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published in
Environmental Science. Nano
2018

DOI (link to publisher)
10.1039/c7en00824d

document version
Publisher's PDF, also known as Version of record

Environmental Science Nano

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Cite this: *Environ. Sci.: Nano*, 2018, 5, 564

No effect of selected engineered nanomaterials on reproduction and survival of the springtail *Folsomia candida*†

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Received 8th September 2017,
Accepted 4th January 2018

DOI: 10.1039/c7en00824d

rsc.li/es-nano

Although the number of studies on engineered nanomaterial (NM) toxicity to soil invertebrates is increasing, only a few studies have reported toxicity of NMs to soil dwelling model species, such as the invertebrate *Folsomia candida*. The main objective of this study was to determine the toxicity of five different engineered NMs (WCCo, CuO, Fe_2O_3, organic pigment and MWCNTs) for the springtail *F. candida*. Copper(II), cobalt and iron chlorides were taken as positive controls. A standardized OECD test was used to measure effects on reproduction and survival, and toxicity was related to metal concentrations in soil and pore water. None of the NMs exerted adverse effects on springtail reproduction and survival at concentrations up to 6400 mg per kg dry soil, whereas the Cu, Co and Fe chlorides resulted in 50% decline in springtail reproduction at 981, 469 and 569 mg metal ion per kg dry soil, respectively. The absence of toxicity of the NMs could partly be explained by the low porewater metal concentrations, suggesting low solubility or slow solubilisation. The fate of engineered NMs in soil is rather complex but needs better understanding to facilitate predicting exposure of soil organisms.

Environmental significance

To date, only a limited number of studies have reported on nanomaterial toxicity effects in soil dwelling model species. Here, we study stress responses of the soil invertebrate *Folsomia candida* exposed to five engineered nanomaterials. This manuscript is novel as it describes chronic exposures of nanomaterials along with their respective metal salt controls. No toxic effects were measured among all nanomaterials up to very high concentrations, which are not realistic in the environment. Thus, our manuscript provides a more realistic view of potential nanomaterial hazards.

1. Introduction

Since the boost of the nanomaterials industry not more than a decade ago, there is increasing concern about the potential entry and impact of engineered nanomaterials (NMs) in the environment.[1–3] Engineered NMs are applied in a great variety of consumer and medical products, such as cosmetics, electronics, pharmaceuticals and textiles.[2,4,5] Keller *et al.* estimated that in 2010 between 206 000 and 309 000 metric tonnes of globally produced NMs were released into the environment, with 8–28% ending up in the soil.[2] Engineered NMs are defined as manufactured substances consisting of particles with sizes smaller than 100 nm in one or more dimensions. They can either be organic (carbon-containing) or inorganic (metal-based).[3,6,7] Investigating the potential environmental risks of new chemicals and materials, such as NMs, is a challenge as they have novel properties that could result in new and unexpected risks.[8,9]

To date, studies on NMs have mainly been performed on aquatic biota (especially on *Daphnia magna*).[10,11] However, an increasing number of studies have reported the effects of NMs on terrestrial soil invertebrates.[12–15]

Folsomia candida is a common soil arthropod that plays an important role in soil ecosystems and is known to be vulnerable to effects of soil contamination. Its reproduction is a sensitive endpoint and together with its short generation time and ease of culturing in the laboratory, the species is often used as a model organism in ecotoxicological studies.[16,17] However, to date, only a few studies have reported the toxicity of NMs to the species. For example, Manzo *et al.* reported that ZnO nanoparticles did not affect survival and reproduction at a test concentration of 230 mg Zn per kg (ref. 18) and Kool *et al.* showed that ZnO nanoparticles did not affect survival but did cause a dose-dependent decrease in reproduction (EC50: 1964 mg Zn per kg dry soil).[17]

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c7en00824d

Since there is a significant gap in the knowledge concerning the ecotoxicity of other NMs, we aimed at assessing five different NMs: multi-walled carbon nanotubes (MWCNTs) (organic), industrial paint pigment (organic), tungsten carbide–cobalt (WCCo) (metal), and two metal oxides, copper oxide (CuO) and iron oxide (Fe_2O_3). For each NM, a standard 28 day toxicity test was performed, with *F. candida* survival and reproduction as the endpoints.[19] To enable comparison of the toxicity of the metal-based NMs with that of the corresponding metal ions, similar concentrations of readily soluble metal species (*i.e.* Cu, Co and Fe chloride salts) were tested in parallel. Previous studies have tried to characterize NMs in soil using electron microscopy. However, Kool *et al.*[17] showed that NMs could only be visualized at very high concentrations, while the route of exposure and the actual form affecting springtails could still not be determined at such concentration levels. This in fact shows how difficult it is to understand the fate and potential effects of NMs in complex matrices like soil. Therefore, in this paper, we determined metal concentrations in the pore water, to get an idea of dissolution and the role of metal ions released from metal-based NMs. Pore water is believed to be the main route of exposure[20] and therefore porewater concentrations may allow for better understanding the exposure and effects of nanomaterials on *F. candida*.

