Toxicokinetics of zinc-oxide nanoparticles and zinc ions in the earthworm Eisenia andrei

Świątek, Zuzanna M.; van Gestel, Cornelis A.M.; Bednarska, Agnieszka J.

published in
Ecotoxicology and Environmental Safety
2017

DOI (link to publisher)
10.1016/j.ecoenv.2017.05.027

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

Ecotoxicology and Environmental Safety 143 (2017) 151–158

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Toxicokinetics of zinc-oxide nanoparticles and zinc ions in the earthworm *Eisenia andrei*

Zuzanna M. Świątek[a,*], Cornelis A.M. van Gestel[b], Agnieszka J. Bednarska[c]

[a] Institute of Environmental Sciences, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland
[b] Department of Ecological Science, Faculty of Earth and Life Sciences, Vrije Universiteit, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands
[c] Institute of Nature Conservation, Polish Academy of Sciences, Mickiewicza 33, 31-120 Kraków, Poland

ARTICLE INFO

Keywords:
Accumulation
Elimination
Metal
Soil
Invertebrate

ABSTRACT

The toxicokinetics of zinc in the earthworm *Eisenia andrei* was investigated following exposure for 21 days to ionic zinc ($ZnCl_2$) or zinc oxide nanoparticles (ZnO-NPs) in Lufa 2.2 soil, followed by 21 days elimination in clean soil. Two concentrations were tested for both $ZnCl_2$ (250 and 500 µg Zn g^{-1}) and ZnO-NPs (500 and 1000 µg Zn g^{-1}), corresponding to EC_{25} and EC_{50} for effects on reproduction. Based on the measured internal Zn concentrations in the earthworms over time of exposure, the kinetics parameters k_a – assimilation rate constant (g_{soil} $g^{-1}{}_{body\ weight}$ day^{-1}) and k_e – elimination rate constant (day^{-1}) were estimated using a one-compartment model for either total Zn concentrations in the soil or porewater Zn concentrations. In the $ZnCl_2$ treatments, k_a was higher for total Zn concentrations in soil, whereas in the ZnO-NP treatments, k_a was higher for porewater Zn concentrations. The value of k_e did not differ between the two Zn forms ($ZnCl_2$ vs ZnO-NPs) for either EC_{50} or EC_{25} when related to total Zn concentrations in soil, but for EC_{50}, k_e related to porewater Zn concentrations was significantly higher for $ZnCl_2$ than for ZnO-NPs. It is concluded that differences in kinetic parameters between treatments were connected with exposure concentrations rather than with the form of Zn. Zinc was efficiently regulated by the earthworms in all treatments: a 2-fold increase in exposure concentration resulted in a less than 2-fold increase in internal concentration, and after transfer to uncontaminated soil the internal Zn concentrations in the earthworms returned to ca 111 µg g^{-1} dw in all treatments.

1. Introduction

The increasing usage of engineered nanoparticles (NPs) is associated with the risk of uncontrolled release into the environment (Caballero-Guzman and Nowack, 2016; Ju-Nam and Lead, 2008). Once released into the environment, NPs can enter wastewater and sewage sludge, which may in turn be used as fertiliser on crop fields. Since ZnO-NPs are among the most widely used nanoparticles, the risk of release into the soil environment is high. However, due to the complex nature of the soil, the exact amount of NPs in the environment is difficult to determine due to various limitations (Caballero-Guzman and Nowack, 2016). After reaching the soil environment, NPs can potentially be taken up by soil-dwelling organisms (García-Gómez et al., 2014). Thus, to date several studies on ZnO-NPs have been performed aimed at examining their influence on soil invertebrates, among them earthworms (Fernández et al., 2014). Most of these studies indicated that ZnO-NPs are not as toxic as the ionic form of this metal (Kwak and An, 2015). Hooper et al. (2011) showed a 50% decrease in reproduction by ZnO-NPs compared to complete inhibition by $ZnCl_2$ treatment when exposing *Eisenia veneta* to 750 µg Zn g^{-1} soil. Moreover, no increased mortality was observed in comparison with control when earthworms were exposed to 0.1, 1, 1000, and 5000 µg Zn g^{-1} of ZnO-NPs in sand for 14 days (Cañas et al., 2011).

Some studies suggest that the toxicity of NPs may be connected with ions released after their dissolution (Heggelund et al., 2014; Kool et al., 2011), rather than with exclusive effect of nanoparticles itself. And recent studies on the metabolomics and genotoxicity of silver nanoparticles (Ag-NPs) confirm that toxicity results from the simultaneous action of nanoparticles and released metal ions (Garcia-Reyero et al., 2014; Li et al., 2015). Probable differences in the pathway and mode of action for ZnO-NPs and Zn^{2+} ions could be indirectly connected with differences in the intracellular compartmentalisation of zinc in the earthworms *Eisenia fetida* exposed to either nano or ionic zinc forms (Li et al., 2011). Li et al. (2011) showed that zinc in ionic form was distributed mainly in cell membranes and tissues, while zinc derived from NPs was stored in organelles and cytosol. Moreover, despite the lower toxicity of ZnO-NPs in comparison with ions, internal Zn concentrations in earthworms were higher for the former (Heggelund

* Corresponding author.
E-mail address: zuza.swiatek@uj.edu.pl (Z.M. Świątek).

http://dx.doi.org/10.1016/j.ecoenv.2017.05.027
Received 15 December 2016; Received in revised form 25 April 2017; Accepted 17 May 2017
Available online 19 May 2017
0147-6513/

et al., 2014; Hooper et al., 2011). Thus, different mechanisms may be responsible for the regulation of Zn concentrations in animals exposed to different forms of the metal.

Many toxicokinetic experiments have been conducted in order to understand the uptake and elimination of different metals by different earthworm species (Giska et al., 2014; Li et al., 2009; Nahmani et al., 2009), including *Eisenia andrei* (Smith et al., 2010). However, the toxicokinetics of Zn after ionic and nanoparticle exposure has rarely been studied in earthworms (Laycock et al., 2016).

Our aim was to compare the toxicokinetics of zinc in the earthworm *E. andrei* after exposure to the metal present in soil in the form of either zinc oxide nanoparticles (ZnO-NPs) or ions derived from a soluble salt ($ZnCl_2$). Two different concentrations, covering the EC_{50} and EC_{25} for effects on earthworm reproduction (Heggelund et al., 2014) were chosen. Based on the measured internal Zn concentrations in the earthworms over time, toxicokinetics parameters, i.e. assimilation and elimination rate constants, were calculated and compared between treatments.

2. Materials and methods

2.1. Test species

Earthworms of the species *E. andrei* were obtained from a laboratory culture at the Department of Ecological Science of Vrije Universiteit in Amsterdam. The earthworms were fed with horse manure free of any pharmaceuticals and cultured at 20 °C in darkness. The experiment used 4-month old adult individuals with well-developed clitellae.

