as zinc, cadmium, lead and copper so far recorded in any soft tissue (Hopkin, 1989; Hopkin et al., 1993; Vijver et al., 2006; Witzel, 1998). Digestive system of isopods is the main route for metal intake and their accumulation of metals is attributed solely to dietary exposure. For these reasons, terrestrial isopods are a good choice in which to compare bioaccumulation of metals from disparate origins.

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attention because of its unique optical, catalytic, semiconducting, piezoelectric, and magnetic properties. Nanosized ZnO is used in products for industrial and cosmetics application, in pigments and coatings, electronic devices, cosmetics, and catalysts.

The aim of this work was an investigation of how different origins of Zn, such as uncoated non-modified ZnO nanoparticles, macropowder ZnO or ZnCl₂ ions in solution form, for example ZnCl₂, influence the bioaccumulation of this metal in a model terrestrial organism, the isopod Porcellio scaber. We hypothesized two distinct scenarios: (1) animals assimilate more Zn when it is added as dissolved ZnCl₂ in comparison to particulate ZnO, due to presumed higher bioavailability of the former, or (2) accumulation patterns for different origins of Zn are similar, because recent toxicity studies with other organisms showed that different origins of Zn do not cause different toxic responses (Franklin et al., 2007; Ma et al., 2009).

2. Materials and methods

2.1. Characterization of ZnO nano- and macro-particles

Two forms of commercially available ZnO particles (Sigma-Aldrich) were investigated. Particles <100 nm in diameter with surface area 15–25 m²/g, referred to here as 'nanoparticles' and ZnO 'macropowder' consisting of particles <1 μm in diameter. Additional characterization of the nanoparticles was performed on the powder as received and also on dispersion in bidistilled water (pH 5.7), which was used to prepare the food for isopod toxicity testing.

After the samples were dried and degassed with nitrogen prior to analysis, BET analysis (Brunauer–Emmet–Teller surface area analysis; Tristar 3000, Micrometrics) was performed to obtain information on the surface area of the solid nanomaterial.

Dispersions of nanoparticles of ZnO (100 mg/L) in ultra-pure, ion free, water (pH 5.7) filtered through a 0.2 μm siewe (Millipore, Billerica, MA, USA) were inspected by dynamic light scattering (DLS) using a 3D-DLS-LSL Spectrometer (LS Instruments, Fribuorg, Switzerland).

Dispersions of nano- and macro-sized ZnO particles in bidistilled water were inspected by transmission electron microscopy (TEM) and scanning electron microscopy (SEM). The dispersions of nanoparticles were put on carbon-coated grids, dried at room temperature and examined by TEM (Philips CM 100). Particles were also inspected by a field emission scanning electron microscope (FE-SEM, Supra 35 VP, Carl Zeiss, Germany), at an accelerating voltage of 1 kV. Prior to the observation, the particles were fixed on a holder with double sided adhesive carbon tape.

2.2. Exposure of isopods to ZnO particles and ZnCl₂

2.2.1. Test organisms

Adult specimens of the terrestrial isopod (P. scaber, Latreille 1804) were collected in July 2006 under the litter layer in a non-polluted garden in Laško, Slovenia. The animals were kept in the laboratory for two months prior to experiments in a soil and a thick layer of partly decomposed hazelnut tree leaves (Corylus avellana) collected in July 2006 under the litter layer in a non-polluted garden in Laško, Slovenia. The animals were kept in the laboratory for two months prior to experiments in a soil and a thick layer of partly decomposed hazelnut tree leaves (Corylus avellana) collected in July 2006 under the litter layer in a non-polluted garden in Laško, Slovenia.

2.2.2. Food preparation

Food was prepared according to the published protocol (Drobne et al., 2009). The ZnO particles at ZnCl₂ (Merck, Germany, 98% purity) were suspended in bidistilled water using a vortex (20 s, 2000 rpm) and prepared freshly for each experiment. The pH of the dispersions was independent on the concentration of Zn and was the same, 5.7, as pure bidistilled water. No surfactants were used. The dispersion (150 μL) per 100 mg of leaf was applied onto the lower hazelnut tree leaf surfaces. Prior to sampling of the suspension, the dispersion was each time vortexed for 5 s. Animals in the control group were fed with the leaves treated with the distilled water only, but otherwise prepared in the same way. Two final concentrations of Zn were applied (2000 or 5000 μg/g dry food).