2. Materials & methods

2.1 Test compounds and spiking of soil

Tungsten carbide–cobalt (WCCo), copper oxide (CuO), multi-walled carbon nanotubes (MWCNT), organic pigment red, Irgazin® (OP) and iron oxide (Fe_2O_3) pigment NMs were provided by the Sustainable Nanotechnologies Project (SUN) and were purchased from different suppliers (for details see Table 1). Particle characterization (Table 1) was performed within the SUN consortium: primary size distribution was measured with transmission electron microscopy (TEM), specific surface area with Brunauer, Emmett and Teller particle size and surface area analysis (BET) and the average agglomeration number (AAN) using dynamic light scattering (DLS). As positive controls copper(II) chloride ($CuCl_2$), cobalt chloride ($CoCl_2{\cdot}6H_2O$) and iron chloride ($FeCl_3{\cdot}6H_2O$) were used (see Table 1 for purities and suppliers). For WCCo, OP, CuO, Fe_2O_3 and MWCNT nanomaterial nominal test concentrations were 0–200–400–800–1600–3200–6400 mg per kg dry soil. Note that for WCCo-NM nominal concentrations were used rather than actual metal concentrations due to the very low amount of cobalt present in the compound. On average, more than 88% of the NM consists of tungsten (W). Furthermore, nanoparticle fraction measurements of the NM were technically not feasible, so that we decided to present WCCo-NM as nominal. $CuCl_2$ was tested at 0–100–200–400–800–1600 mg Cu per kg dry soil, $CoCl_2$ at 0–62.5–125–250–500–1000 mg Co per kg dry soil, and $FeCl_3$ at 0–100–200–400–800–1600 mg Fe per kg dry soil. As engineered NMs often are difficult to disperse in exposure media making it hard to realise a homogeneous distribution, spiking NMs as a suspension as well as dry powder were tested in the present study. For some of the NMs a homogeneous distribution could simply not be achieved by making a suspension, with all the particles either sinking to the bottom or ending up in the top layer of the soil. Spiking soil with NMs as dry powder resulted in better and more homogeneous distributions. The latter method was therefore used for all NMs in the present study. For each test concentration, the corresponding quantity of test compound was mixed in with dry LUFA 2.2 soil (Speyer, Germany, total organic carbon content of 2.09%, pH_{CaCl_2} of 5.5 and a water holding capacity (WHC) of 44%). Subsequently, the soil was moistened with deionized water to 50% of the WHC and mixed once more to ensure a homogeneous distribution of the test compounds. Finally, the spiked soil was divided over replicate test jars and allowed to equilibrate for 1 day before starting the toxicity test.

2.2 Analyses

2.2.1 Metal concentrations in soil and pH measurements. To measure total metal concentrations for the CuO-NM,

Table 1 Characteristics of the nanomaterials and metal salts tested for their toxicity to *Folsomia candida*. Characteristics were determined by partners within the SUN project

Compound	Particle size (TEM) (min–max (average)) (nm)	Surface area (BET)[a] ($m^2\ g^{-1}$) (average ± SD)	Average agglomeration number (AAN)	Purity (%) (from producer)	CAS number	Supplier
WCCo	23–1446 (170)	6.6 ± 0.4	159	<12% Co	12070-12-1 (WC) 744-48-4 (Co)	MBN
CuO	3–35 (12)	47 ± 1.7	77	99	1317-38-0	PlasmaChem
MWCNT	Ø 4–16 (8)	393.3 ± 17.3	NA	90		Nanocyl
OP ($C_{18}H_{10}Cl_2N_2O_2$)	14–151 (43)	94 (from producer)	9	100	84632-65-5	BASF
Fe_2O_3	11–112 (37)	22.6 ± 0.1	39	99	1309-37-1	BASF
$CuCl_2$	NA	NA	NA	98	7758-89-6	Merck
$CoCl_2{\cdot}6H_2O$	NA	NA	NA	98–102	7791-13-1	J.T. Baker
$FeCl_3{\cdot}6H_2O$	NA	NA	NA	98–102	10025-77-1	Sigma-Aldrich

NA = not available, SD = standard deviation.[a] Number of replicates is not specified by the SUN consortium. BET = Brunauer, Emmett and Teller particle size and surface area analysis. TEM = transmission electron microscopy. WCCo = tungsten carbide–cobalt, CuO = copper oxide, MWCNT = multi-walled carbon nanotubes, OP = organic pigment red (Irgazin®).