2.2. Soil spiking procedure

Loamy sand soil (LUFA-Speyer 2.2, Germany) was used. Soil came from two deliveries with pH_{CaCl2} 5.4 ± 0.2, total organic carbon content 1.59 ± 0.13%, cation exchange capacity 9.7 ± 0.4 meq. 100 g^{-1}, and maximum water holding capacity (WHC) 43.5 ± 2.8% (w/w). The experiment used 30 nm ZnO-NPs as powder without any surfactant or coating (Nanosun Zinc Oxide P99/30, Microniser, Australia). Transmission electron micrographs and particle size distribution, determined by Waalewijn-Kool et al. (2012), showed that the primary particle size of the nanoparticles was in agreement with the values provided by the manufacturer. Full details and results of particle characterization can be found in Waalewijn-Kool et al. (2012). Soil spiked with zinc chloride salt ($ZnCl_2$, Merck, Germany) was used to represent treatments with ionic Zn. Two concentrations of ZnO nanoparticles (nominal: 500 and 1000 µg Zn g^{-1} dry soil, designated as ZnO-NP 500 and ZnO-NP 1000, respectively), two concentrations of $ZnCl_2$ (nominal: 250 and 500 µg Zn g^{-1} dry soil, designated as $ZnCl_2$ 250 and $ZnCl_2$ 500, respectively), and one control without added zinc were tested. Test compounds were introduced into soil using different methods. Four days before starting the exposure (in order to obtain equilibration), soil was spiked with $ZnCl_2$ as aqueous solutions prepared with an amount of water sufficient to achieve a soil moisture content of 50% of WHC. After spiking, to achieve homogenous distribution, the soil was mixed with a kitchen robot. Shortly before starting the exposure (day 0), ZnO nanoparticles were added to a small portion of dry soil (i.e. 250 g which was 10% of the soil used per treatment) as dry powder and then mixed by shaking 4–6 min in a closed jar. Subsequently, the remaining soil (2300 g) and enough water to reach a soil moisture content equivalent to 50% of WHC were added and homogenised carefully with the kitchen robot under a fume hood. Thus, a shorter equilibration time was used for treatments with ZnO nanoparticles than for $ZnCl_2$ to take into account differences in their solubility (Romero-Freire et al., 2017) and to ensure that the earthworms from the ZnO-NP treatments were exposed mainly to zinc in the form of nanoparticles.

2.3. Toxicokinetics experiment

The toxicokinetics test followed OECD Guideline 317 (OECD, 2010), with 21 days of exposure in Zn contaminated soil (uptake phase) followed by 21 days of elimination in control soil (elimination phase, also described in the literature as the decontamination phase). Three replicates (i.e. glass jars filled with approximately 60 g of wet soil) were prepared per sampling point for each Zn treatment and control. Food was added at the beginning of each phase by mixing 7 mg dry weight of horse dung per 1 g dry weight of soil prior to introducing the soil into the test jars. Earthworms, one individual per jar, were randomly allocated to treatments. Before starting the exposure (day 0) and after 1, 2, 4, 7, 10, 14, 17, and 21 days (uptake phase) and 22, 23, 25, 28, 31, 35, 38, and 42 days (elimination phase), three individuals were sampled from each Zn treatment. At days 0, 7, 14, 21, 28, 35, 38, and 42, three individuals were sampled from the control treatment. Once a week, soil moisture content was checked by weighing the jars, and moisture loss was replenished with deionised water when necessary. The jars were also aerated by this procedure. At each sampling point, the collected earthworms were rinsed with tap water, blotted dry on filter paper, and weighed to the nearest 0.0001 g. Then, the animals were kept individually in Petri dishes lined with moistened filter paper to void the gut content. After 24 h, the earthworms were rinsed, blotted dry and weighed again, then killed by freezing at −20 °C.

2.4. Chemical analysis

Frozen animals were freeze-dried for 48 h and then weighed to the nearest 0.0001 g. Soil samples collected before the exposure (day 0) from each treatment were dried for 48 h at 50 °C. For analysis of the total zinc concentrations, both individual earthworms and soil samples (ca 100 mg dried soil) were digested in 2 mL of a 4:1 mixture of HNO_3 (65% p.a., Sigma-Aldrich) and HCl (37% p.a., Sigma-Aldrich). Digestion was performed using Teflon bombs, closed tightly and placed in an oven (CEM MDS 81D) at 140 °C for 7 h. To determine the level of analytical precision, three blanks and three samples of a certified reference material (for earthworms: *Dolt-4 Dogfish Liver*, National Research Council of Canada, with a certified Zn concentration of 116 ± 6 µg g^{-1}; for soil: *ISE sample 989 of River Clay* from Wageningen, the Netherlands, with a certified Zn concentration of 1060 µg g^{-1}) were run with the samples. After digestion, the samples were complemented with demineralised water (8 mL) and Zn concentrations were analysed using flame Atomic Absorption Spectrometry (AAS) (Perkin Elmer AAnalyst 100) and expressed in µg g^{-1} dry weight (dw). Measured zinc concentrations in the reference materials were within ± 0.5% and ± 3.5% of the certified concentrations for the *Dolt-4 Dogfish Liver* and *ISE sample 989 of River Clay*, respectively.

At the beginning (day 0) and end (day 21) of the uptake phase, three soil samples (ca 49g) per treatment were taken, saturated to 100% WHC and equilibrated for 48 h. Soil porewater was collected via centrifugation (Centrifuge Falcon 6/300 series, CFC Free) for 45 min with a relative force (RCF) of 2000 × *g* over two round filters (cat. no. 1001-047, Ø 47 mm) and a 0.45 µm membrane filter (cellulose nitrate, cat. no. 10401112, Lot G9942878, Ø 47 mm) placed inside the centrifuge tubes. Approximately 10 mL of soil porewater per sample was collected and analysed using flame AAS (Perkin Elmer AAnalyst 100) for Zn concentration. Additionally, Zn concentration was determined after ultrafiltration from an aliquot of the porewater: to obtain particle-free extracted porewater, samples were centrifuged in a 3 kDa ultrafiltration device (Amicon Ultra-15 Filters, Millipore) for 45 min at 3000 × *g*. The ultrafiltration was only done for soil samples taken at day 0.

To measure the pH_{CaCl2} of the test soils, samples (ca 5g dw) were taken at days 0, 7, 14, and 21 and shaken in plastic tubes with 25 mL 0.01 M $CaCl_2$ for 2 h at 2000 rpm. After overnight settling of the floating particles, pH was measured using a pH-meter (inoLab® pH 7110, WTW).

Soil organic matter content was determined as loss on ignition. Soil samples were taken at days 0 and 21, dried at 50 °C in the stove for 24 h, and subsequently ashed in an ashing oven by increasing the temperature from 200 °C (first hour) to 400 °C (second hour), and finally up to 500 °C (an additional 6 h).

2.5. Data analysis

To describe Zn interaction between soil and porewater, the soil-solution partition coefficient (K_d) was calculated as the ratio of the total Zn concentration measured in soil (μg g^{-1} dry soil) and the corresponding porewater Zn concentration (μg mL^{-1}).

The development of internal Zn concentrations in the earthworms with time was analysed with a one-compartment first-order model proposed by Skip et al. (2014). Different modifications of the original model were tested with either (1) single k_a and single k_e fitted to both phases together, (2) separate estimation of the model parameters: k_{a1} and k_{e1} for the uptake phase and k_{a2} and k_{e2} for the elimination phase, or (3) k_a and k_e fitted only to the uptake phase. The models were compared according to R^2 values adjusted for degrees of freedom (R^2_{adj}). The equation for the last model is as follows:

$$C_{(t)} = C_0 \times e^{-k_e \times t} + C_{exp}\frac{k_a}{k_e}(1 - e^{-k_e \times t})$$

where:

$C_{(t)}$ – internal Zn concentration at time t (μg g^{-1} dw); C_0 – internal Zn concentration in the earthworms at $t=0$ (μg g^{-1} dw), given in the model explicitly as the average concentration measured in earthworms (132 μg g^{-1} dw) before starting the exposure (day 0); C_{exp} – measured exposure concentration in the uptake phase, either in the soil (μg g^{-1} dw soil) or porewater (μg mL^{-1}); k_a – assimilation rate constant (g_{soil} $g^{-1}_{body\ weight}$ day^{-1} or $mL_{porewater}$ $g^{-1}_{body\ weight}$ day^{-1}); k_e – elimination rate constant (day^{-1}). The following symbols were used for kinetics parameters estimated based on total Zn concentrations measured either in the soil: $k_{a\text{-}S}$, $k_{e\text{-}S}$ or in the porewater: $k_{a\text{-}PW}$, $k_{e\text{-}PW}$. Equations were fitted using the Marquardt method. The kinetics parameters were checked for significance using asymptotic 95% confidence intervals, which were also used to compare the parameters between treatments. Using 95% confidence intervals for comparisons between treatments is a conservative approach, assuming a significant difference when the confidence intervals for two treatments do not overlap. If the confidence intervals do overlap, it does not necessarily mean, however, that the parameters cannot be considered significantly different.