Significant differences between the control and exposed groups of animals were determined by Kruskal–Wallis analysis and Mann–Whitney U-test (p < 0.05) using Statgraphics software (Statgraphics Plus for Windows 4.0, Statistical Graphics, Herndon, VA, USA). Homogeneity of variance was tested with Levene’s test.

3. Results

3.1. Characterization of nano- and micro-ZnO particles

The BET-estimated specific surface area for nanosized ZnO powder was 12.39 ± 0.0270 m²/g and a single particle was calculated to have a diameter of 84.9 nm. The size of particles was in accordance with supplier’s information, but the measured surface area was smaller than specified. Using DLS analysis of aqueous ZnO nano-dispersion, a wide distribution of particles sizes was observed and the average hydrodynamic diameter of particles was found to be 614 nm.

TEM images of aqueous dispersion of ZnO nanoparticles showed considerable particle aggregation with the particles of variable sizes from some tens of nanometers up to a few hundred nanometers. The shape of the particles was spherical to ellipsoidal (Photo 1).

Scanning electron microscopy revealed that macropowder ZnO was composed of particles of different sizes ranging from some tens of nanometers up to a micrometer (Photo 2). ZnO nanoparticles were significantly smaller than particles of macropowder ZnO. However, here also large particles up to a few 100 nm in size were observed (Photo 3). Both TEM and SEM measurements revealed that nanoparticles were considerably larger than the nominal size (100 nm) specified by the supplier.

3.2. Survival and feeding rate of isopods

Some of exposed isopods died while molting because the animals are very sensitive when in this physiological state and there is a very high probability of their death upon physical disturbances during the molting. For the control group, those fed 2000 μg
macro-ZnO/g dry weight, 2000 μg nano-ZnO/g dry weight, 2000 μg ZnCl₂/g dry weight, 5000 μg macro-ZnO/g dry weight, 5000 μg nano-ZnO/g dry weight, and 5000 μg ZnCl₂/g dry weight, the number of dead animals was 1, 3, 1, 1, 2, 2, and 3, respectively. Thus seven or more animals per treatment were available for analysis at the end of the experiment.

Feeding rate, determined at the end of experiments as mg ingested food per dry weight of animals per day was not affected as a result of elevated concentrations of Zn in the food. Feeding intensity in each treatment also was unchanged during the experiment, since the amount of collected feces was the same each week. Animals fed on food with higher concentrations of ZnCl₂ had higher assimilation efficiency than those fed with 2000 μg/g of Zn provided as ZnCl₂. Such differences were not observed with other sources of Zn.

3.3. Zinc accumulation

3.3.1. Zinc concentration in the food and zinc exposure dose

The actual measured concentrations of Zn in the remnants of leaves varied by more than 10% from the nominal concentrations, therefore the actual measured values and not the nominal, applied values were used to calculate the exposure dose. Zn exposure doses in the case of 2000 μg/g of Zn had low variability (coefficient of variation up to 25%), but at higher concentrations data were more variable (coefficient of variation up to 48%) (Fig. 1). Despite high variation the ratio between the measured high and low concentration were 2.5 as for the nominal ones (5000 and 2000 μg/g) (Fig. 2).

3.3.2. Zinc concentration in the animals

We found no differences in the Zn body burden between control animals and those exposed to 2000 μg/g of Zn in the food (Fig. 3). Animals exposed to 5000 μg/g of Zn accumulated statistically significantly more Zn. No differences between animals feed on the same concentration but with three different sources of Zn (nano-, bulk-particles, ion) were observed.