$CuCl_2$, WCCO-NM, $CoCl_2$, Fe_2O_3-NM and $FeCl_3$ treatments, soil samples were dried for 24 h at 60 °C. Approximately 130 mg of dried soil (three replicates per treatment) were digested in 2 ml of a mixture of concentrated HNO_3 and concentrated HCl (4 : 1 by vol.). All mixtures were placed in Teflon bombs, tightly closed and digested for 7 hours in an oven (CEM MDS 81-D) at 140 °C. After digestion, the solution was diluted to 10 ml and analysed by flame atomic absorption spectrometry (AAS) (Perkin Elmer AAnalyst 100). As reference material ISE sample 989 (River Clay) from Wageningen University, The Netherlands, was used to check for the accuracy of the analytical procedure (ISE, 2007). Fe (±SD; n = 4) and Cu levels (±SD; n = 2) in the ISE reference material were 86 ± 3.49% and 90 ± 0.34% of the certified values, respectively. Measured Co levels (±SD; n = 2) were 20 ± 0.25 mg per kg dry soil, but for Co no certified concentrations were available for ISE sample 989.

For all compounds, soil pH_{CaCl_2} values were measured at the start and at the end of the toxicity tests. For each treatment, three replicates were prepared by adding 24 ml of 0.01 M $CaCl_2$ to 6 grams of moist soil. Samples were shaken for 2 hours at 200 rpm and after settling of the soil particles, pH of the supernatant was recorded using a WTW PH7110 meter.

2.2.2 Metal concentrations in soil pore water. At the start and the end of the toxicity tests, pore water from the CuO-NM, $CuCl_2$, WCCo-NM, $CoCl_2$, Fe_2O_3-NM and $FeCl_3$ spiked soils was collected after saturation of 28 g soil with 5 ml deionized water and equilibration for 1 week followed by centrifugation (Centrifuge Falcon 6/300 series, CFC Free). Soils were centrifuged in tubes with two paper filters (S&S 597 Ø 47 mm, pore size 11 μm) and a 0.45 μm cellulose-nitrate membrane filter (S&S Ø 47 mm) using a relative force of 2000 g for 45 minutes (method described by Waalewijn-Kool *et al.*[21]). Approximately 5 ml soil pore water per sample was collected and subsequently analysed by flame AAS (Perkin Elmer AAnalyst 100).

2.2.3 Toxicity tests. The parthenogenetic springtail *Folsomia candida* ("Denmark strain", VU Amsterdam) was used as a model organism. Cultures were kept in a climate room at 16 ± 0.5 °C and a 16/8 h light/dark regime. To obtain synchronized animals, mature adults were allowed to lay eggs in plastic containers with a moist bottom of plaster of Paris for two days. Juveniles hatched from these eggs form a synchronized cohort suitable for experiments. A 28 day toxicity test with juveniles of 10–12 days old was performed for each test chemical following OECD guideline 232.[19] Five replicate 100 ml glass jars were prepared for each concentration and control. Ten animals were introduced into each test jar with 30 grams of moist soil and sufficient food supply (dried baker's yeast). Each jar was closed with a plastic screw top. Once a week jars were aerated, moisture loss was replenished with deionized water and animals were fed. After 28 days, springtails were extracted from soil by adding 100 ml of deionized water to each test jar, gently stirring and transferring them to a plastic beaker, allowing springtails to float on the surface. Pictures were taken to later count all animals with the software program ImageJ to determine survival (number of adults) and reproduction (number of juveniles). All toxicity tests were performed in a climate room at 20 ± 0.5 °C, 75% relative humidity and a 16 : 8 h light : dark regime.

2.3 Data analysis

Effect concentrations that reduced reproduction by 50% (EC50) compared to the untreated controls were determined using a logistic dose response model; corresponding 95% confidence intervals were calculated by using nonlinear regression analysis in IBM SPSS Statistics 23 software.[22] Effect concentrations that reduced survival by 50% (LC50) were estimated using the trimmed Spearman–Karber method.[23] One-way analysis of variance (ANOVA) followed by Dunnett's comparison post-hoc test ($P < 0.05$) was used to test for differences between controls and treatments.

Sorption of Co, Cu and Fe to the test soil was determined using the measured soil porewater and total soil concentrations. The Freundlich isotherm was used:

$$C_S = K_f C_w^n$$

where, C_s = concentration in soil (mg Co, Cu or Fe per kg dry soil) K_f = Freundlich sorption constant (l kg^{-1}) C_w = concentration in the pore water (mg Co, Cu or Fe per l) n = shape parameter of the Freundlich isotherm

Estimates for K_f and n were obtained by linear regression on a logarithmic scatter plot of C_s *versus* C_w.