All models were estimated using Statgraphic Centurion XVI version 16.2.04. Based on assimilation and elimination rate constants, bioaccumulation factor ($BAF = k_a/k_e$) and steady-state concentration ($C_{SS} = (k_a/k_e) \times C_{exp}$) were calculated for each Zn treatment.

3. Results

3.1. Soil properties

Soil pH_{CaCl2} varied between treatments and exposure days (Table 1). At day 0, after 4 days of equilibration, pH values for both treatments with ionic Zn were a bit lower (pH = 5.9 and pH = 5.7 for $ZnCl_2$ 250 and $ZnCl_2$ 500, respectively) than for the control (pH = 6.0). At day 0 the soil spiked with ZnO-NPs had slightly higher pH_{CaCl2} (6.2 and 6.5 for ZnO-NP 500 and ZnO-NP 1000, respectively) than for the control. After 14 days of exposure, soil pH did not change in the $ZnCl_2$ 500 and ZnO-NP 500 treatments, while in the remaining treatments the decrease did not exceed 0.2 units. A considerable drop in pH was noticed only at the end of the uptake phase in all treatments except for $ZnCl_2$ 500. In general, pH values were higher for the ZnO-NP treatments than for the treatments with ionic Zn. Average soil organic matter content was similar for all treatments and ranged from 4.0% to 4.2% at day 0 and from 3.8% to 4.0% at day 21 (Table S1).

3.2. Soil total and porewater concentrations

The measured Zn concentrations in the soil were in accordance with the nominal ones (Table 2). The Zn concentration in control soil was 40.4 ± 8 μg g^{-1} dw (mean ± SD; n = 3). Average (n = 3) zinc concentrations in the porewater for $ZnCl_2$ 250 and both ZnO-NP treatments were similar, at 4.0–6.0 μg mL^{-1} for day 0 and 3.4–7.7 μg mL^{-1} for day 21, whereas porewater Zn concentrations for the $ZnCl_2$ 500 treatment were an order of magnitude higher than for the other treatments. In all treatments, the porewater Zn concentrations changed only slightly over the 21-day exposure period. Porewater Zn concentrations after ultrafiltration measured at day 0 ranged from 56% of the values before filtration for both ZnO-NP treatments to 85% and 94% for $ZnCl_2$ 250 and $ZnCl_2$ 500, respectively (Table 2).

3.3. Partition coefficients

Partition coefficients (K_d) describing the release of Zn into the porewater were much lower for the treatments with $ZnCl_2$ than for those with ZnO-NPs. After 21 days of exposure, K_d decreased noticeably for ZnO-NP 1000 while in ZnO-NP 500 a slight increase of K_d with time could be noticed (Fig. 1).

3.4. Toxicokinetics

All earthworms gained weight during the study and no mortality occurred. An initial rapid increase in internal Zn concentrations, followed by a decrease to some equilibration already within the uptake phase and by further decrease in the elimination phase, was observed in earthworms from all treatments (Fig. 2). Average (± standard deviation, SD) internal Zn concentrations reached the highest level for $ZnCl_2$ 500 (221 ± 83 μg g^{-1} dw) and ZnO-NP 500 (230 ± 53 μg g^{-1} dw) on the second day of the exposure. For the lowest ionic treatment ($ZnCl_2$ 250) and highest nanoparticle treatment (ZnO-NP 1000), the highest

Table 1
pH_{CaCl2} (average ± standard deviation, n = 3) of Lufa 2.2 soil spiked with different concentrations of $ZnCl_2$ or ZnO nanoparticles (ZnO-NPs), measured during the uptake phase in a toxicokinetics study with the earthworm *Eisenia andrei*.

Treatment	Nominal Zn concentration (μg g^{-1} dw)	pH in soil			
		T = 0	T = 7	T = 14	T = 21
Control	**0**	6.0 ± 0.01	6.1 ± 0.04	5.8 ± 0.06	5.4 ± 0.06
$ZnCl_2$	**250**	5.9 ± 0.04	5.8 ± 0.03	5.7 ± 0.03	5.6 ± 0.01
	500	5.7 ± 0.02	5.7 ± 0.02	5.7 ± 0.04	5.8 ± 0.005
ZnO-NPs	**500**	6.2 ± 0.01	6.3 ± 0.06	6.2 ± 0.01	5.9 ± 0.01
	1000	6.5 ± 0.004	6.5 ± 0.04	6.4 ± 0.02	6.1 ± 0.01

Table 2
Average (± standard deviation; n = 3) zinc concentrations measured in Lufa 2.2 soil, soil porewater (PW), and porewater after ultrafiltration (U) (n = 2) spiked with different concentrations of $ZnCl_2$ or ZnO nanoparticles (ZnO-NPs) and in control soil. Soil porewater was collected at day (T) 0 and day 21 (last day of the exposure phase) in the uptake phase of a toxicokinetics experiment with the earthworm *Eisenia andrei*. Ultrafiltration was done at day 0. % – percentage of total Zn in porewater recovered after ultrafiltration.

Treatment	Zn concentration in soil (μg g^{-1})		Zn concentration in soil porewater (μg mL^{-1})			
	Nominal	Actual	T = 0			T = 21
			PW	U	%	PW
Control	**0**	40.4 ± 8	0.3 ± 0.3	0.03 ± 0.01	12	0.04 ± 0.01
$ZnCl_2$	**250**	287 ± 11	6.0 ± 0.1	5.1 ± 0.01	85	7.7 ± 0.3
	500	520 ± 56	53.7 ± 5.9	50.5 ± 7.8	94	33.4 ± 2.4
ZnO-NPs	**500**	511 ± 39	4.0 ± 0.1	2.2 ± 0.3	56	3.4 ± 0.5
	1000	940 ± 82	5.5 ± 0.3	3.1 ± 0.3	56	7.3 ± 0.8

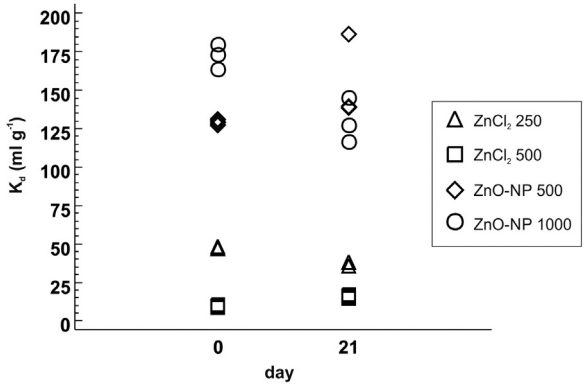

Fig. 1. Partition coefficients (n = 3) (*Kd* in mL g^{-1}) of Zn in Lufa 2.2 soil spiked with two different concentrations of $ZnCl_2$ or ZnO nanoparticles (in μg Zn g^{-1} dw). *Kd* is expressed as the total Zn concentration in soil divided by the Zn concentration in porewater, and was determined before the exposure (day 0) and after 21 days of the exposure in a toxicokinetics study with earthworms (*Eisenia andrei*).

average internal Zn concentrations were reached on the fourth day of exposure (196 ± 25 μg g^{-1} dw and 332 ± 34 μg g^{-1} dw, respectively). Then, the internal Zn concentrations gradually decreased in the earthworms from all treatments. After transferring the animals to clean soil, a rapid decrease was observed, to on average, 111 ± 11 μg Zn g^{-1} dw (mean ± SD, n = 94, calculated for the whole elimination phase and all treatments together).

