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Accumulation and toxicity of metal oxide nanoparticles in a soft-sediment estuarine amphipod

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ABSTRACT

Estuarine and marine sediments are a probable end point for many engineered nanoparticles (ENPs) due to enhanced aggregation and sedimentation in marine waters, as well as uptake and deposition by suspension-feeding organisms on the seafloor. Benthic infaunal organisms living in sediments encounter relatively high concentrations of pollutants and may also suffer toxic effects of ENPs. We tested whether three heavily used metal oxide ENPs, zinc oxide (ZnO), copper oxide (CuO), and nickel oxide (NiO) were toxic to an estuarine amphipod, *Leptocheirus plumulosus*. We used results from 10-day laboratory bioassays to estimate potential demographic impacts of ENP exposure. We also evaluated fate and transport pathways of the ENPs in the experiments to elucidate routes of uptake and exposure. Dissolved Zn was found in sediment pore water and overlying water samples at 10 fold the concentrations of Cu or Ni, a pattern indicative of the relatively high dissolution rate of ZnO ENPs compared with CuO and NiO ENPs. Accumulation of metals in amphipod tissues increased with exposure concentrations for all three ENPs, suggesting possible exposure pathways to higher taxa. Amphipods accumulated $\leq 600 \mu\text{g Zn g}^{-1}$ and $1000 \mu\text{g Ni g}^{-1}$. Amphipod mortality increased with ZnO and CuO concentrations, but showed no significant increase with NiO to concentrations as high as $2000 \mu\text{g g}^{-1}$. The median lethal concentration in sediment (LC_{50}) of ZnO was $763 \mu\text{g g}^{-1}$ and $868 \mu\text{g g}^{-1}$ for CuO ENPs. Our results indicate that ZnO and CuO ENPs, but not NiO ENPs, are toxic to *L. plumulosus* and that ZnO toxicity primarily results from Zn ion exposure while CuO toxicity is due to nanoparticle exposure.

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1. Introduction

Their small size imparts engineered nanoparticles (ENPs) with unique electrochemical properties that make them useful for advanced industrial materials and processes as well as consumer applications. The same properties, however, also raise concerns about ENP behavior and toxicity in the environment. The increased use and production of ENPs over the last decade has led to their release in aquatic environments (Klaine et al., 2008; Keller et al., 2013), and potential negative impacts of ENPs to aquatic fauna and flora have been shown for bacteria (Adams et al., 2006), freshwater

plants and algae (Franklin et al., 2007; Perreault et al., 2010; Saison et al., 2010), marine phytoplankton (Miller et al., 2010, 2012; Gong et al., 2011), as well as freshwater and marine invertebrates (Zhu et al., 2009; Hanna et al., 2013) and fish (Griffitt et al., 2007; Lin et al., 2011; Cedervall et al., 2012). Metal oxide ENPs are of particular concern because they are used in many household products including sunscreens, cosmetics, and bottle coatings, as well as in antifouling paints and construction materials (Klaine et al., 2008; Lee et al., 2010; Keller et al., 2013), and so have a high potential for impacting human and environmental health and accumulating in aquatic environments.

Biological risks of ENP exposure and toxicity in marine environments are probably highest for organisms that live on the seafloor, especially in soft sediments, which make up a large proportion of bottom habitat in estuaries and oceans. Many ENPs and especially metal oxide ENPs aggregate rapidly to the micron scale in seawater and settle from the water column (Keller et al., 2010),

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where they can accumulate in sediments (Buffet et al., 2013). Once in sediments, they may be bound by particulate organic matter (POM), buried, and broken down or transformed by physical and biogenic processes, including bacterial decomposition, bioturbation, or digestion (Farré et al., 2009). Commonly used metal oxide ENPs composed of ZnO, TiO₂, and CeO₂ have rates of aggregation and sedimentation that can vary with ENP concentrations, the ionic strength of water, the amount of suspended natural organic matter (NOM) (Keller et al., 2010), as well as light intensity and temperature (Zhou et al., 2012). For example, ZnO ENPs that enter marine environments will settle (Keller et al., 2010) and dissolve rapidly (Fairbairn et al., 2011), thus exposing infaunal organisms to toxic Zn ions in pore water, or epibenthic animals to dissolved ions at the sediment–water interface.