3. Results

3.1 Metal concentrations in soil

Total metal (Cu, Co and Fe) concentrations in the test soil ranged between 85 and 120% of the added total concentrations for CuO-NM, $CuCl_2$, $CoCl_2$, Fe_2O_3-NM and $FeCl_3$ (Table S1–S4 in the ESI†). LUFA 2.2 control soil contained on average 0.83 and 1.5 mg Co per kg dry soil (WCCo-NM and $CoCl_2$ test, respectively), 4.4 and 4.7 mg Cu per kg dry soil (CuO-NM and $CuCl_2$ test, respectively) and 3591 and 3526 mg Fe per kg dry soil. This is in line with the Lufa 2.2 supplier's guide indicating average concentrations of 1.3 ± 0.1 mg Co per kg, 3.4 ± 0.4 mg Cu per kg and 4286 ± 27 mg Fe per kg. On average 7% of the nominal WCCo-NM concentration added was retrieved as Co in soil, which agrees with the Manufacturers' information (Table 1). For Fe_2O_3-NM and $FeCl_3$, total iron concentrations in soil were corrected for the high background iron levels in the control Lufa 2.2 soil (mean value: 3591 mg Fe per kg dry soil), so all results are expressed on the basis of added iron concentrations.

3.2 Soil pH

Soil pH_{CaCl_2} of control soils ranged from 5.99 to 6.37 at the start of the tests and decreased to 5.65–5.80 after 28 days of exposure (Tables S5–S7†). Soil pH_{CaCl_2} slightly increased with

increasing WCCo-NM concentration (Table S5†), and decreased for $FeCl_3$ (Table S6†) and $CoCl_2$ (Table S7†). The pH was not affected by Fe_2O_3-NM, OP-NM and MWCNT-NM (Table S5†). Soil pH values also did not change with increasing CuO-NM concentrations at T = 0 (Table S5†), but at T = 28 a slight dose-related increase was seen suggesting pH did not decrease with time at higher exposure concentrations. $CuCl_2$ caused a dose-related pH decrease at T = 0, which was no longer seen at T = 28 (Table S6†), in this case due to an increase at high and a decrease at low concentrations.

In summary, soil pH_{CaCl_2} of control soils did not differ from treatments for Fe_2O_3-NM, OP-NM and MWCNT-NM, but did differ from soil with increasing WCCo-NM concentrations (*i.e.* slight increase of pH with increasing metal concentrations). For all tested chlorides, soil pH_{CaCl_2} decreased with increased metal concentration at T = 0, however this dose-related decrease of pH was no longer seen for $CoCl_2$ and $CuCl_2$ at T = 28.

3.3 Metal concentrations in soil pore water and sorption

Metal concentrations in pore water increased with exposure concentration for all compounds measured, except for Fe_2O_3-NM. Concentrations of 1.20–3.88 mg Co per l and 7.70–366 mg Co per l were measured for WCCo-NM (Table S8†) and $CoCl_2$ (Table S9†), respectively, corresponding with a solubility of 0.24–0.43% and 1.40–15.5% of the measured total cobalt concentrations. Iron concentrations ranging from 0.04–0.13 mg Fe per l (0.002–0.007%) were found for soil spiked with Fe_2O_3-NM (Table S10†) and from 0.04–129 mg Fe per l (0.004–3.58%) for $FeCl_3$ (Table S11†). Copper concentrations in the pore water ranged from 0.41–2.31 mg Cu per l (0.003–0.02%) for soil spiked with CuO-NM (Table S12†) and from 0.15–17.1 mg Cu per l (0.01–0.44%) for soil spiked with $CuCl_2$ (Table S13†). Sorption of the metals added as chloride salts (Fig. 1) could be described well with a Freundlich isotherm (Table 2). Because isotherms were based on only three data points, R^2 values were high (>0.940) for all measured salts.

3.4 Toxicity

Survival of *Folsomia candida* in LUFA 2.2 soil spiked with up to 6400 mg per kg dry soil of either CuO, WCCo, Fe_2O_3, OP or MWCNT nanomaterials and $CuCl_2$ was not affected and comparable to the controls (*i.e.* 99%, 96%, 80%, 99%, 99% and 91%, respectively). Survival was affected by $FeCl_3$ and $CoCl_2$ with LC50s of 849 mg Fe per kg dry soil (95% CI 748–962) and 622 mg Co per kg dry soil (95% CI 556–695), respectively, based on measured added or total concentrations, respectively.

On average, 957 juveniles (Coefficient of variance (CV) 27%) were found in controls from the $CuCl_2$ test, 830 (CV 13%) in controls from the $CoCl_2$ test and 290 (CV 32%) in controls from the $FeCl_3$ test. Reproduction was not affected by any of the nanomaterials tested at concentrations up to 6400 mg per kg dry soil (Fig. 2 and 3). Although a decreasing trend in reproduction is visible in the WCCo-NM treatment, reduction was less than 50% at the highest test concentration making it impossible to calculate a reliable EC50. The metal

