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CeO_2 nanoparticles induce no changes in phenanthrene toxicity to the soil organisms *Porcellionides pruinosus* and *Folsomia candida*

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ABSTRACT

Cerium oxide nanoparticles (CeO_2 NPs) are used as diesel fuel additives to catalyze oxidation. Phenanthrene is a major component of diesel exhaust particles and one of the most common pollutants in the environment. This study aimed at determining the effect of CeO_2 NPs on the toxicity of phenanthrene in Lufa 2.2 standard soil for the isopod *Porcellionides pruinosus* and the springtail *Folsomia candida*. Toxicity tests were performed in the presence of CeO_2 concentrations of 10, 100 or 1000 mg Ce/kg dry soil and compared with results in the absence of CeO2 NPs. CeO_2 NPs had no adverse effects on isopod survival and growth or springtail survival and reproduction. For the isopods, LC50s for the effect of phenanthrene ranged from 110 to 143 mg/kg dry soil, and EC50s from 17.6 to 31.6 mg/kg dry soil. For the springtails, LC50s ranged between 61.5 and 88.3 mg/kg dry soil and EC50s from 52.2 to 76.7 mg/kg dry soil. From this study it may be concluded that CeO_2 NPs have a low toxicity and do not affect toxicity of phenanthrene to isopods and springtails.

1. Introduction

Engineered nanoparticles (NPs) are defined as industrially produced materials in which >50% of the particles have a size distribution ranging from 1 to 100 nm at least in one of their dimensions (European Commission, 2011). New applications of NPs are constantly being developed, resulting in the introduction to the market of diverse products containing NPs (Roco et al., 2011). Within commercially available NPs, CeO_2 NPs have drawn attention due to their potential use as fuel additive in diesel (Cassee et al., 2011; Johnson and Park, 2012). CeO_2 NPs enhance the oxidation rate and decrease the emission of particulate matter during combustion (Jung et al., 2005). Diesel fuel additives show promising improvements for emission reductions (Park et al., 2008). Nevertheless, the potential release of CeO_2 NPs in combination with other emission products in the environment is still unknown. Also mixture toxicology of CeO_2 NPs and organic pollutants in soil has not been studied so far. Predictions of CeO_2 NPs in soils showed an environmental concentration of 0.016 mg/kg in (20-m) areas next to urban roads in the United Kingdom, after 7 years of deposition and are expected to increase up to 0.04 mg/kg after 12 years of deposition (Johnson and Park, 2012). Adverse effect concentrations, however, have shown to be several orders of magnitude above predicted concentrations in the environment (Batley et al., 2013; Johnson and Park, 2012; Park et al., 2008). Studies have suggested that CeO_2 NPs could work as an antioxidant or free radical scavenger leading therefore to a low toxicity (Colon et al., 2010; Schubert et al., 2006; Xia et al., 2008). By comparing CeO_2 NPs and ZnO NPs, Xia et al. (2008) found different mechanisms of toxicity in cellular responses. While ZnO and TiO_2 generated reactive oxygen species (ROS) leading to cell death, CeO_2 NPs showed a protective response by suppressing ROS production.

CeO_2 NPs are likely to co-exist with polycyclic aromatic hydrocarbons (PAHs), as both are released during the combustion process of diesel fuel (Hendren et al., 2011; Park et al., 2008; Scheepers and Bos, 1992; Tavares Jr. et al., 2004). Nanoparticles have a proportionately very large surface area and this surface can have a high affinity for organic chemical combustion products such as PAHs (Moore, 2006). Few toxicity studies have assessed the effects of the combination of NPs and PAHs (Baun et al., 2008; Cui et al., 2011; Hu et al., 2008; Yang and Watts, 2005). Hu et al. (2008) showed that, fullerene C_{60} decreased the bioavailability of phenanthrene by decreasing the freely dissolved concentration in water. Still, phenanthrene sorbed to C_{60} was found to be available

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to the algae *Raphidocelis subcapitata* (previously known as *Pseudokirchneriella subcapitata)* and the crustacean *Daphnia magna* (Baun et al., 2008).