Marine amphipods are a diverse group of small crustaceans that occupy a wide range of aquatic habitats, including coastal soft-sediment habitats, where they are important prey for many invertebrates (Martin et al., 1989), fish (Duffy and Hay, 2000), birds (Hicklin and Smith, 1984) and mammals (Nerini and Oliver, 1983; Dauby et al., 2005). Feeding strategies of amphipods include predation (Oliver et al., 1982), suspension feeding (Caine, 1977), grazing (Zimmerman et al., 1979), and scavenging (Thurston, 1979), as well as combinations of these strategies. Many amphipods are bioturbators that excavate, turn over, and modify marine sediment, and in the process bury surface materials, resuspend sediment and sediment-bound materials and oxygenate subsurface pore waters (Krantzberg, 1985). Increasing oxygen concentrations in subsurface layers in sediment can oxidize metal compounds and release metal ions, and also promote the growth and metabolism of diverse microbial communities, which further influence metal diagenesis and mobility (Hargrave, 1970). Amphipods are susceptible to contaminants because they ingest sediment-bound materials, or, in the case of metal compounds, are exposed to toxic metal ions when the metals dissolve. Due to their relatively high sensitivity to contaminants and their ecological importance, amphipods are frequently used to test sediment toxicity (Schlekat et al., 1992; Lenihan and Oliver, 1995; DeWitt et al., 2001). Nevertheless, few tests of nanomaterial toxicity with amphipods are published, and of these only two were conducted in sediments (Fabrega et al., 2011; Mwangi et al., 2011).

We examined the solubility, uptake, and toxicity of ZnO, CuO, and NiO ENPs in soft-sediment, estuarine microcosms containing *Leptocheirus plumulosus* in 10 day experiments in order to evaluate the toxicity and predominant routes of exposure and uptake of these high-production nanomaterials. *L. plumulosus* is both a filter and deposit feeder and therefore may be exposed both to metal ions resulting from dissolution and intact ENPs that are ingested as suspended particles or associated with sediments (DeWitt et al., 1992). ZnO, CuO, and NiO ENPs all have the potential to be released into the environment due to increased use in cosmetics (Pitkethly, 2004; Klaine et al., 2008), thin films as antimicrobial agents (Cioffi et al., 2005), and fuel and solar cells (Ahmad et al., 2006). While all three ENPs are of similar size, they differ in terms of dissolution rates and thus the potential to cause metal ion-related toxicity. ZnO ENPs dissolve rapidly in seawater (Keller et al., 2010), and nano-ZnO toxicity appears primarily due to exposure to Zn²⁺ (Miller et al., 2010; Wong et al., 2010). Therefore, we tested the hypothesis that amphipod mortality would be closely correlated with Zn²⁺ concentration in sediment pore waters. We also tested the hypothesis that the toxicity of nano-CuO and nano-NiO was due to exposure and possible ingestion of ENPs because the materials dissolve very slowly in aqueous media (Baek and An, 2011; Buffet et al., 2011). Prior work attributed nano-CuO toxicity in a marine polychaete to ingestion of CuO ENPs, not exposure to Cu²⁺ (Buffet et al., 2013). Alternatively, biogeochemical conditions in sediments, including oxidizing conditions caused by bioturbation and sulfide

concentration, might enhance dissolution, exposing amphipods to elevated metal ions in pore water (Di Toro et al., 1992; Lee et al., 2000).

2. Materials and methods

2.1. Test organisms

L. plumulosus (Family: Aoridae) were obtained from Aquatic Biosystems (Fort Collins, CO, USA) and cultured in polystyrene bins containing 2 l of aerated, filtered seawater adjusted to 17 ppt salinity with deionized water, and a 1 cm thick layer of sediment. The animals were kept on a 16 h light and 8 h dark cycle at 20 °C. All tools, culture bins, and cups were washed in a 5% HNO₃ acid bath and rinsed thoroughly with deionized water prior to use to avoid metal contamination. Sediment was obtained from a local estuary (Goleta Slough, Goleta, CA, USA), sieved through 500 µm mesh, and then rinsed with 17 ppt salinity filtered seawater prior to use. The sediment was a fine to medium sand comprised of approximately 9% silt/clay. Three samples of this sediment were analyzed for total organic carbon (TOC) by drying and acidifying samples with 10% HCl to remove inorganic carbon. An elemental analyzer (CE-440 CHN/O/S, Exeter Analytical Incorporated, North Chelmsford, MA, USA) was then used to determine total carbon. Mean TOC was 0.25 ± 0.02%. Partial water changes were conducted three times per week where approximately 50% of culture water was removed from culture bins and replaced with 17 ppt filtered seawater. Salinity was checked and adjusted by adding deionized water with gentle mixing to maintain a 17 ppt concentration. Amphipods were fed finely ground fish flakes (TetraMin, Blacksburg, VA, USA) after each water change.