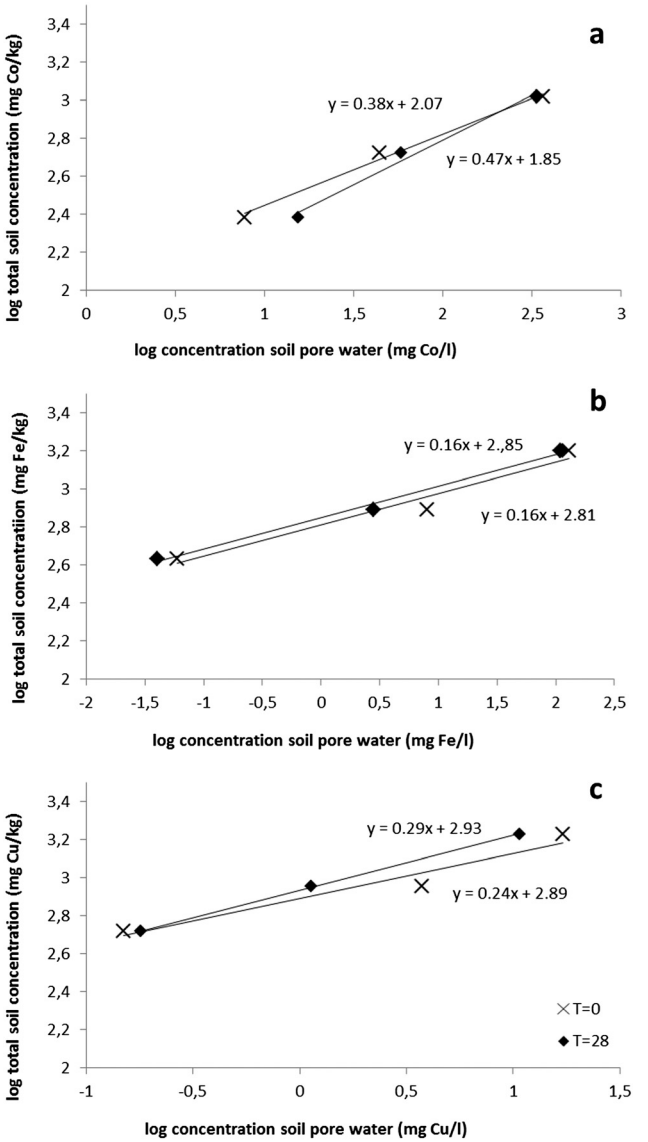

Fig. 1 Measured total metal concentrations in soil as a function of metal concentrations in soil pore water. Lines and equations represent the fit of the Freundlich isotherm to the data for the sorption of Co (panel a, $CoCl_2$), Fe (panel b, $FeCl_3$) and Cu (panel c, $CuCl_2$) in spiked Lufa 2.2 soil at T = 0 (X) and T = 28 days (◆).

Table 2 Freundlich sorption parameters (K_f and in between brackets n) for the binding of three elements to Lufa 2.2 soil as derived from concentrations in pore water extracted from soil freshly spiked with chloride salts of the three metals (T = 0) or after 28 days of incubation (T = 28). See Fig. 1 for the Freundlich isotherms

	Sorption constant K_f	
	l kg^{-1} (n)	l kg^{-1} (n)
Metal salt	T = 0	T = 28
Cobalt (Co)	117 (0.38)	70 (0.47)
Iron (Fe)	646 (0.16)	706 (0.16)
Copper (Cu)	774 (0.24)	858 (0.29)

chlorides, however, decreased springtail reproduction in a dose-dependent manner (Fig. 4) with EC50 values of 981 (95% CI 787–1174) mg Cu per kg dry soil, 469 (405–533) mg Co per kg dry soil and 569 (370–769) mg Fe per kg dry soil for $CuCl_2$, $CoCl_2$ and $FeCl_3$, respectively, based on measured total (Cu, Co) or added (Fe) concentrations. Porewater-based EC50s and LC50s for the effects of $CuCl_2$, $CoCl_2$ and $FeCl_3$ were 3.81 (1.43–6.19) and >13.9 mg Cu per l, 45.4 (35.7–55.2) and 86.7 (68.8–108) mg Co per l, and 4.33 (CI could not be calculated) and 12.2 (4.7–31.4) mg Fe per l, respectively.

4. Discussion

Results obtained in this study showed that the tested nanomaterials had little or no effects on *F. candida* survival and reproduction, while soluble metal salts corresponding with these nanomaterials did affect these endpoints at similar total concentrations. The differences in porewater metal concentrations between nanomaterials and metal chloride treatments may explain the difference in toxicity.