Phenanthrene is a major component of diesel exhaust particles and therefore a common pollutant in the environment. Phenanthrene concentrations in soil vary between urban and unpolluted rural or forest areas, with urban soils presenting higher levels due to anthropogenic emissions. However, a large variation in phenanthrene concentrations in soils can be found. Phenanthrene concentrations were estimated at ~500 μg/kg in urban soils (Wild and Jones, 1995), ~700 μg/kg in street dust particles in Copenhagen (Johnsen et al., 2006), and only 43 μg/kg in urban areas of Tarragona County (Nadal et al., 2011). In unpolluted areas in the UK and Norway, phenanthrene concentrations ranged between 42 to 54 μg/kg (Nam et al., 2008), while in Tarragona County 7 μg/kg was found (Nadal et al., 2011).

No data on combined effects of CeO_2 NPs and phenanthrene is available in the literature for soil organisms. And for the isopod *Porcellionides pruinosus* no data on the toxicity of phenanthrene is available at all. This study therefore aimed at evaluating the influence of CeO_2 NPs on the toxicity of phenanthrene to two soil organisms, the isopod *P. pruinosus* and the springtail *Folsomia candida*. For that purpose, feeding inhibition and reproduction tests were performed with *P. pruinosus* and *F. candida*, respectively. These organisms were chosen because they represent two common groups of organisms found in terrestrial environments, and they present different routes of exposure. Springtails are mainly exposed to dermal uptake from the pore water (Smit and Van Gestel, 1998), while isopods are mainly exposed by oral uptake of soil particles (Vijver et al., 2006).

Three scenarios are possible for the outcome of mixture toxicity tests with phenanthrene and CeO_2 NPs. In a first scenario, the toxicity of the mixture of phenanthrene and CeO_2 NPs is additive, with CeO_2 NPs showing almost no toxicity from the mixture of phenanthrene and CeO_2 NPs compared to phenanthrene alone. In a second scenario CeO_2 NPs act as a synergist and increase toxicity of phenanthrene. Nanoparticles may act as a Trojan horse by enhancing the transport of phenanthrene to intracellular receptors (Farkas et al., 2012), which could lead to a higher toxicity of the mixture. In the third scenario CeO_2 NPs could act as an antagonist and reduce the toxicity of phenanthrene. This could, for instance, be achieved by binding of phenanthrene to the NPs preventing it from entering the cells (Walker et al., 2012).

2. Materials and methods

2.1. Organisms

2.1.1. Porcellionides pruinosus

Specimens of the isopod *P. pruinosus* were collected from an unpolluted field in Coimbra (Portugal). The animals were cultured in plastic boxes (24×24 cm^2) containing potting soil at 20 ± 2 °C and 16/8 h photoperiod, and fed with alder (*Alnus glutinosa*) leaves. The animals were kept in lab conditions for at least one month before exposure.

2.1.2. Folsomia candida

F. candida ("Berlin strain"; VU University, Amsterdam) was cultured in transparent plastic boxes (250 cc) with a moist bottom of plaster of Paris containing charcoal (plaster of Paris: charcoal 12:1 (w/w)) at 20 ± 1 °C at a light/dark regime of 12/12 h. Toxicity tests were initiated with juveniles of the same age (10–12 days) that were obtained by synchronizing the egg laying of the culture animals, fed with dried baker's yeast (Dr. Oetker, Leeuwarden, The Netherlands).

2.2. Soil and pH measurements

Standard Lufa 2.2 natural soil (LUFA-Speyer 2.2, Germany) was used as test soil. This soil is characterized as sandy loam with an organic carbon content of 2.3 ± 0.2%, pH (0.01 M $CaCl_2$) of 5.6 ± 0.4, cation exchange capacity (CEC) of 10.0 meq/100 g and water-holding capacity (WHC) of 46.5%.

Soil pH_{CaCl2} of the test soils was measured at the beginning of the toxicity tests. Spiked soils (5 ± 0.1 g) were shaken with 25 ml 0.01 M $CaCl_2$ solution for 2 h. After settlement of the particles, the pH of the soil solution was recorded using a Consort P907 meter.