2.2. Solubility of ENPs

ZnO ENPs were obtained from Meliorum Technologies (Rochester, NY, USA) and characterized for size, morphology and chemical composition (Godwin et al., 2009; Keller et al., 2010). ZnO ENPs were spheroid, 100% zincite, and 20–30 nm in diameter. CuO and NiO ENPs were obtained from Sigma–Aldrich (St. Louis, MO, USA). CuO characterization has not been published by our group but these ENPs were found to be irregularly shaped, 84.8 ± 2.7% pure (impurities include Na, Ca, Si, and Mg), and 200–1000 nm in diameter, using the same methods as those for ZnO characterization. NiO was characterized previously and described as being irregularly shaped with no detectable impurities and had a primary size of 13.1 ± 5.9 nm (Zhang et al., 2012).

We previously reported dissolution rates for ZnO ENPs in seawater (Fairbairn et al., 2011) and conducted the same experiments for CuO and NiO here. Stock suspensions of 1000 mg l^{−1} were prepared by adding ENPs to deionized water and sonicating for 30 min (Branson model 2510 sonic bath, Danbury, CT). Stock suspensions were then diluted to 10 mg l^{−1} in filtered (0.45 µm) 34 ppt salinity seawater, which has an ionic strength of 0.707 M. These suspensions were placed on a motorized roller at 60 rpm for constant mixing. Temperature was maintained at 22 °C. Aliquots of the suspensions were withdrawal at specified time intervals (minutes to hours for ZnO, days to weeks for CuO and NiO), placed in Amicon Ultra-15 Ultracel 3 centrifuge tubes (3 kDa cutoff ≈ 0.9 nm, Millipore, Billerica, MA), and centrifuged for 30 min at 4000 × g. The filtrate was sampled and analyzed for Zn, Cu, and Ni using inductively coupled plasma atomic emission spectroscopy (ICP-AES, Thermo ICAP 6300, Thermo Fisher Scientific). Samples were run in triplicate, and blank and standard solutions were run every 10 samples.

2.3. Toxicity of ENPs

Toxicity of ENPs to *L. plumulosus* was determined by performing 10 day tests with sediment, modified from Fisher et al. (2000). The 10 day tests were performed on adult amphipods in polyethylene cups containing 25 ml of wet sediment and 60 ml of 17 ppt salinity filtered (0.45 μm) seawater, which were kept at the same light and temperature conditions as culture bins, however, no feed was provided during the exposure. ENPs were mixed into the sediment of each container using a glass stir rod for 1 min to obtain ENP concentrations in the sediment of 0, 500, 1000, 1500, and 2000 $\mu\text{g g}^{-1}$ dry weight. Water was then gently added to avoid suspending the sediment. Adult amphipods were obtained from culture bins by removing amphipods that remained on a 500 μm sieve and adding them to containers immediately after adding water. Containers were then covered and an aerator was added to each container, placed approximately 3 cm above the sediment surface to avoid suspension of ENPs or sediment. Four replicates of each concentration were run, with 10 amphipods in each container.

2.4. Water, sediment, and tissue metals

After 10 days, overlying water was collected by gently pouring water in to centrifuge tubes. Live amphipods were then immediately collected by gently searching through the sediment with forceps and rinsed in deionized water. All sediment was collected using a spatula and placed in centrifuge tubes. Sediment was then centrifuged at 2500 $\times g$ for 10 min and the supernatant (pore water) was collected via pipette in centrifuge tubes. Live amphipods and sediment were dried at 60 °C for 72 h. Overlying water and pore water samples were acidified to 5% acid using trace metal grade HNO_3 (Fisher Scientific, Pittsburgh, PA, USA). Amphipods and 100 mg of sediment were weighed and digested in trace metal grade HNO_3 at 60 °C for 2 h and diluted to 5% acid with purified water. Samples were analyzed for Zn, Cu, and Ni using ICP-AES. Zn was analyzed at 206.2 nm, Cu at 324.7 nm, and Ni at 341.4 nm.