Different modifications of the original model were tested as described in the Materials and Methods. Because the equation with single k_a and single k_e fitted to both phases together yielded a very poor fit, in the next step the model parameters k_{a1} and k_{e1} for the uptake phase and k_{a2} and k_{e2} for the decontamination phase were estimated separately. Even though this modification explained 60–75% of the total variance, all estimated parameters were nonsignificant (Table S2), making comparison between treatments impossible. Finally, the model with single k_a and single k_e was fitted to the internal Zn concentrations measured in the uptake phase only. The highest assimilation rate constant and the lowest elimination rate constant estimated based on total Zn concentrations in the soil ($k_{a\text{-}S}$, $k_{e\text{-}S}$) were found for the $ZnCl_2$ 250 treatment; the lowest $k_{a\text{-}S}$ and the highest $k_{e\text{-}S}$ were found for ZnO-NP 1000 (Table 3). The asymptotic 95% confidence intervals around $k_{a\text{-}S}$ for the different treatments did not overlap, indicating significant differences at $p < 0.05$. However, this comparison should be treated with caution, because the narrowness of the estimated confidence intervals can stem from the specific pattern of toxicokinetics, i.e. the rapid accumulation of Zn in the first 2 days of exposure followed by the

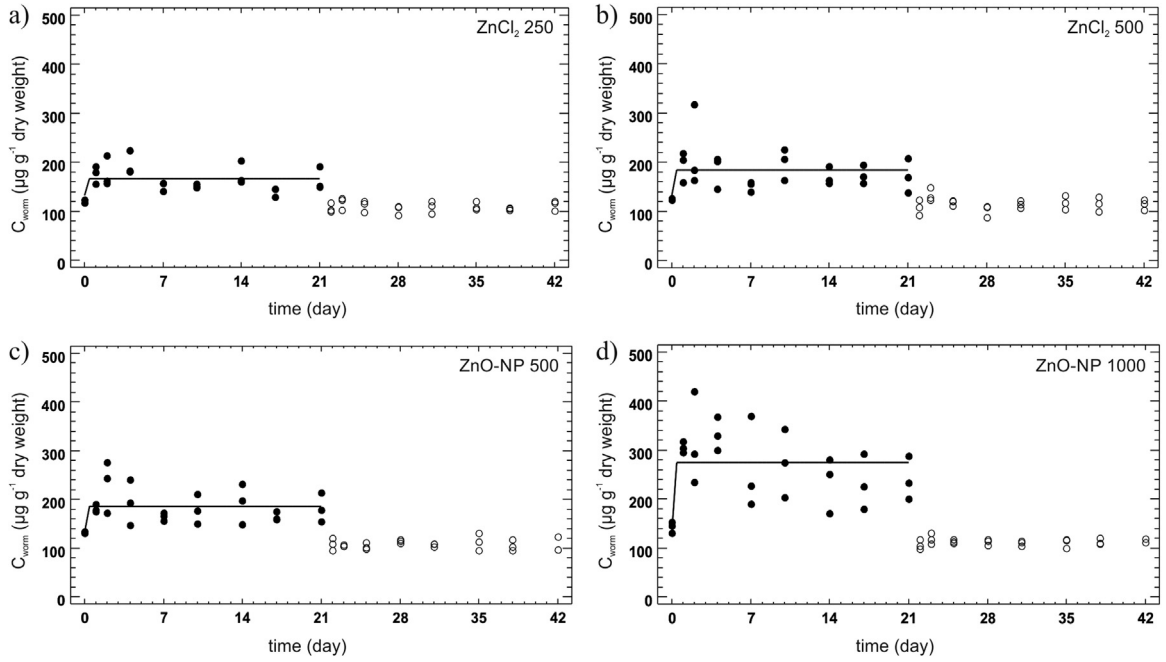

Fig. 2. Uptake and elimination kinetics of Zn in *Eisenia andrei* exposed to Lufa 2.2 soil contaminated with different concentrations (μg Zn g^{-1} dw) of $ZnCl_2$ (a, b) and ZnO nanoparticles (c, d) in relation to total Zn concentration in the soil. Dots represent measured internal Zn concentrations (solid dots – uptake phase; open dots – elimination phase). Kinetics curves (solid line) were estimated according to a one-compartment model applied to the 21-day uptake phase only. See Table 3 for the estimated model parameters.

Table 3
Parameters describing the uptake and elimination kinetics of Zn in the earthworm *Eisenia andrei* exposed in Lufa 2.2 soil contaminated with different concentrations of $ZnCl_2$ or ZnO nanoparticles (ZnO-NPs) (see Fig. 2). Kinetics parameters are related to total Zn concentrations measured in the soil (C_{exp}). Assimilation ($k_{a\text{-}S}$) and elimination rate constants ($k_{e\text{-}S}$) are given with corresponding asymptotic 95% confidence intervals (*CI*). Also included are the bioaccumulation factor ($BAF = k_{a\text{-}S}/k_{e\text{-}S}$), steady-state concentration ($C_{SS} = C_{exp}\, k_{a\text{-}S}/k_{e\text{-}S}$), and R-squared adjusted for degrees of freedom (R^2_{adj}). Kinetics parameters were estimated by fitting a one-compartment model to data for the uptake phase of the experiment.

Treatment	Nominal Zn concentration (μg g^{-1})	Estimated parameters						
		$k_{a\text{-}S}$ (g soil g^{-1} body weight day^{-1})	*CI*	$k_{e\text{-}S}$ (day^{-1})	*CI*	*BAF*	C_{SS} (μg g^{-1} dry body weight)	R^2_{adj}
$ZnCl_2$	**250**	11.3	(11.3060–11.3062)[a]	19.4	(18.3–20.6)[a]	0.58	167	0.18
	500	8.1	(8.0534–8.0538)[b]	22.8	(20.9–24.8)[bc]	0.35	183	0.12
ZnO-NPs	**500**	7.1	(7.1396–7.1399)[c]	19.6	(18.1–21.1)[ab]	0.36	186	0.19
	1000	7.0	(7.0025–7.0039)[d]	24.0	(21.7–26.3)[c]	0.29	274	0.29

[a,b,c,d] – these letters next to *CI* values indicate no differences between treatments for the parameter, i.e., the asymptotic 95% confidence intervals overlapped.

Table 4
Parameters describing the kinetics of Zn in the earthworm *Eisenia andrei* exposed in Lufa 2.2 soil contaminated with different concentrations of $ZnCl_2$ or ZnO nanoparticles (ZnO-NPs) with kinetics parameters related to the porewater Zn concentrations determined at day 0 (C_{exp}). The assimilation ($k_{a\text{-}PW}$) and elimination rate constants ($k_{e\text{-}PW}$) are given with the corresponding asymptotic 95% confidence intervals. Also given are the bioaccumulation factor ($BAF = k_{a\text{-}PW}/k_{e\text{-}PW}$), steady-state concentration ($C_{SS} = C_{exp}\, k_{a\text{-}PW}/k_{e\text{-}S}$), and R-squared adjusted for degrees of freedom (R^2_{adj}). Kinetics parameters were estimated by fitting a one-compartment model to data for the uptake phase of the experiment.

Treatment	Nominal Zn concentration (μg g^{-1})	Estimated parameters						
		$k_{a\text{-}PW}$ (mL porewater g^{-1} body weight day^{-1})	*CI**	$k_{e\text{-}PW}$ (day^{-1})	*CI*	*BAF*	C_{SS} (μg g^{-1} dry body weight)	R^2_{adj}
$ZnCl_2$	**250**	531	(531–531)[a]	19.1	(18.0–20.3)[a]	27.8	167	0.18
	500	86.7	(86.7–86.7)[b]	25.4	(23.2–27.6)[b]	3.4	183	0.12
ZnO-NPs	**500**	892	(892–892)[c]	19.2	(17.7–20.6)[a]	46.5	186	0.19
	1000	899	(899–899)[d]	18.0	(16.3–19.8)[a]	49.9	274	0.29

[a,b,c,d] – these letters next to values indicate no differences between treatments for the parameter, i.e., the asymptotic 95% confidence intervals overlapped.