2.3. Test compounds and spiking of the soil

Phenanthrene supplied by Sigma-Aldrich (98% purity) was used. CeO_2 NP powder was manufactured by Antaria with a primary particle size of approximately 10–50 nm. Fig. 1 shows the

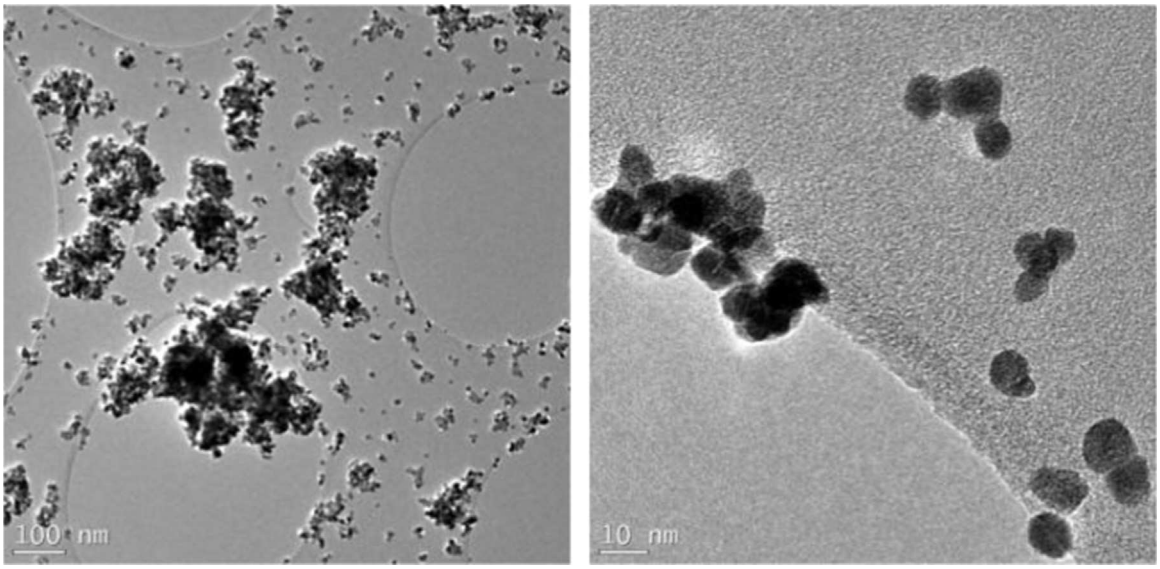

Fig. 1. Transmission Electron Micrographs of CeO_2 NPs, when deposited on a carbon coated Cu TEM grid after dispersion of 1 mg/ml in deionised water and sonicated for 30 s in a low power ultrasonic bath.

nanoparticles when deposited on a carbon coated Cu TEM (Transmission Electron Microscopy) grid after dispersion of 1 mg/ml in deionised water and sonication for 30 s in a low power ultrasonic bath.

Soil exposures consisted of three CeO_2 NPs concentrations, namely 10, 100 and 1000 mg Ce/kg dry soil and six concentrations of phenanthrene, namely 15, 30, 60, 120, 240, and 480 mg/kg dry soil for isopods and 5, 10, 20, 40, 80 and 160 mg/kg dry soil for springtails. Only three concentrations of CeO_2 NPs were tested, based on the lack of effects on reproduction that CeO_2 NPs have shown in an earlier study with earthworms at 10,000 mg Ce/kg dry soil (Lahive et al., 2014). Phenanthrene concentrations were chosen based on previous studies for springtails (Droge et al., 2006). Since no previous work on effects of phenanthrene to isopods via soil was available, a broader concentration range was tested.