2.5. Statistical analysis

We tested whether ENPs dissolved into pore water and overlying water during our experiment and whether amphipods accumulated metals during the exposure using ordinary least squares (OLS) multiple regression models. We predicted that increased ENP concentrations would result in increased dissolved metals in pore water, overlying water, and the tissues of surviving amphipods. To test these predictions we constructed multiple OLS models as follows:

$$Y = \alpha + \beta_1 \text{Conc} + \varepsilon \quad (1)$$

where Y is the metal concentration in the pore water, overlying water, or in amphipod tissues, α is the metal concentration of the control groups, β_1 is the change in Y with an increase in ENP concentration, Conc is the ENP concentration, and ε is the error not explained by the model.

The median lethal concentrations, LC_{50} , of ENPs were estimated using a logit regression model. The model equation was as follows:

$$\text{Logit}(p_d) = \ln \left(\frac{p_d}{1 - p_d} \right) = \beta_0 + \beta_1 d + \varepsilon \quad (2)$$

where p_d is the probability of dying, β_0 is the log odds of dying when the dose, d , is 0, and β_1 is the change in log odds of dying with a unit increase in the dose. We tested whether the probability of dying varied mainly as a function of sediment metal content – a proxy for ENP concentration – or pore water metal content. We predicted that mortality would be due mainly to dissolution for

Fig. 1. Percent dissolution of ZnO (●), CuO (▼), and NiO (■) in seawater over time. All suspensions were prepared at 1000 mg l^{-1} in deionized water and diluted to 10 mg l^{-1} in seawater containing 10 mg l^{-1} alginate. ZnO dissolved very rapidly, followed by NiO, and very little CuO dissolved after 90 days. ZnO data from Fairbairn et al. (2011).

ZnO ENPs but that mortality for amphipods exposed to CuO or NiO ENPs would be due to the sediment metal content. To test these predictions we adapted Eq. (2) as follows:

$$\text{Logit}(p_d) = \ln \left(\frac{p_d}{1 - p_d} \right) = \beta_0 + \beta_1 \text{Sed} + \beta_2 \text{PW} + \varepsilon \quad (3)$$

where p_d is the probability of dying, β_0 is the log odds of dying when the dose, d , is 0, β_1 is the change in log odds of dying with a unit increase in the sediment metal content, β_2 is the change in log odds of dying with a unit increase in pore water metal content, and ε is the error not explained by the model. All statistical tests were performed using R (The R Foundation for Statistical Computing, version 2.10.1).

3. Results

3.1. Solubility of ENPs

ENPs had drastically different dissolution rates in seawater: more than 65% of ZnO dissolved within 3 days (Fairbairn et al., 2011), while 21% of NiO dissolved after 28 days, and only 1% of CuO dissolved after 90 days (Fig. 1). These data confirmed that ZnO ENPs dissolve much faster than CuO or NiO ENPs in seawater but also indicate that NiO ENPs dissolve faster than CuO ENPs. While the majority of ZnO ENP dissolution took place within the first hour after addition to seawater, NiO ENPs dissolved over several weeks, and CuO slowly dissolved over several months.

3.2. Toxicity of ENPs

Mortality of amphipods increased in a dose dependent manner with ZnO and CuO ENP exposure, however NiO ENPs did not affect mortality in our study (Fig. 2). LC_{50} for ZnO and CuO ENPs in sediment was $763 \pm 64 \mu\text{g g}^{-1}$ and $868 \pm 89 \mu\text{g g}^{-1}$ dry weight, respectively. Mortality increased significantly with pore water Zn concentrations, but not sediment-bound Zn, while for Cu, mortality increased significantly with sediment-bound Cu, but not pore water Cu (Table S1). The LC_{50} values based on the pore water metals from these same exposures were $0.50 \pm 0.05 \mu\text{g Zn ml}^{-1}$ and $0.17 \pm 0.02 \mu\text{g Cu ml}^{-1}$. Mean mortality of amphipods in control sediment was $17 \pm 5\%$ and remained $\leq 20\%$ in all concentrations of NiO ENPs used.

Fig. 2. Mean mortality $\pm$ 1 SE of *L. plumulosus* after 10 day exposure to ZnO (●), CuO (▽), or NiO (■) ENPs in sediment ($n=4$ microcosms, each with 10 amphipods). LC_{50} for ZnO and CuO ENPs is $763 \pm 64 \mu\text{g g}^{-1}$ and $868 \pm 89 \mu\text{g g}^{-1}$, respectively, which correspond to pore water concentrations of $0.50 \pm 0.05 \mu\text{g ml}^{-1}$ for Zn and $0.17 \pm 0.06 \mu\text{g ml}^{-1}$ for Cu. Mean mortality for all NiO ENP exposures was $\leq 20\%$.