3.3.3. Zn assimilation efficiency (AE)

No differences in Zn assimilation efficiency were observed among animals fed on the same concentration but with three different origins of Zn (Fig. 4). Statistically significant differences were observed between 2000 and 5000 μg/g of ZnO nanoparticles.

3.3.4. Bioaccumulation factors (BAFs)

No differences in BAF were observed between animals fed with three different sources of Zn (nano-, bulk-particles, ion) at the same concentration. However, bioaccumulation was higher at lower con-
Fig. 1. Total consumed amount of Zn in isopods *Porcellio scaber* after feeding for 4 weeks on ZnCl₂ (2000 Cl₂ and 5000 Cl₂), ZnO nanoparticles (2000 and 5000 n) and ZnO macropowder (2000 and 5000 M) dosed food. Nominal exposure concentrations of Zn are provided on x-axis.

Fig. 2. Concentration of measured Zn on leaves when 2000 and 5000 μg/g dry leaf of ZnCl₂ (2000 Cl₂ and 5000 Cl₂), ZnO nanoparticles (2000 and 5000 n) and ZnO macropowder (2000 and 5000 M) were applied.

Fig. 3. Measured concentration of Zn in total body of isopods *Porcellio scaber* after feeding for 4 weeks on ZnCl₂ (2000 Cl₂ and 5000 Cl₂), ZnO nanoparticles (2000 and 5000 n) and ZnO macropowder (2000 and 5000 M) dosed food. Nominal exposure concentrations of Zn are provided on x-axis.

Fig. 4. Zinc assimilation efficiency of isopods *Porcellio scaber* after feeding for 4 weeks on ZnCl₂ (2000 Cl₂ and 5000 Cl₂), ZnO nanoparticles (2000 and 5000 n) and ZnO macropowder (2000 and 5000 M) dosed food. Nominal exposure concentrations of Zn are provided on x-axis.

Fig. 5. Bioaccumulation factors for Zn in isopods *Porcellio scaber* after feeding for 4 weeks on ZnCl₂ (2000 Cl₂ and 5000 Cl₂), ZnO nanoparticles (2000 and 5000 n) and ZnO macropowder (2000 and 5000 M) dosed food. Nominal exposure concentrations of Zn are provided on x-axis.

The results obtained in this study showed that the amount of assimilated Zn by terrestrial isopod *P. scaber*, the bioaccumulation factors and Zn assimilation efficiency at a single concentration were similar regardless of the source of the Zn, either particulate ZnO or ZnCl₂. The amount of assimilated Zn and BAF were dose-dependent, while Zn assimilation efficiency was, with slight exceptions of ZnO nanoparticles, the same for both concentrations.

In our study, the average Zn assimilation efficiency by isopods was 10% (95% confidence interval 7.14–12.8). This is in agreement with previous reports that 7.2–16% of assimilated zinc is acquired from food when isopods were fed with ZnCl₂ from 450 to 5400 μg/g Zn in the food for 14 days (Zidar et al., 2003). This means, that isopods are probably not able to assimilate more than up to 16% of ingested Zn, regardless of the available exposure concentration.
In studies regarding metal assimilation, feeding behavior of animals is usually recorded. In the present study, difference in food assimilation efficiency between 5000 μg/g ZnCl₂ and 2000 μg/g ZnCl₂ was observed, similarly as previously reported by Drobne and Hopkin (1995), however feeding rate was similar in all experimental groups. Therefore we think that Zn assimilation was not influenced by the feeding rate of isopods.

The most frequently used approach to provide a relative assessment of metal bioavailable from the environment is a calculation of a bioaccumulation factor (Gál et al., 2008). The average BAF in our study (0.20 ± 0.088) was similar to previously reported BAF values (0.10–0.26) in isopods exposed to 300–3000 μg/g Zn in the food for 10 days (Bibič et al., 1997). BAFs in animals fed on the same concentration but with three different sources of Zn (nano-, bulk-particles, ion) were the same, indicating that the bioavailability of Zn was the same regardless of its source.