CeO_2 NPs were added to a large batch of dry soil to reach nominal concentrations of 10, 100 and 1000 mg Ce/kg dry soil. A total of 120 and 200 g of soil were used in each treatment for the toxicity test with isopods and springtails, respectively. Phenanthrene was dissolved in acetone ($\geq$ 99% purity, Sigma-Aldrich) and added to a small amount of the CeO_2 NP spiked soil first, i.e. corresponding with 20 and 50 g dry soil for the isopod and springtail tests, respectively. Then, soil samples were equilibrated in closed pots for 24 h. After equilibration, the pots were opened to let the acetone evaporate overnight. Then, the remaining soil spiked with CeO_2 NPs, i.e. 100 and 150 g dry soil for the isopod and springtail test, respectively, was added to the soil spiked with phenanthrene. Soils were mixed thoroughly with a kitchen spoon and Milli-Q water was added to obtain a moisture content of 23.3% (w/w), corresponding to 50% of the WHC. Water and acetone controls were also prepared.

2.4. Toxicity tests

Toxicity tests were conducted with both compounds (CeO_2 NPs and phenanthrene) simultaneously with combined exposures under a full factorial design. A feeding inhibition test was conducted with isopods, using 8 replicates per treatment. For the springtails, a reproduction test was conducted with 5 replicates per treatment.

2.4.1. Porcellionides pruinosus

Adult isopods (15–25 mg), both male and non-gravid females, were selected for the feeding inhibition test (Silva et al., 2014; Tourinho et al., 2013). The animals were exposed individually in plastic boxes containing 10 g of moist soil. For each treatment, 8 replicates were used. The boxes were incubated at 20 $\pm$ 1 °C and a light/dark regime of 12/12 h. Alder leaves previously cut in disks (Ø 10 mm) were offered as food ad libitum. Soil moisture content was adjusted after 7 days by weighing the test containers. Survival, food consumption (mg food/mg isopod) and changes in biomass (% of fresh weight) were evaluated after 14 days.

2.4.2. Folsomia candida

The ISO guideline 11267 for testing chemical effects on the reproduction of springtails was followed (ISO, 1999). Tests were conducted in 100 ml glass jars containing 30 g moist soil and five replicates for each treatment were prepared. At the start of the test, 10 synchronized animals were transferred into each test jar. The jars were filled randomly and before introduction, the animals were checked under the microscope for a healthy appearance. The animals were fed a few grains of dried baker's yeast (Dr. Oetker). The jars were incubated in a climate room at 20 $\pm$ 1 °C and a light/dark regime of 12/12 h. Once a week, soil moisture content was checked by weighing the jars, and moisture loss was replenished with Milli-Q water when necessary. The jars were also aerated by this procedure.

After four weeks, the jars were sacrificed for determination of springtail survival and reproduction. Each jar was emptied into a 200 ml beaker glass and 100 ml Milli-Q water was added. The mixture was stirred carefully to let all the animals float to the surface. The number of adults and juveniles were counted manually after taking a picture of the water surface using a digital camera (Olympus, C-5060).

2.5. Data analysis

Concentrations causing 50% mortality (median lethal concentrations or LC50s) of *P. pruinosus* were calculated by the Trimmed Spearman–Karber method (Hamilton et al., 1977). Food consumption ratio (Cr) and biomass change (B) were calculated as:

$$Cr = (W_{li} - W_{lf})/W_{isop}$$

$$B = \left(W_{isopf} - W_{isop}\right)/W_{isop} * 100$$

where, Cr is the consumption ratio (mg leaf/mg isopod), W_{li} the initial leaf weight (mg dw), W_{lf} the final leaf weight (mg dw), W_{isop} the initial isopod weight (mg fw), B the biomass change (%), and W_{isopf} the final isopod weight (mg fw).

Isopod consumption ratio was analyzed by a two-way Analysis of Variance (ANOVA) followed by Fisher LSD post-hoc test, after log-transformation. Data homoscedasticity and normality were tested by Levene's test and the Kolmogorov–Smirnov test, respectively. EC50 values for effect on isopod biomass change were calculated applying a 4-parameter logistic model ($Y = Y_{min} + (Y_{max} - Y_{min})/(1 + (X/EC50)^{-b})$). To assess effects of CeO_2 NPs, biomass of isopods exposed only to CeO_2 NPs was analyzed by one-way ANOVA. LC50 for effects on survival and EC10 and EC50 values for effects on springtail reproduction were estimated applying the logistic model of Haanstra et al. (1985). A generalized likelihood ratio test (Sokal and Rohlf, 1995) was applied to compare LC50, EC10 and EC50 values obtained for each treatment. Exceptions were the LC50 values for isopods, in which differences were assessed using the overlap of 95% confidence intervals. All calculations were performed in SPSS Statistics 20.