* The upper and lower asymptotic 95% confidence intervals are not different even at the third decimal place which is the method's accuracy in Statgraphic Centurion XVI.

stabilization of the earthworm body concentrations in the uptake phase. Only parameters within a very narrow range are able to describe such a pattern, hence the estimated confidence intervals are extremely narrow, despite the variance between individual data points (Table 3). The value for $k_{e\text{-}S}$ for the $ZnCl_2$ 250 was significantly lower than those for $ZnCl_2$ 500 and ZnO-NP 1000, which did not differ between each other; $k_{e\text{-}S}$ for ZnO-NP 500 was significantly lower than that for ZnO-NP 1000 (Table 3). Kinetics parameters estimated based on the measured porewater Zn concentrations are presented in Table 4. Similar to $k_{a\text{-}S}$, $k_{a\text{-}PW}$ also differed significantly between all treatments, but again notice the narrow confidence intervals. Regarding the elimination rate constant ($k_{e\text{-}PW}$), only $ZnCl_2$ 500 differed significantly from other treatments. The percentage of the total variance of internal Zn concentrations explained by the models and fitted either to the total Zn concentrations in soil or Zn concentrations measured in porewater was similar: R^2_{adj} values ranged from 0.12 for $ZnCl_2$ 500 to 0.29 for ZnO-NP 1000 treatment.

4. Discussion

The present study showed that zinc, irrespective of form (NPs vs ions) or exposure concentration, is regulated efficiently by the earthworm *E. andrei* (Fig. 2). Differences between ionic Zn and ZnO-NP treatments as well as between different exposure concentrations were found for the assimilation rate constant (but see the limitations described in Methods section 2.5). The elimination kinetics of Zn differed only between exposure concentrations for either ionic Zn or ZnO-NP treatments when related to total Zn concentrations in soil and for ionic Zn only when related to Zn porewater concentrations. Thus, it is the exposure concentration rather than the form of Zn which influences the process of Zn regulation by earthworms: with increasing exposure concentration, Zn was accumulated to higher levels by earthworms and was eliminated from their bodies more rapidly.

4.1. Zinc availability in soil

The characteristics that determine the fate of nanoparticles in the environment comprise intrinsic and extrinsic factors (Caballero-Guzman and Nowack, 2016). The intrinsic factors relate to inherent properties of NPs such as size, shape, or coating (Garner and Keller, 2014), which were determined before our experiment. The extrinsic factors refer to the characteristics of the system, e.g. pH and organic matter content (Cornelis et al., 2014), which were monitored during the exposure. The measured total soil and porewater Zn concentrations enabled estimates of partition coefficients (K_d) for comparison of the availability of Zn to earthworms between different treatments. Previous studies showed that K_d is most affected by total metal concentration, pH, and organic matter content (Sauvé et al., 2000), with pH being the most important factor regulating zinc availability in soil (McBride et al., 1997; Rutkowska et al., 2015). In our study, partition coefficients were much lower for $ZnCl_2$ than for ZnO-NP treatments (Fig. 1), most probably because of different physicochemical properties of studied compounds. Romero-Freire et al. (2017) also found that K_d values in LUFA 2.2 soil contaminated with either $ZnCl_2$ or ZnO-NPs differed when measured after ageing for 1, 3, 56 and 168 days. The authors suggested that lower porewater Zn concentrations obtained for ZnO-NP than for $ZnCl_2$ treatments, especially at the beginning of the experiment, indicated agglomeration of NPs and their staying in particulate form. During the exposure, slight decreases in K_d and pH values were observed for the $ZnCl_2$ 250 treatment, resulting in a slight increase in Zn concentrations in the porewater. Conversely, in the $ZnCl_2$ 500 treatment, pH values were, in general, the lowest among all treatments and did not change much during the exposure. The $ZnCl_2$ 500 treatment was also characterised by the lowest K_d values at the beginning and end of the exposure. Relatively low K_d values for both $ZnCl_2$ treatments indicate high solubility of Zn, which may be due to the lower adsorptive capacity of the soil solid surfaces and pronounced competition between Zn and other cations, such as H^+ ions (Rutkowska et al., 2015).

In accordance with previous studies, Zn concentrations in the porewater were lower for ZnO-NP than for $ZnCl_2$ treatments (Heggelund et al., 2014; Kool et al., 2011) and increased only slightly during 21 days of exposure in ZnO-NP 1000. The increase of porewater Zn concentration over time was previously demonstrated by Waalewijn-Kool et al. (2013). These authors observed that after three months of equilibration, zinc concentrations in porewater were 4 and 8 times higher, respectively, than those measured directly after spiking soil with uncoated ZnO-NPs at 400 and 800 mg Zn kg^{-1} (Waalewijn-Kool et al., 2013). In our experiment we used the same nanoparticles as Waalewijn-Kool et al. (2013), but measured porewater Zn concentration after much shorter exposure time. We found higher porewater Zn concentrations after 21 days of exposure for the ZnO-NP 1000 treatment than for the ZnO-NP 500 treatment. It is possible that at the higher exposure concentration more NPs could have interacted with different components of the soil, affecting their speciation and transformation and resulting in dissolution and ion release (Cornelis et al., 2014). This might also have led to a saturation effect, i.e. not all ions could find binding sites and, as a consequence, we found higher Zn concentrations in the porewater for ZnO-NP 1000 than for ZnO-NP 500. However, our ultrafiltration results contradict those obtained by Waalewijn-Kool et al. (2013). Directly after spiking soil with different concentrations of uncoated ZnO-NPs, Waalewijn-Kool et al. (2013) found more than 90% of the total porewater zinc concentration in ultrafiltrates (indicating that only 10% of Zn stayed in the NP form), in comparison with only 56% recorded for the ZnO-NPs treatments in our study. More zinc – above 80% of the total Zn concentration – was found in the porewater following ultrafiltration for $ZnCl_2$ treatments (Table 2). It must be stressed, however, that, Waalewijn-Kool et al. (2013) collected porewater, and thus ultrafiltrates, after one week of saturation of the soil, while in our study only 48-h equilibration was used. Thus, the time of equilibration after the soil saturation might have an impact on speciation and dissolution of ZnO-NPs.

4.2. Toxicokinetics

In many studies on earthworms, models for Zn toxicokinetics either could not be fitted at all or parameters could not be estimated properly (Ardestani et al., 2014). The main reason is that zinc, as an essential metal, is regulated efficiently by earthworms, even when they are exposed to concentrations exceeding those in their natural environment (Giska et al., 2014; Lock and Janssen, 2001a). In most cases, regardless of soil type (artificial/field), either initial rapid accumulation and then elimination as early as within the exposure period (Smith et al., 2010; Vijver et al., 2005) or steady state within 2–3 days (Peijnenburg et al., 1999) were observed. In another study, Zn uptake and elimination were barely observable in earthworms, even though Zn exposure concentrations in soil greatly exceeded natural background concentrations, making it impossible to fit the classic one-compartment model (Giska et al., 2014). Similarly, in our study the classic one-compartment model could not be fitted to the data from both phases simultaneously, and was thus fitted only to the uptake phase. The model with two different sets of parameters, k_{a1} and k_{e1} for the uptake phase and k_{a2} and k_{e2} for the elimination phase (Skip et al., 2014), could not be fitted satisfactorily to our data due to its complexity; the model required estimation of so many parameters that it was not possible to obtain meaningful confidence intervals, and thus significance levels, with the available data (Table S2).