Our hypothesis was that animals would assimilate more Zn when it is added as ZnCl₂ in comparison to particulate ZnO, due to presumed higher bioavailability – the extent to which a metal in a source is free for uptake and to interact with an organism – of the former. Contrary to our expectations, the present results showed that the bioaccumulation of Zn in isopods is independent on its source. At the moment, the most probable explanation for this is based on the dissolution of Zn from ZnO particles. Dissolution of the highly soluble ZnO presumably results in Zn²⁺ and Zn(OH)⁺ ions (Brunner et al., 2006; Auffan et al., 2009). Franklin et al. (2007) demonstrated that 19% of the nominal total concentration of Zn (100 mg/L) dissolved from nanoparticulate ZnO or bulk ZnO using a dialysis membrane with very fine (approximately 1 nm) pores, permeable to only Zn ions or small Zn complexes. The rate of dissolution is considered to be proportional to the specific surface area, and the dissolved fraction is characteristic of a specific surface area with a faster dissolution of ZnO nanoparticles that particles whose size is on the order of microns. However, other factors such as aggregation of nanoparticles can change this relationship and the dependence of aggregation on the dissolution behavior of particles is not well understood (Borm et al., 2006; Yang and Xie, 2006; Franklin et al., 2007). If it is assumed that the dissolution rate of Zn from particulate ZnO in isopods was as high as 19% as reported by Franklin et al. (2007), then the bioavailable fraction of dissolved Zn would be high enough to reach the isopods’ assimilation ability of Zn (16%). This could explain the same bioaccumulation potential of different origins of Zn for isopods.

Our data on similarity of bioaccumulation levels of different Zn forms are in agreement with data on the toxicity of different types of Zn. Toxicity studies performed on freshwater algae Pseudokirchneriella subcapitata revealed comparable toxicities of ZnO (30 nm in diameter), bulk ZnO and dissolved ZnCl₂ salts (Franklin et al., 2007). Similar results were obtained for this species exposed to nano-ZnO (50–70 nm), bulk ZnO and ZnSO₄ (Arujoa et al., 2009). No differences in toxicity of dissolved ZnCl₂ salts and 1.5 nm ZnO nanoparticles were also found for the nematode Caenorhabditis elegans (Ma et al., 2009).

At the moment, very little is known about expected quantities of nanosized ZnO in environment, therefore the concentrations of Zn²⁺ tested in the present study were derived from our previous work on ZnCl₂ (Drobne and Hopkin, 1995; Bibič et al., 1997). Such high concentrations of Zn²⁺ are still present in some industrially polluted areas (Perez-Lopez et al., 2008). In the future, high environmental levels of ZnO nanoparticles are expected due to predicted frequent use in cosmetics and pharmaceuticals. Based on current knowledge, we can assume, that Zn²⁺ or small inorganic complexes will dissolve from ZnO (Franklin et al., 2007), thus higher pollution with Zn²⁺ is expected.

In studies of the biological potential of nanoparticles, physico-chemical characterization of particles is among the most difficult and complex issues. Among a battery of available techniques is visual inspection of particles (Hassellöv et al., 2008). Among these techniques, transmission electron microscopy (TEM) is most commonly applied for particle characterization. Our study showed that scanning electron microscopy (SEM) is perhaps an even better choice, because it can reveal characteristics which are not evident from TEM micrographs. For example, the ZnO macropowder as well as nanosized ZnO tested in this work had a broad size distribution range which could not be assessed by TEM. In addition, the advantage of SEM is that it allows observation of nanoparticles in a wide range of magnifications and enables precise 3D shape investigation.

We conclude that bioaccumulation of particulate ZnO by isopods follows most probably from Zn dissolution from Zn particles. In the case of isopods, the bioavailable fraction in all three Zn sources was high enough to reach the assimilation capacity, therefore no differences in Zn assimilation efficiency and BAF were seen among animals fed with food dosed with nano-ZnO, ZnO macropowder or ZnCl₂. This suggests that internal distribution and clearance of assimilated metal from the body after transition to clean food would be similar among animals fed with different sources of Zn. Research into this aspect is currently underway.

Conflicts of interest

There are none.

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