3. Results

3.1. Soil properties

Soil pH_{CaCl2} was not affected by the addition of CeO_2 NPs or phenanthrene and ranged between 5.40 and 5.49 for *F. candida* and between 5.53 and 5.71 for *P. pruinosus.* TEM images showed that primary particle size was ~10–50 nm, and mostly small (< 100 nm) and medium sized agglomerates/aggregates (100–300 nm) were found as well as some large (micron sized) agglomerates.

3.2. Toxicity to Porcellionides pruinosus

No mortality was observed in the control soil, while one out of eight isopods died (13%) in the acetone control. No mortality was observed for isopods exposed to CeO_2 NPs up to 1000 mg Ce/kg dry soil. Phenanthrene had a dose-related effect on isopod survival, with LC50s ranging from 110 to 143 mg/kg dry soil with no significant effect of CeO_2 NPs as shown by the overlap of the 95% confidence intervals (Table 1).

Because high mortality (> 50%) was observed at phenanthrene concentrations above 120 mg/kg dry soil, the surviving animals

Table 1
LC50 and EC50 values with 95% confidence interval for the toxicity of phenanthrene at different concentrations of CeO_2 (mg Ce/kg dry soil) to *Porcellionides pruinosus* exposed to Lufa 2.2 soil for 14 days. LC50 values were obtained with the Trimmed Spearman–Karber (TSK) method (Hamilton et al., 1977) and EC50 values by logistic regression.

Compound(s)	LC50 (mg Phe/kg dry soil)	EC50 (mg Phe/kg dry soil)
Phenanthrene (Phe)	137 (101–187)	18.8[a] –
Phe + 10 mg Ce/kg	110 (79–153)	26.1[a] –
Phe + 100 mg Ce/kg	128 (92–176)	17.6[a] –
Phe + 1000 mg Ce/kg	143 (107–190)	31.6[a] –

– Data did not allow calculating reliable 95% confidence intervals.

[a] Indicate significant differences between EC50 values according to a generalized likelihood-ratio test ($X^2_{df} > 3.84$; $p < 0.05$).

Isopod biomass was not affected by CeO_2 NPs, and no difference between animals exposed to CeO_2 NPs and control was found (One-way ANOVA, $p > 0.05$). Biomass decreased in a dose-related manner with increasing phenanthrene concentration (Fig. 2B). EC50 values for phenanthrene toxicity in the presence of CeO_2 NPs ranged from 17.6 to 31.6 mg/kg dry soil, and were not significantly different from each other according to a generalized likelihood-ratio test ($X^2_{df=1} < 3.84$, $p < 0.05$) (Table 1).

3.3. Toxicity to Folsomia candida

The mortality of adult springtails in the water control was 20% and in the acetone control 14%. The average number of juveniles in the control soil was 322 and in the acetone control 391. The coefficient of variation for reproduction was 18.9% in the control and 14.3% in the acetone control. According to ISO guideline 11267 all test validity criteria were met (ISO, 1999).

Survival and reproduction of *F. candida* were reduced in a dose-dependent manner by phenanthrene. The fits obtained with a logistic model for the effect of phenanthrene in the presence of different concentrations of CeO_2 NPs on the survival and reproduction are shown in Fig. 3.