4.2.1. Uptake and elimination kinetics related to total Zn concentrations in the soil

The differences in k_a (in $g_{soil}\ g^{-1}{}_{body\ weight}\ day^{-1}$) were significant between all treatments, although more pronounced for ionic zinc ($k_{a\text{-}S}$ $ZnCl_2$ 250 = 11.3 and $k_{a\text{-}S}$ $ZnCl_2$ 500 = 8.1) than for nanoparticles ($k_{a\text{-}S}$ ZnO-NP 500 = 7.1 and $k_{a\text{-}S}$ ZnO-NP 1000 = 7.0), indicating that k_a depends on both the exposure concentration and form of Zn in the exposed soil. However, $k_{e\text{-}S}$ was different only for the earthworms exposed to different effect concentrations (EC_{25} vs EC_{50}) within both ionic and NP treatments. The lack of differences in $k_{e\text{-}S}$ between the same nominal exposure concentrations ($ZnCl_2$ 500, ZnO-NP 500) suggests similar elimination kinetics regardless of the form of Zn in soil. Because elimination rates were the same for treatments with different forms of Zn in the Lufa 2.2 soil and, at the same exposure concentration, k_e depended rather on internal concentration in the earthworms, it is probable that Zn in the body of the earthworms was present in a single (i.e. ionic) form regardless of its form in the test soil. A similar conclusion was drawn previously for the potworm *Enchytraeus crypticus* exposed to different forms of Ag by Topuz and van Gestel (2015), who suggested that the form in which Ag was present in the test organisms may have been the same regardless of the different Ag compounds (citrate- or polyvinylpyrrolidone-coated Ag-NPs or $AgNO_3$) in the sand-solution test medium. Moreover, ZnO nanoparticles along with Ag and CuO nanoparticles, are the most soluble ones (Bondarenko et al., 2016). In a multi-laboratory evaluation of 15 bioassays, Bondarenko et al. (2016) showed that toxicity patterns of ZnO to different organisms were almost identical for respective metal salts, suggesting that the toxicity of ZnO was due to dissolution. On the other hand, an in-depth study of Ag NP internalisation in the earthworm *Lumbricus rubellus* (after 7 days of exposure to 500 μg g^{-1} as Ag-NPs), demonstrated that the NPs presumably were taken up by the animals as intact Ag-NPs via the endocytosis pathway (Diez-Ortiz et al., 2015). Taking into account the results obtained by Diez-Ortiz et al. (2015) we cannot exclude the possibility that some nanoparticles had been internalized and/or regulated by earthworms in their pristine form. Thus the lack of differences in the elimination rate could be the result of either similar or different mechanisms of regulation for ions and NPs, but these mechanisms cannot be distinguished from each other on the organismal level and only an in-depth study done at cellular level would help to identify the exact mechanism. In our study, regulation of different forms of Zn depended on exposure concentration: the higher the exposure concentration, the lower the value of $k_{a\text{-}S}$ and the higher $k_{e\text{-}S}$. Exposure concentration-dependent kinetics was observed previously in the cricket *Gryllus assimilis* exposed to elevated concentrations of ionic Zn (164–2620 mg Zn kg^{-1}) via food (Bednarska et al., 2015).

When comparing our results with other studies on Zn toxicokinetics in earthworms, both the uptake and elimination rates derived from this study were one or two orders of magnitude higher than previously reported values. For instance, Nahmani et al. (2009) derived uptake rate constants of 0.06 and 0.02 $g_{soil}\ g^{-1}{}_{body\ weight}\ day^{-1}$, and elimination rate constants of 0.37 and 0.09 day^{-1} when *E. fetida* were exposed to field soil with total Zn concentrations of 1050 and 902 μg Zn g^{-1}, respectively. Smith et al. (2010), who exposed *E. andrei* to 106 μg Zn g^{-1} in artificial soil spiked with zinc chloride, also obtained low values for $k_{a\text{-}S}$ (0.44 $g_{soil}\ g^{-1}{}_{body\ weight}\ day^{-1}$) and $k_{e\text{-}S}$ (0.45 day^{-1}). To the best of our knowledge, to date only one toxicokinetics study has been performed on earthworms with ZnO-NPs, showing that accumulation and elimination kinetics were the same for both forms of zinc in the test soil (Laycock et al., 2016). Laycock et al. (2016) investigated the kinetics of isotopically enriched zinc (dissolved ^{68}Zn and ^{68}ZnO-NPs) applied to soil at a concentration of 5 μg of enriched ^{68}Zn per gram of soil. Since the exposure lasted only 72 h, the kinetics parameters obtained by Laycock et al. (2016) are not comparable with our study. Nevertheless, from the calculated kinetics, the authors estimated a BAF, for a 30-day exposure period, of 3.4 for the treatment with ^{68}ZnO-NPs and 4.9 for dissolved ^{68}Zn. The previously mentioned study by Smith et al. (2010) reported a BAF of 0.98, and even lower values were found in our study (between 0.29 and 0.58). Because Zn, as an essential metal, is regulated efficiently by earthworms, BAF should decrease with increasing exposure concentration (Lock and Janssen, 2001b). The steady-state Zn concentrations (C_{ss}) in the earthworms (from 167 to 274 μg g^{-1}) at the end of the uptake phase were similar to the values

obtained by Nahmani et al. (2009) for earthworms exposed to either 1050 or 902 µg Zn g^{-1} dry soil (either 167 µg g^{-1} or 162 µg g^{-1} dry body weight, respectively). The fact that a 2-fold increase in exposure concentration resulted in a less than 2-fold increase in internal concentration confirmed the efficient regulation of Zn by the earthworms. Although higher internal Zn concentrations in the NP than in the ionic treatments had already been observed in earthworms exposed to elevated ZnO-NP levels and explained by direct internalisation of ZnO-NPs by the organism (Heggelund et al., 2014; Hooper et al., 2011), we found no such differences in our study: the values of C_{ss} for ZnO-NP 500 and $ZnCl_2$ 500 treatments were very similar.

4.2.2. Uptake and elimination kinetics related to Zn concentration in porewater

The actual risk of NPs exposure in soil is connected with the bioavailability of the NPs and/or the released metal ions (Cornelis et al., 2014). The bioavailable fraction of the metal in soil is mainly related to its concentration in the liquid phase (Van Gestel, 1997). Therefore, we calculated kinetic parameters based not only on total Zn concentrations in soil, but also on porewater concentrations. Similar to the results for $k_{a\text{-}S}$, $k_{a\text{-}PW}$ also differed significantly between all treatments; the biggest difference was found between $ZnCl_2$ 500, for which $k_{a\text{-}PW}$ was the lowest, and the other treatments. At the same time, the $k_{e\text{-}PW}$ for $ZnCl_2$ 500 was the lowest, and only this treatment differed significantly from the other treatments. Generally, the lowest pH values in soil were found for the $ZnCl_2$ 500 treatment; this might reduce sorption and promote competition between metal ions and cations, leading to higher porewater concentrations (Alloway, 2013). Even though the Biotic Ligand Model (BLM) was not formally tested in our study, the assimilation rate for $ZnCl_2$ 500, 6–10 times lower than for the other treatments, is in agreement with the BLM principle (Ardestani et al., 2015): competition with other cations (i.e. H^+) might reduce the binding of Zn to biotic ligands and, as a consequence, limit uptake of metal by soil organisms, thus reduced assimilation could occur. For the ZnO-NP treatments the reverse effect might occur: since porewater Zn concentrations were lower in both NP treatments, lower uptake was expected. However, higher pH values, and therefore less competition between Zn and other ions for binding sites, might have contributed to high uptake rates related to porewater concentrations in the NP treatments. It should be noted, however, that Zn enters an earthworm's body by means of oral ingestion and through the skin. For example, Vijver et al. (2003) exposed L. *rubellus* for 6 days to field-contaminated soils (2092 or 732 µg Zn g^{-1} dw) and showed that 21–30% of Zn uptake was attributed to the oral route. When *L. rubellus* was exposed to labelled ^{68}Zn (5 µg g^{-1} soil) in nanoparticle form, only 5% of the total uptake was attributed to the dermal route (Laycock et al., 2016). Thus, uptake cannot be linked exclusively to porewater concentrations, as is done in BLM (Li et al., 2008; Thakali et al., 2006), which assumes that uptake occurs only via porewater and therefore a metal reaches earthworms mostly through the body wall and only partly with ingested soil which also contains porewater phase. The rest however, is digested from the solid part of the soil matrix.