4.1 Toxicity of metal-based nanomaterials in relation to soil properties

For all metal and metal oxide particles the soil pH_{CaCl_2} ranged from 5.6 to 6.6 (except for $FeCl_3$) and did not show strong increases or decreases with increasing soil concentrations. According to Fountain & Hopkin, *F. candida* shows the highest reproduction at a pH of around 5.6.[16] For $FeCl_3$ a strong dose-dependent decrease of the soil pH was seen (Table S6†) with a pH around 4.6 at 800 mg Fe per kg dry soil and 3.5 at 1600 mg Fe per kg dry soil. These lower pH values could also have contributed to the significant decrease in reproduction that was observed. In this study, porewater metal concentrations were measured at two time points (T = 0 and T = 28 days), but no substantial differences were seen in this short period. Porewater Co, Cu and Fe concentrations were considerably lower in the NM treated soils and were not concentration-dependent, unlike the porewater metal concentrations for $CoCl_2$, $CuCl_2$ and $FeCl_3$ (Tables S8–S13†). For example, cobalt porewater concentrations from soil spiked with the highest concentration of WCCo-NM (6400 mg WCCo per kg) were almost 2-fold lower (3.88 mg Co per l) than measured in soils spiked with the lowest concentration of $CoCl_2$. Porewater-based EC50 for $CoCl_2$ was 86.7 (68.8–108) mg Co per l, which is in line with a previous study performed in Lufa 2.2 with $CoCl_2$ and *F. candida*, showing an EC50 of 174 (86–350) mg Co per l.[24] Assuming that toxicity of WCCo-NM would especially be due to dissolution of Co (which would partly explain toxicity since both the particles themselves and tungsten (W) could also contribute to toxicity), the absence of toxicity could at least partly be explained from the low dissolved metal concentrations of these NMs. For CuO-NM dissolved copper levels in the pore water from soils spiked with 6400 mg Cu per kg were still lower (2.31 mg Cu per l) than the porewater-based EC50 for $CuCl_2$ that was found. Bicho *et al.* found total Cu concentration in the soil solution for $CuCl_2$ to be similar or up to 3-fold higher than for CuO-NM at concentrations of 200–400 mg Cu per kg dry soil, indicating increased toxicity to enchytraeids exposed to the copper salt.[25] Iron porewater concentrations from soil spiked with the highest concentration of Fe_2O_3-NM (6400 mg Fe per kg) were more than 36 times lower (0.12 mg Fe per l) than the porewater-based EC50 that was found for $FeCl_3$.

These results all indicate that the NMs tested in this study showed lower solubility or slower solubilisation than the metal salts and the low dissolved metal concentrations in the pore water may thus explain the absence of toxicity for the NMs tested. These results are in line with previous studies that indicated lower solubility and/or slower dissolution and absence of toxicity for NMs, compared to similar concentrations of readily soluble metal species.[17,18,26,27] This could all be explained by the release of metal ions after dissolution of the nanomaterials[21,28] and *via* aggregation and agglomeration processes, which affect fate, behaviour and bioavailability of NMs in the environment.[7,29]

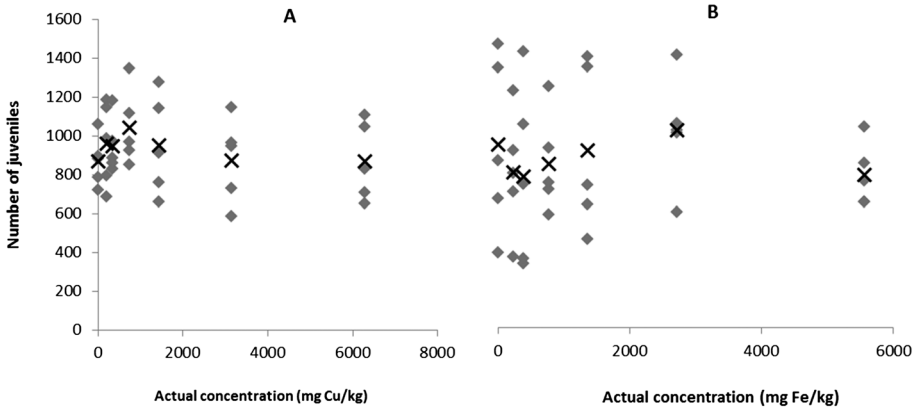

Fig. 2 Effect of CuO (A) and Fe_2O_3 (B) nanomaterials (NM) on the reproduction (number of juveniles) of *Folsomia candida* after 28 d exposure in LUFA 2.2 soil. X = mean number of juveniles. Measured total exposure concentrations of Cu (A) and added exposure concentrations of Fe (B) in the soil are provided on the x-axis.