Table 2 summarizes the EC10, EC50 and LC50 values for the

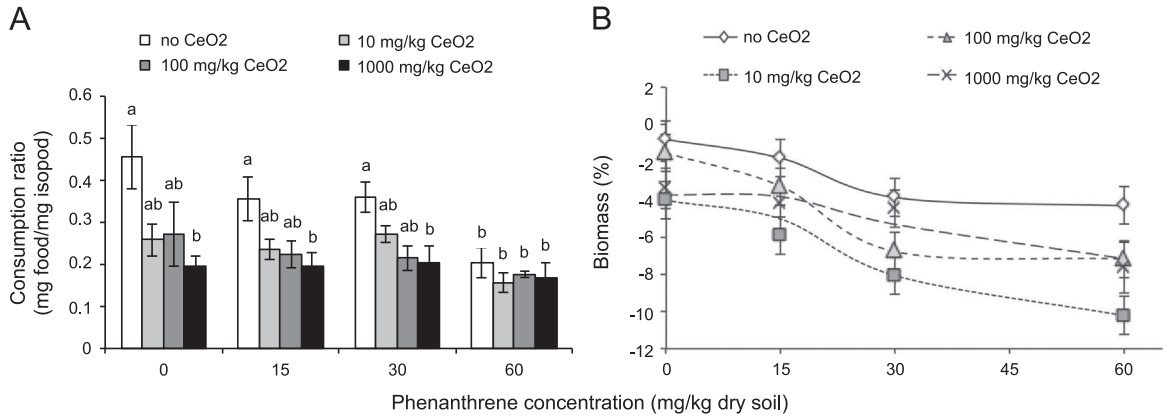

Fig. 2. Consumption ratio (A) and biomass (B) of the isopod *Porcellionides pruinosus* exposed for 14 days to phenanthrene in the presence of CeO_2 NPs in Lufa 2.2 soil. Lines represent fit obtained with a four-parameter logistic model. Biomass is expressed as % of gain/loss of mass relatively to the weight of the organisms in the beginning of the test.

were excluded from further analysis for consumption ratio and biomass change. Consumption ratio was affected by CeO_2 NPs and by phenanthrene (Two-way ANOVA, Fisher LSD test, $p < 0.05$) (Fig. 2A). No significant interaction was found between CeO_2 NPs and phenanthrene (Two-way ANOVA, $p > 0.05$).

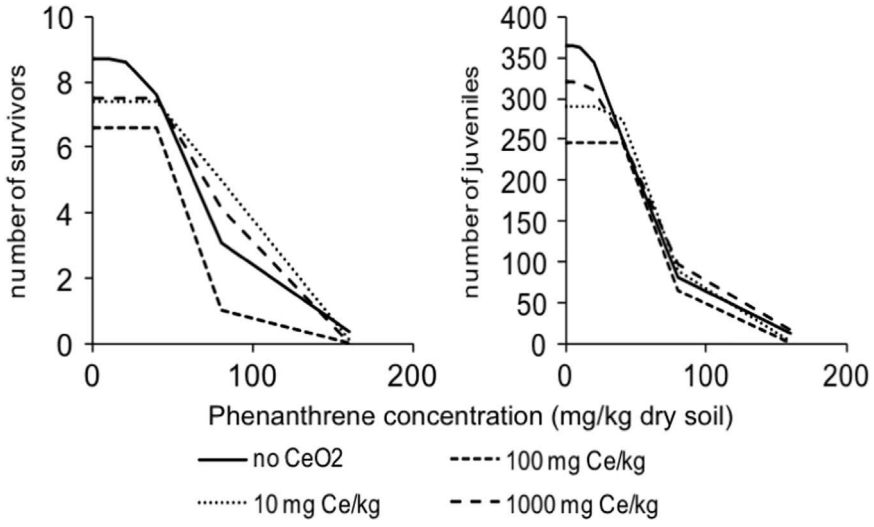

Fig. 3. Dose–response curves obtained with a logistic model for the effect of phenanthrene in the presence of CeO_2 NPs in Lufa 2.2 soil on the survival (left) and reproduction (right) of *Folsomia candida* after 28 days exposure.

Table 2
EC10, EC50 and LC50 values with 95% confidence interval, for the toxicity of phenanthrene (Phe) in the presence of CeO_2 NPs to *Folsomia candida* after 28 days in Lufa 2.2 soil.