5. Conclusion

Assimilation but not elimination rate constants differed significantly between different forms of Zn (ZnO-NPs vs Zn ions) when the kinetics model was fitted to total soil Zn concentrations. Regulation of different forms of Zn depended on exposure concentration; in general, the higher the exposure concentration, the lower the value of $k_{a\text{-}S}$ and the higher $k_{e\text{-}S}$. Lower assimilation rate constants at higher exposure concentrations may be connected with intrinsic factors related to characteristics of the compounds (i.e. solubility), extrinsic factors related to soil properties (i.e. pH), and the physiology of the animals.

Acknowledgments

The authors thank R. A. Verweij for technical support in the experiment.

Financial support was provided by the National Science Centre, Poland, project PRELUDIUM (2015/17/N/NZ8/01576). Z. Świątek was supported by the project 'Launching interdisciplinary doctoral studies programme in ecology in English and increasing the didactic potential of the staff of the Institute of Environmental Sciences at the Jagiellonian University' (POKL.04.01.01-00-053/09) and Jagiellonian University (DS 758).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2017.05.027.

References

Alloway, B.J., 2013. Bioavailability of elements in soil. In: Essentials of Medical Geology. Springer, Netherlands, Dordrecht, pp. 351–373. ⟨http://doi.org/10.1007/978-94-007-4375-5_15⟩.

Ardestani, M.M., Van Straalen, N.M., Van Gestel, C.A.M., 2015. Biotic ligand modeling approach: synthesis of the effect of major cations on the toxicity of metals to soil and aquatic organisms. Environ. Toxicol. Chem. 34, 2194–2204. http://dx.doi.org/10.1002/etc.3060.

Ardestani, M.M., Van Straalen, N.M., Van Gestel, C.A.M., 2014. Uptake and elimination kinetics of metals in soil invertebrates: a review. Environ. Pollut. 193, 277–295. http://dx.doi.org/10.1016/j.envpol.2014.06.026.

Bednarska, A.J., Opyd, M., Żurawicz, E., Laskowski, R., 2015. Regulation of body metal concentrations: toxicokinetics of cadmium and zinc in crickets. Ecotoxicol. Environ. Saf. 119, 9–14. http://dx.doi.org/10.1016/j.ecoenv.2015.04.056.

Bondarenko, O.M., Heinlaan, M., Sihtmäe, M., Ivask, A., Kurvet, I., Joonas, E., Jemec, A., Mannerström, M., Heinonen, T., Rekulapelly, R., Singh, S., Zou, J., Pyykkö, I., Drobne, D., Kahru, A., 2016. Multilaboratory evaluation of 15 bioassays for (eco) toxicity screening and hazard ranking of engineered nanomaterials: FP7 project NANOVALID. Nanotoxicology 5390, 1–14. http://dx.doi.org/10.1080/17435390.2016.1196251.

Caballero-Guzman, A., Nowack, B., 2016. A critical review of engineered nanomaterial release data: are current data useful for material flow modeling? Environ. Pollut. 213, 502–517. http://dx.doi.org/10.1016/j.envpol.2016.02.028.

Cañas, J.E., Qi, B., Li, S., Maul, J.D., Cox, S.B., Das, S., Green, M.J., 2011. Acute and reproductive toxicity of nano-sized metal oxides (ZnO and TiO2) to earthworms (*Eisenia fetida*). J. Environ. Monit. 13, 3351. http://dx.doi.org/10.1039/c1em10497g.

Cornelis, G., Hund-Rinke, K., Kuhlbusch, T., Van den Brink, N., Nickel, C., 2014. Fate and bioavailability of engineered nanoparticles in soils: a review. Crit. Rev. Environ. Sci. Technol. 44, 2720–2764. http://dx.doi.org/10.1080/10643389.2013.829767.

Diez-Ortiz, M., Lahive, E., Kille, P., Powell, K., Morgan, A.J., Jurkschat, K., Van Gestel, C.A.M., Mosselmans, J.F.W., Svendsen, C., Spurgeon, D.J., 2015. Uptake routes and toxicokinetics of silver nanoparticles and silver ions in the earthworm *Lumbricus rubellus*. Environ. Toxicol. Chem. 34, 2263–2270. http://dx.doi.org/10.1002/etc.3036.

Fernández, M.D., Alonso-Blázquez, M.N., García-Gómez, C., Babin, M., 2014. Evaluation of zinc oxide nanoparticle toxicity in sludge products applied to agricultural soil using multispecies soil systems. Sci. Total Environ. 497–498. http://dx.doi.org/10.1016/j.scitotenv.2014.07.085.

García-Gómez, C., Fernández, M.D., Babin, M., 2014. Ecotoxicological evaluation of sewage sludge contaminated with zinc oxide nanoparticles. Arch. Environ. Contam. Toxicol. 67, 494–506. http://dx.doi.org/10.1007/s00244-014-0070-2.

Garcia-Reyero, N., Kennedy, A.J., Escalon, B.L., Habib, T., Laird, J.G., Rawat, A., Wiseman, S., Hecker, M., Denslow, N., Steevens, J.A., Perkins, E.J., 2014. Differential effects and potential adverse outcomes of ionic silver and silver nanoparticles in vivo and in vitro. Environ. Sci. Technol. 48, 4546–4555. http://dx.doi.org/10.1021/es4042258.

Garner, K.L., Keller, A.A., 2014. Emerging patterns for engineered nanomaterials in the environment: a review of fate and toxicity studies. J. Nanopart. Res. 16, 2503. http://dx.doi.org/10.1007/s11051-014-2503-2.

Giska, I., Van Gestel, C.A.M., Skip, B., Laskowski, R., 2014. Toxicokinetics of metals in the earthworm *Lumbricus rubellus* exposed to natural polluted soils – relevance of laboratory tests to the field situation. Environ. Pollut. 190, 123–132. http://dx.doi.org/10.1016/j.envpol.2014.03.022.

Heggelund, L.R., Diez-Ortiz, M., Lofts, S., Lahive, E., Jurkschat, K., Wojnarowicz, J., Cedergreen, N., Spurgeon, D., Svendsen, C., 2014. Soil pH effects on the comparative toxicity of dissolved zinc, non-nano and nano ZnO to the earthworm *Eisenia fetida*. Nanotoxicology 8. http://dx.doi.org/10.3109/17435390.2013.809808.

Hooper, H.L., Jurkschat, K., Morgan, A.J., Bailey, J., Lawlor, A.J., Spurgeon, D.J., Svendsen, C., 2011. Comparative chronic toxicity of nanoparticulate and ionic zinc to the earthworm *Eisenia veneta* in a soil matrix. Environ. Int. 37, 1111–1117. http://dx.doi.org/10.1016/j.envint.2011.02.019.

Ju-Nam, Y., Lead, J.R., 2008. Manufactured nanoparticles: an overview of their chemistry, interactions and potential environmental implications. Sci. Total Environ. 400, 396–414. http://dx.doi.org/10.1016/j.scitotenv.2008.06.042.

Kool, P.L., Ortiz, M.D., Van Gestel, C.A.M., 2011. Chronic toxicity of ZnO nanoparticles, non-nano ZnO and $ZnCl_2$ to *Folsomia candida* (Collembola) in relation to bioavailability in soil. Environ. Pollut. 159, 2713–2719. http://dx.doi.org/10.1016/j.envpol.2011.05.021.

Kwak Il, J., An, Y.-J., 2015. Ecotoxicological effects of nanomaterials on earthworms: a review. Hum. Ecol. Risk Assess. Int. J. 21, 1566–1575. http://dx.doi.org/10.1080/10807039.2014.960302.