3.3. Water, sediment, and tissue metals

Pore water and overlying water concentrations of Zn, Cu, and Ni from bioassay containers increased with increasing concentrations of ZnO, CuO, and NiO ENPs after 10 days (Table S2), and did so in a generally linear manner (Fig. 3A and B). At our highest exposure concentration, mean Zn concentration in pore water was $2.00 \pm 0.4 \mu\text{g l}^{-1}$ and in overlying water was $1.31 \pm 0.1 \mu\text{g l}^{-1}$. Mean Cu concentration in pore water was $0.37 \pm 0.1 \mu\text{g l}^{-1}$ and in overlying water was $0.32 \pm 0.1 \mu\text{g l}^{-1}$. Mean Ni concentration in pore water was $0.17 \pm 0.02 \mu\text{g l}^{-1}$ and in overlying water was $0.55 \pm 0.2 \mu\text{g l}^{-1}$. Metal ion concentrations in both pore water and overlying water samples from control treatments were $<0.02 \mu\text{g ml}^{-1}$. At our highest exposure concentration, sediment metal concentrations averaged $901 \pm 120 \mu\text{g Zn g}^{-1}$, $1098 \pm 37 \mu\text{g Cu g}^{-1}$, and $851 \pm 176 \mu\text{g Ni g}^{-1}$ dry weight. Control sediment contained mean concentrations of $88 \pm 3 \mu\text{g Zn g}^{-1}$, $90 \pm 7 \mu\text{g Cu g}^{-1}$, and $12 \pm 2 \mu\text{g Ni g}^{-1}$. Concentration of Zn, Cu, and Ni in amphipods increased linearly with the concentration of each ENP (Fig. 3C). Amphipods in control groups had $123 \pm 9 \mu\text{g Zn g}^{-1}$, $148 \pm 10 \mu\text{g Cu g}^{-1}$, and $26 \pm 2 \mu\text{g Ni g}^{-1}$ dry weight. When exposed to $2000 \mu\text{g g}^{-1}$, the highest exposure concentration, amphipods had $585 \pm 9 \mu\text{g Cu g}^{-1}$ and $1028 \pm 44 \mu\text{g Ni g}^{-1}$ dry weight. We could not measure Zn in groups exposed to ZnO ENPs $\geq 1500 \mu\text{g g}^{-1}$ due to low survival of amphipods at these concentrations.

4. Discussion

We tested the hypotheses that ZnO ENPs were toxic to amphipods due mainly to dissolution and that CuO and NiO ENPs were toxic due mainly to ingestion of ENPs. Our results show that ZnO and CuO ENP toxicity is nearly equivalent, despite Cu being much more toxic to amphipods than Zn in seawater and sediment bioassays (King et al., 2006a). This pattern likely resulted from the greater dissolution rate of ZnO ENPs compared with CuO ENPs, and thus greater exposure of amphipods to Zn ions than Cu ions. Although NiO ENPs were not toxic at the concentrations tested, Ni LC_{50} values were $>3000 \mu\text{g l}^{-1}$ for *Hyaella azteca* (Keithly et al., 2004) and $>30,000 \mu\text{g l}^{-1}$ for *Allorchestes compressa* (Ahsanullah, 1982), suggesting very high concentrations are needed to illicit a toxic response. However, amphipods had higher concentrations of

Fig. 3. Mean Zn (●), Cu (▽), or Ni (■) concentrations $\pm$ 1 SE in overlying water (A), sediment pore water (B), and amphipod tissues (C) after 10 day exposure to ZnO, CuO, or NiO ENPs in estuarine sediment ($n=4$ microcosms, each with 10 amphipods). Lines are ordinary least squares regression fits (for equations see Table S1). Zn was found in higher concentrations than Cu or Ni in water samples but amphipod tissues accumulated more Ni than Zn or Cu. We could not measure Zn accumulation in groups exposed to ZnO ENPs $\geq 1500 \mu\text{g g}^{-1}$ due to low survival.

Ni than Zn or Cu in our study, suggesting that Ni may be transferred to higher trophic levels to a greater extent than Zn or Cu.