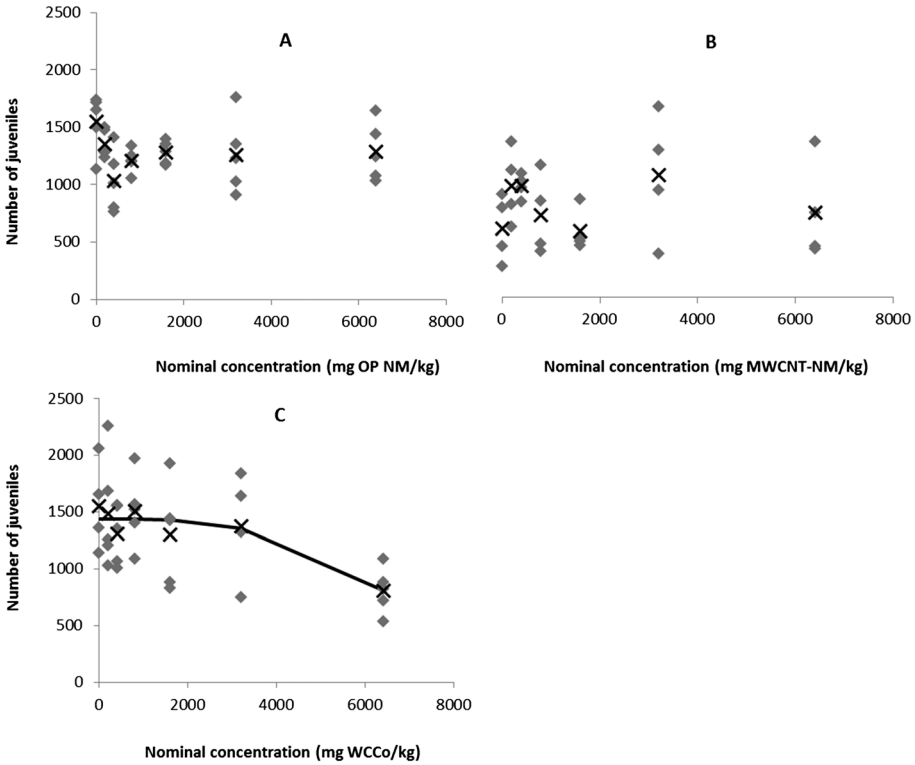

Fig. 3 Effect of organic pigment (A), MWCNT (B) and WCCo (C) nanomaterials (NM) on the reproduction (number of juveniles) of *Folsomia candida* after 28 d exposure in Lufa 2.2 soil. *X* = mean number of juveniles. Nominal exposure concentrations are provided on the *x*-axis. Line shows fit obtained with a logistic model for WCCo-NM (C).

Waalewijn-Kool *et al.* already showed the effect of changes in the soil with an increased release of Zn from ZnO nanoparticles with decreasing pH for soils containing 100 to 1600 mg Zn per kg dry soil and that release of Zn continued after one year.[21] Díez-Ortiz *et al.* even found a significantly ($p < 0.05$) increased toxicity of Ag nanoparticles to *E. fetida* in time ($EC50_{reproduction}$ of 1420 mg Ag per kg dry soil after 1 week *versus* 34 mg Ag per kg dry soil in soil aged for 52 weeks) and suggested that this was due to Ag ion dissolution.[30] The authors concluded that environmental risks of nanoparticles could not properly be assessed with short-term exposures. Both studies show the importance and necessity of assessing long-term NM behaviour as it may determine environmental risk.

4.2 Toxic effects of organic nanomaterials

The organic pigment red (Irgazin®) nanomaterials (OP-NMs) did not affect the survival or reproduction of *F. candida*. Currently, literature about the toxicity of organic pigment is almost absent. In 1980, Anliker & Clarke described organic pigments to not present major ecological problems in aquatic environments, due to their low solubility in water.[31] They reported that it would be highly unlikely for organic pigment particles to end up in the open seawater, as they would be removed by sedimentation or adsorption to sewage sludge. Recently, Hofmann *et al.* investigated the inhalation effects of organic pigment red in rats and found no adverse effects after exposure, although histopathological examination revealed the presence of pigment particles in the lungs.[32]

In our study, we could also clearly see that *F. candida* had ingested the organic pigment red nanoparticles, as the midgut coloured red (Fig. S1†), but no effect on its survival or reproduction was observed at 6400 mg per kg dry soil. This may suggest that the organic pigment NM does not pass the gut epithelium and thus cannot affect metabolic processes. Currently, mechanistic investigations that describe the potential effects of engineered NM uptake in invertebrate guts on metabolic processes are scarce.[33,34] Therefore, further research is needed to confirm this hypothesis.

In our study we also did not observe any effects of MWCNTs on *F. candida* survival or reproductive output. Toxic effects of carbonaceous materials such as CNTS and fullerenes have mainly been described for aquatic organisms (algae, bacteria, crustaceans and fish),[35–38] while only few studies have focused on the ecotoxicity of CNTs in terrestrial invertebrates. For example, Scott-Fordsmand *et al.* exposed the earthworm *Eisenia veneta* to double-walled CNTs (DWCNTs) and found no effect on body mass or survival at concentrations up to 495 mg per kg dry food.[39] However, reproduction was affected at concentrations above 37 mg per kg dry food. Bioaccumulation studies with single-walled CNTs (SWCNTs) and multi-walled CNTS showed no effects on the survival of the earthworm *E. fetida* when exposed to