Laycock, A., Diez-Ortiz, M., Larner, F., Dybowska, A., Spurgeon, D., Valsami-Jones, E., Rehkämper, M., Svendsen, C., 2016. Earthworm uptake routes and rates of ionic Zn and ZnO nanoparticles at realistic concentrations, traced using stable isotope labeling. Environ. Sci. Technol. 50, 412–419. http://dx.doi.org/10.1021/acs.est.5b03413.

Li, L.-Z., Zhou, D.-M., Peijnenburg, W.J.G.M., Van Gestel, C.A.M., Jin, S.-Y., Wang, Y.-J., Wang, P., 2011. Toxicity of zinc oxide nanoparticles in the earthworm, *Eisenia fetida* and subcellular fractionation of Zn. Environ. Int. 37, 1098–1104. http://dx.doi.org/10.1016/j.envint.2011.01.008.

Li, L., Wu, H., Peijnenburg, W.J.G.M., Van Gestel, C.A.M., 2015. Both released silver ions and particulate Ag contribute to the toxicity of AgNPs to earthworm *Eisenia fetida*. Nanotoxicology 9, 792–801. http://dx.doi.org/10.3109/17435390.2014.976851.

Li, L., Zhou, D., Wang, P., Peijnenburg, W.J.G.M., 2009. Kinetics of cadmium uptake and subcellular partitioning in the earthworm *Eisenia fetida* exposed to cadmium-contaminated soil. Arch. Environ. Contam. Toxicol. 57. http://dx.doi.org/10.1007/s00244-009-9296-9.

Li, L.Z., Zhou, D.M., Luo, X.S., Wang, P., Wang, Q.Y., 2008. Effect of major cations and pH on the acute toxicity of cadmium to the earthworm *Eisenia fetida*: implications for the biotic ligand model approach. Arch. Environ. Contam. Toxicol. 55, 70–77. http://dx.doi.org/10.1007/s00244-007-9100-7.

Lock, K., Janssen, C.R., 2001a. Modeling zinc toxicity for terrestrial invertebrates. Environ. Toxicol. Chem. 20, 1901–1908. http://dx.doi.org/10.1002/etc.5620200907.

Lock, K., Janssen, C.R., 2001b. Zinc and cadmium body burdens in terrestrial oligochaetes: use and significance in environmental risk assessment. Environ. Toxicol. Chem. 20, 2067–2072. http://dx.doi.org/10.1002/etc.5620200928.

McBride, M., Sauvé, S., Hendershot, W., 1997. Solubility control of Cu, Zn, Cd and Pb in contaminated soils. Eur. J. Soil Sci. 48, 337–346. http://dx.doi.org/10.1111/j.1365-2389.1997.tb00554.x.

Nahmani, J., Hodson, M.E., Devin, S., Vijver, M.G., 2009. Uptake kinetics of metals by the earthworm *Eisenia fetida* exposed to field-contaminated soils. Environ. Pollut. 157, 2622–2628. http://dx.doi.org/10.1016/j.envpol.2009.05.002.

OECD, 2010. Test No. 317: Bioaccumulation in terrestrial oligochaetes. OECD Guidelines for the Testing of Chemicals. OECD Guidelines for the Testing of Chemicals, Section 3. OECD Publishing. ⟨http://doi.org/10.1787/9789264090934-en⟩.

Peijnenburg, W.J.G.M., Baerselman, R., de Groot, A.C., Jager, T., Posthuma, L., Van Veen, R.P.M., 1999. Relating environmental availability to bioavailability: soil-type-dependent metal accumulation in the Oligochaete *Eisenia andrei*. Ecotoxicol. Environ. Saf. 44, 294–310. http://dx.doi.org/10.1006/eesa.1999.1838.

Romero-Freire, A., Lofts, S., Martín Peinado, F.J., Van Gestel, C.A.M., 2017. Effects of aging and soil properties on zinc oxide nanoparticle availability and its ecotoxicological effects to the earthworm Eisenia andrei. Environ. Toxicol. Chem. 36, 137–146. http://dx.doi.org/10.1002/etc.3512.

Rutkowska, B., Szulc, W., Bomze, K., Gozdowski, D., Spychaj-Fabisiak, E., 2015. Soil factors affecting solubility and mobility of zinc in contaminated soils. Int. J. Environ. Sci. Technol. 12, 1687–1694. http://dx.doi.org/10.1007/s13762-014-0546-7.

Sauvé, S., Hendershot, W., Allen, H.E., 2000. Solid-solution partitioning of metals in contaminated soils: dependence on pH, total metal burden, and organic matter. Environ. Sci. Technol. 34, 1125–1131. http://dx.doi.org/10.1021/es9907764.

Skip, B., Bednarska, A.J., Laskowski, R., 2014. Toxicokinetics of metals in terrestrial invertebrates: making things straight with the one-compartment principle. PLoS One 9. http://dx.doi.org/10.1371/journal.pone.0108740.

Smith, B.A., Egeler, P., Gilberg, D., Hendershot, W., Stephenson, G.L., 2010. Uptake and elimination of cadmium and zinc by *Eisenia andrei* during exposure to low concentrations in artificial soil. Arch. Environ. Contam. Toxicol. 59, 264–273. http://dx.doi.org/10.1007/s00244-009-9459-8.

Thakali, S., Allen, H.E., Di Toro, D.M., Ponizovsky, A.A., Rooney, C.P., Zhao, F.-J., McGrath, S.P., 2006. A terrestrial biotic ligand model. 1. Development and application to Cu and Ni toxicities to barley root elongation in soils. Environ. Sci. Technol. 40, 7085–7093. http://dx.doi.org/10.1021/es061171s.

Topuz, E., Van Gestel, C.A.M., 2015. Toxicokinetics and toxicodynamics of differently coated silver nanoparticles and silver nitrate in *Enchytraeus crypticus* upon aqueous exposure in an inert sand medium. Environ. Toxicol. Chem. 34, 2816–2823. http://dx.doi.org/10.1002/etc.3123.

Van Gestel, C.A.M., 1997. Scientific basis for extrapolating results from soil ecotoxicity tests to field conditions and the use of bioassays. In: Ecological Risk Assessment of Contaminants in Soil. Springer, US, Boston, MA, pp. 25–50. ⟨http://doi.org/10.1007/978-1-4615-6361-7_2⟩.

Vijver, M.G., Vink, J.P.M., Jager, T., Wolterbeek, H.T., Van Straalen, N.M., Van Gestel, C.A.M., 2005. Biphasic elimination and uptake kinetics of Zn and Cd in the earthworm *Lumbricus rubellus* exposed to contaminated floodplain soil. Soil Biol. Biochem. 37, 1843–1851. http://dx.doi.org/10.1016/j.soilbio.2005.02.016.

Vijver, M.G., Vink, J.P.M., Miermans, C.J.H., Van Gestel, C.A.M., 2003. Oral sealing using glue: a new method to distinguish between intestinal and dermal uptake of metals in earthworms. Soil Biol. Biochem. 35, 125–132. http://dx.doi.org/10.1016/S0038-0717(02)00245-6.

Waalewijn-Kool, P.L., Diez Ortiz, M., Van Gestel, C.A.M., 2012. Effect of different spiking procedures on the distribution and toxicity of ZnO nanoparticles in soil. Ecotoxicology 21, 1797–1804. http://dx.doi.org/10.1007/s10646-012-0914-3.

Waalewijn-Kool, P.L., Diez Ortiz, M., Van Straalen, N.M., Van Gestel, C.A.M., 2013. Sorption, dissolution and pH determine the long-term equilibration and toxicity of coated and uncoated ZnO nanoparticles in soil. Environ. Pollut. 178, 59–64. http://dx.doi.org/10.1016/j.envpol.2013.03.003.