Although Zn and Cu are known to be toxic to marine invertebrates, few studies have examined toxicity of these metals in the ENP form, and even fewer have compared the toxicity of these ENPs. Estuarine amphipods accumulated Zn and exhibit toxic responses when exposed to ZnO ENPs, although these impacts did not differ from ionic or bulk forms of Zn (Fabrega et al., 2011). Marine worms and clams exposed to CuO ENPs also accumulated Cu and showed toxic effects and these impacts differed only slightly from ionic or bulk forms of Cu (Buffet et al., 2013). However, ZnO ENPs were found to be 17 times more toxic to algae than CuO ENPs (Aruoja et al., 2009). This information contrasts with previous work showing that ionic Cu is 3–6 times more toxic than Zn to amphipods

(King et al., 2006a) and stresses the need to understand ENP chemistry and dissolution kinetics as well as taxon-specific sensitivity when considering potential environmental consequences of ENPs. Although our LC₅₀ estimates for ZnO and CuO ENPs fall within the range reported in the literature for dissolved metal ions, these ranges span several orders of magnitude due to differences in laboratory procedures, sediment characteristics, and the species of amphipod tested (Bat and Raffaelli, 1998; King et al., 2006b). LC₅₀ estimates from 96 h tests in water are typically more precise than sediment bioassay estimates, probably due to variability in sediment composition and heterogeneity in dispersion of contaminants through the sediment. LC₅₀ values from water tests, moreover, are similar to pore water LC₅₀ estimates from 10 day sediment bioassays (Lee et al., 2004). Reported LC₅₀ estimates for Zn and Cu in 96 h water tests are 0.9 µg Zn ml⁻¹ (Lee et al., 2004) and 0.1 µg Cu ml⁻¹ (Güven et al., 1999). These estimates are very similar to our pore water results and suggest that the similarities between Zn and Cu toxicity are due to the combined effect of low dissolution of CuO ENPs and greater toxicity of Cu compared to Zn. ENP or metal ion toxicity can potentially result from adherence to body surfaces or uptake via respiration or ingestion (Klaine et al., 2008) followed by membrane or protein disruption, or from production of reactive oxygen species (ROS). These injuries impact electron transport, respiration, and the reduction of energy supplies (Klaine et al., 2008), as well as physiological injuries that influence demographic responses, including growth, reproduction, and survival (Buffet et al., 2013).

Metal concentrations in amphipods increased with exposure to all three metal oxide ENPs in this study, indicating the potential for the transfer of either ionic metals or ENPs within marine food webs. The uptake of metals by estuarine invertebrates is assumed to be via passage across permeable membranes for dissolved forms (Rainbow, 2007) and ingestion for particulate forms (Luoma, 1989). In our study, the majority of the ENPs remained in particulate form or bound to sediment particles, as shown by the low concentrations of metals in the water relative to the amount of ENPs added. Therefore, ingestion was likely a major route of uptake in our experiments. Amphipods are known to ingest up to three times their body weight in sediment per day (Schlekat et al., 2000). Uptake of quantum dots via ingestion was shown previously for *L. plumulosus* (Jackson et al., 2012). Amphipods are an important food source for many marine birds, fish, and invertebrates and thus may contribute to the accumulation of these contaminants in other organisms that may otherwise not be exposed to them. Further work is needed to understand the trophic transfer of ENPs in aquatic ecosystems.

Sediment is thought to be a major sink of metal oxide ENPs due to aggregation, binding (Klaine et al., 2008; Keller et al., 2010), and deposition by benthic suspension feeders, e.g. mussels (Montes et al., 2012). Our study suggests that for organisms living within estuarine or marine sediments, ENPs that dissolve readily are potentially more toxic than those which remain sediment bound, even when the metal in the readily-dissolved material is less toxic than the metal in the slowly-dissolving material. However, filter feeding organisms may be more susceptible to ENPs that dissolve rapidly while deposit feeders may be more affected by slow dissolving ENPs. Furthermore, slowly-dissolving ENPs may build up to a greater degree in sediment, leading to higher levels of contamination in the long term. In our study, the impacts were similar for *L. plumulosus* exposed to ZnO or CuO ENPs, but for organisms with different feeding habits, this would probably differ. Amphipods play a key role in estuarine and coastal ecosystems as prey and bioturbators, and our study suggests that ENPs that build up in sediments over time will be accumulated by amphipods and reduce their survival, which would directly impact higher trophic levels by reducing their food supply and exposing them to ENPs. Future work in this field should focus on the fate and transport of ENPs

in natural media containing organisms with a variety of ecological traits, including feeding modes, as well as the potential for trophic transfer and biomagnification in higher taxa.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.aquatox.2013.09.019>.

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