Compound(s)	LC50 (mg Phe/kg)	EC50 (mg Phe/kg)	EC10 (mg Phe/kg)
Phenanthrene (Phe)	67.7[a] (58.7–76.7)	52.2[a] (38.3–66.1)	24.8[a] (10.3–39.2)
Phe + 10 mg Ce/kg	88.3[b] (49.0–128)	68.5[a] (50.6–86.5)	45.2[ab] (12.1–78.2)
Phe + 100 mg Ce/kg	74.8[a] –	76.7[a] –	69.7[b] –
Phe + 1000 mg Ce/kg	65.1[ab] (49.3–80.9)	60.8[a] (45.8–75.8)	29.4[ab] (13.3–45.5)

– Data did not allow calculating reliable 95% confidence intervals.

[ab] Indicate significant differences between EC50 or EC10 values according to a generalized likelihood-ratio test ($X^2_{df} > 3.84$; $p < 0.05$).

toxicity of phenanthrene for *F. candida* in the absence and presence of CeO_2 NPs. LC50 values ranged from 65.1 to 88.3 mg/kg dry soil, EC50s from 52.2 to 76.7 mg/kg dry soil and EC10s from 24.8 to 69.7 mg/kg dry soil. According to a likelihood-ratio test, the LC50 for phenanthrene with 10 mg Ce/kg dry soil was significantly lower than that of phenanthrene alone ($X^2_{df=1}=5.28$) and phenanthrene with 100 mg Ce/kg ($X^2_{df=1}=7.02$, $p<0.05$). The other LC50s were not significantly different from each other. EC50s did not significantly differ from each other according to the likelihood-ratio test. The EC10 for the toxicity of phenanthrene alone was significantly lower than that for phenanthrene with 100 mg Ce/kg ($X^2_{df}=4.04$, $p<0.05$), while the other EC10 values were not significantly different from each other.

4. Discussion

Combined exposure to CeO_2 NPs and phenanthrene is likely to occur in the environment, because both compounds are present in diesel fuel. The use of CeO_2 NPs provides advantages in reducing emission during fuel combustion. This study showed that the release of CeO_2 NPs with phenanthrene into the environment does not lead to an enhanced toxicity of the latter to soil arthropods. It also showed that CeO_2 NPs have low toxicity to soil arthropods.

Many components are influencing CeO_2 behavior and its interaction with phenanthrene in soils. Soil organic matter content, for example, is an important factor affecting CeO_2 NP behavior (Batley et al., 2013; Cornelis et al., 2011; Zhao et al., 2012). Organic matter content controls CeO_2 NP bioavailability, as it determines the partitioning of CeO_2 NPs between the soil and the soil solution (Zhao et al., 2012). Cornelis et al. (2011) have shown that CeO_2 NPs are predominantly negatively charged in soils due to the adsorption of phosphate and organic molecules. As a result, positively charged clays attract CeO_2 NPs. The dissolution of Ce from CeO_2 NPs was very low and no levels of Ce could be detected after ultrafiltration (Cornelis et al., 2011). In our study, the organic carbon content of Lufa 2.2 soil (2.3%) may have resulted in a low release of ionic Ce and in a strong binding of CeO_2 NPs to organic matter. This could have resulted in low bioavailability and explain the low toxicity of the CeO_2 NPs.

The binding of phenanthrene to CeO_2 NPs in soil remains uncertain. In water, it has been shown that the binding of phenanthrene to NPs can be explained by electron donor–acceptor interactions (Fang et al., 2008; Farkas et al., 2012). In spiked soils, however, the characterization of NPs, and especially the binding of an organic pollutant to metallic nanoparticles, is extremely difficult, and therefore not included in this study.