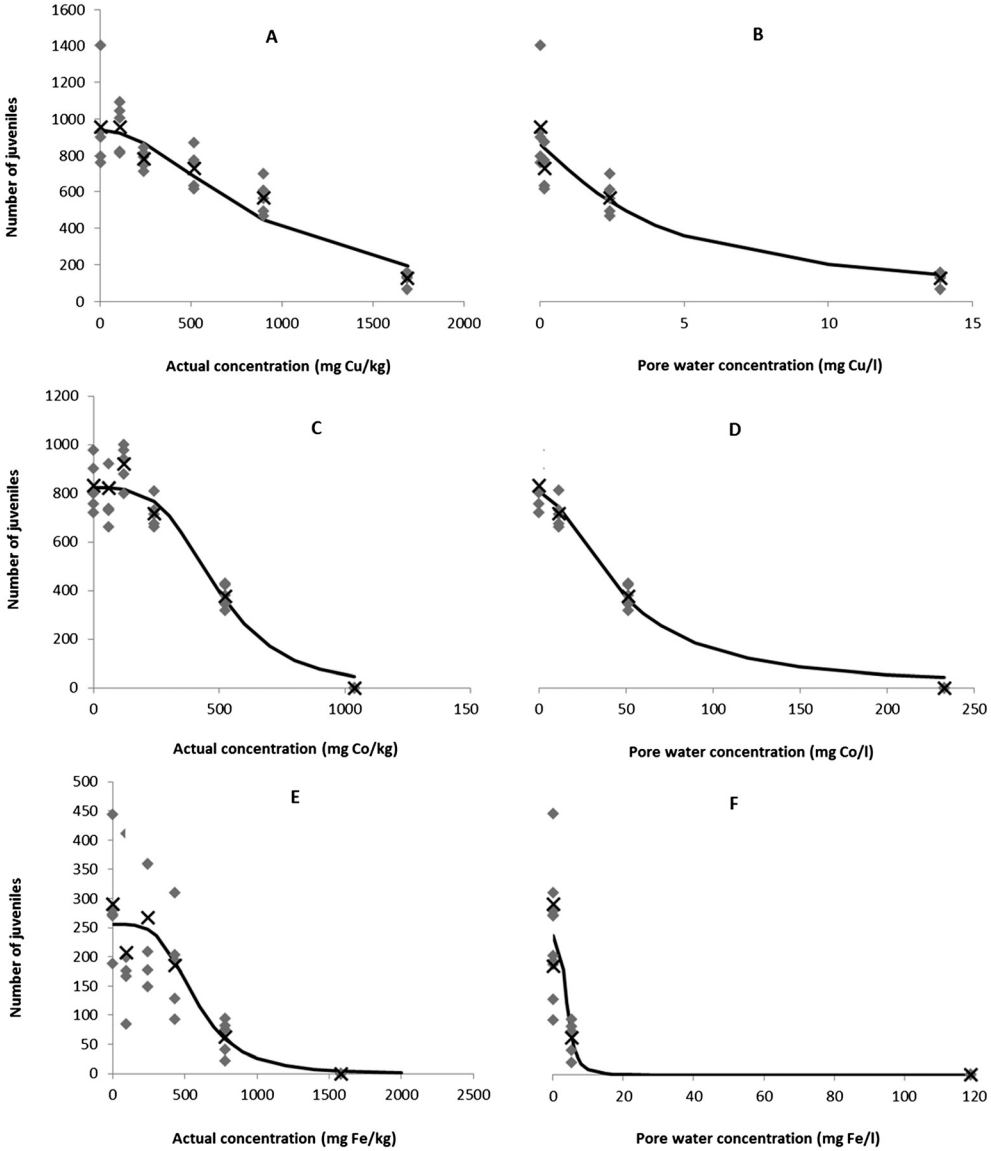

Fig. 4 Effect of $CuCl_2$ (A and B), $CoCl_2$ (C and D) and $FeCl_3$ (E and F) on the reproduction (number of juveniles) of *Folsomia candida* after 28 d exposure in Lufa 2.2 soil. *X* = mean number of juveniles. Left: Reproduction related to actual exposure concentrations in the soil; right: reproduction related to soil pore water concentrations. Line shows fit obtained with a logistic dose–response model.

concentrations up to 3000 mg kg^{-1} in soil.[38] A study on *Drosophila melanogaster* fed with up to 1000 mg CNT nanomaterial per kg food reported no effects on survival and development, despite the presence of CNTs within the organisms. However, direct exposure to CNT powder did negatively affect grooming behaviour, locomotion and survival.[40]

Conclusion

We determined, for the first time, the toxicity of WCCo, CuO, Fe_2O_3, OP and MWCNT nanomaterials to the springtail *F. candida*. Standard 28 day toxicity tests with these nanomaterials did not show any adverse effects on springtail survival and reproductive success, not even at concentrations as high as 6400 mg per kg dry soil. We showed that, in the case of metal-based NMs, this could at least partly be explained from the low porewater metal concentrations, suggesting low solubility or slow solubilisation. Since fate of engineered NMs is rather complex and effects upon long-term, multigenerational exposure cannot be excluded, more research is necessary to better predict exposure of soil organisms.

Conflicts of interest

The authors declare no competing financial interest.

Acknowledgements

This work was supported by the project on Sustainable Nanotechnologies (SUN) that received funding from the European

Union Seventh Framework Programme (FP7/2013) under Grant Agreement No. 604305.

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