So far, studies on the toxicity of CeO_2 NPs to soil invertebrates were mainly restricted to the nematode *Caenorhabditis elegans* and recently a study of CeO_2 NPs toxicity for the earthworm *Eisenia fetida* has been published (Lahive et al., 2014). Zhang et al. (2011) showed that *C. elegans* survival was affected by a concentration of 1 nM. Due to the low dissolution of CeO_2 NPs into ionic Ce forms in nematode growth media, the authors suggested that uptake of nanosized Ce occurred. Toxicity of CeO_2 NPs with different functionalized surfaces, including positive, negative and neutral charges, has been reported in moderately hard reconstituted water (MHRW) (Collin et al., 2013). Positively charged CeO_2 NPs were found to be more toxic and showed higher bioaccumulation in *C. elegans* than negatively charged or neutral NPs. Moreover, the authors also found that the presence of humic acid decreased the toxicity of CeO_2 NPs and had a greater influence on toxicity than the NP surface charge (Collin et al., 2013). For earthworms, however, no effects on survival and reproduction were observed up to 10,000 mg/kg dry soil, although Ce body concentration increased in a dose-related manner with soil Ce concentration, and exceeded 100 µg/g dw at the higher exposures (Lahive et al., 2014). Because they did observe histological changes in Ce-exposed animals, Lahive et al. (2014) did not exclude a long-term effect of CeO_2 NPs. The latter study is in agreement with our findings, where CeO_2 NPs did not show toxicity to the isopod *P. pruinosus* and the collembolan *F. candida* at high exposure concentrations. This could be explained by the much lower bioavailability of CeO_2 NPs in soils than in liquid exposure media (i.e., lower dissolution of CeO_2 NPs), and suggests that CeO_2 NPs pose little risk to soil invertebrates.

Phenanthrene was toxic to both organisms tested. This study is the first report on phenanthrene toxicity to the isopod *P. pruinosus*. A previous study evaluated the toxicity of phenanthrene to other isopod species using contaminated food (Van Brummelen et al., 1996). The authors found no effect on the survival of *Porcellio scaber* and *Oniscus asellus*, and a slight reduction in growth for *O. asellus* after exposure for 16 (*P. scaber*) and 47 weeks (*O. asellus*) to phenanthrene at 706 mg/kg dry food (Van Brummelen et al., 1996). For the earthworm *Eisenia veneta* 28-day LC50 and EC50 values of 134 and 94 mg/kg dry soil, respectively were reported for the effects on growth (Sverdrup et al., 2002). This LC50 was similar to the values estimated in the present study for *P. pruinosus* (110–143 mg/kg dry soil). The EC50 values regarding the isopod biomass (17.6–31.6 mg/kg dry soil) however suggest that *P. pruinosus* was more sensitive to phenanthrene than the earthworms. In addition, CeO_2 NPs did not change the toxicity pattern induced by phenanthrene regarding the biomass change of *P. pruinosus*. Food consumption ratio in isopods was found to decrease with both increasing phenanthrene and CeO_2 NP concentrations (Fig. 2A). However, a decrease in food consumption may be no good evidence of toxicity as isopods may be able to survive without feeding for very long periods of time (Donker, 1992). In the present study, even though food consumption significantly decreased in some treatments, it did not result in biomass loss. Perhaps, it may need more time before reduced food consumption leads to mass changes.

Springtail survival and reproduction were reduced in a dose dependent manner by phenanthrene. LC50 values of 65–88 mg/kg dry soil, EC50 values of 52–77 mg/kg dry soil and EC10 values of 25–70 mg/kg dry soil were comparable to the toxicity data reported before (Droge et al., 2006). No effect of the CeO_2 NPs on springtail survival and reproduction was observed, and the different CeO_2 NP levels did not influence the toxicity of phenanthrene.

The data supports our first hypothesis, in which the joint toxicity could be expressed only by phenanthrene toxicity as CeO_2 did not have any effect by itself nor did it affect phenanthrene toxicity up to concentrations of 1000 mg Ce/kg dry soil. This study, however, is limited by lack of information on the characterization

of CeO_2 NPs (e.g., particle size distribution, zeta potential) and bioavailability (i.e., partitioning between soil and pore water) of the test compounds. We suggest further studies to determine the behavior of the mixture of CeO_2 NPs with phenanthrene and evaluate the environmental factors affecting it.

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