Quantitative modelling of the response of earthworms to metals
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Chapter III

Modelling Cadmium And Nickel Toxicity To Earthworms With The Free Ion Approach

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Abstract

The use of the Free Ion Approach to quantify the toxic effects of Cd and Ni to the earthworms *Lumbricus rubellus* and *Aporrectodea longa* exposed in soils of different types was explored. Median lethal concentration (LC50) of Cd (expressed as the total concentration in soil) varied by approximately 11 and 28 fold for *L. rubellus* and *A. longa*, respectively. For Ni, these values were 50 and 38, respectively. For the two earthworm species, no significant influence of cations (H^+, Ca^{2+}, Mg^{2+}, K^+, and Na^+) on Cd^{2+} toxicity was observed, while Mg^{2+} was found to significantly alleviate Ni^{2+} toxicity. The Free Ion Activity Model, which is a special case of the Free Ion Approach with no impact of cations, sufficiently described the variability in Cd^{2+} toxicity across soils but failed in predicting Ni^{2+} toxicity. The Free Ion Approach, in which the protective effects of Mg^{2+} were included, explained 89% and 84% of the variations in LC50$\{Ni^{2+}\}$ (expressed as free ion activity) for *L. rubellus* ($\log LC50\{Ni^{2+}\} = 1.18\log\{Mg^{2+}\} - 0.52$) and *A. longa* ($\log LC50\{Ni^{2+}\} = 0.51\log\{Mg^{2+}\} - 2.16$), respectively. Prediction error was within a factor of 2 for both Cd^{2+} and Ni^{2+} toxicity, indicating the applicability of the Free Ion Approach for predicting toxicity of these two metals. Although extrapolation of the Free Ion Approach across metals still needs more research efforts, this approach, as an alternative for the biotic ligand model, provides a feasible framework for site-specific risk assessment.

3.1 Introduction

Toxicity of metals to soil organisms frequently deviates across different soil types. For example, up to a factor of 11 and 50 differences in median effect concentrations (EC50) of Cu were found for *Eisenia fetida* cocoon production and *Folsomia candida* juvenile production, respectively, in 19 European soils (Criel et al., 2008). The EC50 of Ni for barley root elongation reached a maximum variation of 37-fold in 16 soils (Rooney et al., 2007). Data interpretation is complicated as the effects are metal- or species-specific. Furthermore, many soil properties are more or less correlated with each other (Wang et al., 2011a), making it difficult to distinguish the real source of an effect from the interdependent factors. Many empirical models have been developed to relate metal toxicity to typical soil properties, such as pH, organic matter content, and cation exchange capacity (CEC) (Criel et al., 2008; Oorts et al., 2006; Rooney et al., 2007). The predictive ability of these models seems to be reasonably well. However, the mechanisms underlying such empirical relationships are not fully revealed.

In the past years, many studies have been performed attempting to understand the exact mechanisms that induce differences in bioavailability and toxicity. An important development in this respect is the finding that the free metal ion is the dominant species that can be taken up by the organisms and subsequently induces toxicity. This triggered the development of the free ion activity model (FIAM) (Campbell, 1995; Morel, 1983). After it was shown that uptake and effect of a metal are highly influenced by other coexisting cations (e.g., H^+, Ca^{2+}, and Mg^{2+}), the FIAM was extended into the biotic ligand model (BLM) in order to incorporate both metal speciation and the effects of competition with other cations (De Schamphelaere and Janssen, 2002; Plette et al., 1999). The BLM concept is based on the mechanistic assumption that metal toxicity depends on the amount of metal ion bound to active sites within the organism (i.e., biotic ligand, BL), while protons and other cations can alleviate metal toxicity by competitive binding to the BL (De Schamphelaere and Janssen, 2002).

In the aquatic environment, the BLM has shown the advantage of being generally valid and applicable, as it relies on fundamental thermo-dynamical rather than fitting parameters (Di Toro et al., 2001). However, the applicability of BLM theory to soils has rarely been evaluated. Several BLMs have been developed for soil animals such as a Cu-BLM for the earthworm *Aporrectodea caliginosa* (Steenbergen et al., 2005), a Cd-BLM for the earthworm *Eisenia fetida* (Li et al., 2008), and a Co-BLM for the enchytraeid *Enchytraeus albidus* (Lock et al., 2006). BLMs were also used to assess the effects of metals on plants (Li et al., 2009a; Lock et al., 2007). All these BLMs were developed using solution (or solution-sand) as exposure medium instead of real soil, as it is difficult to univariately manipulate soil porewater properties and to estimate reliable and representative BLM parameters. Thakali and co-workers (Thakali et al., 2006a; 2006b) reported the development and application of BLMs for predicting Cu and Ni toxicity to invertebrates, plants and microbial processes in soils. They found that it is always necessary to empirically fix one or more parameters of the BLM before fitting the binding constants for each cation. Therefore, the derived stability constants should not be regarded as conventional stability constants but rather as parameters that summarize the processes underlying the observed relationships between metal toxicity and protective cations.

Recently, based on empirical studies and BLM theory, Lofts and co-workers (Lofts et al., 2004; 2013) have proposed an alternative method, the Free Ion Approach to describe the variation in the toxic effects of a metal among soils of varying compositions, with the variation in soil porewater pH and concentrations of other protective cations (e.g., Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}). This approach, requiring fewer parameters than the BLM, has been successfully used to model Cu toxicity to soil invertebrates (including earthworms) and plants (Lofts et al., 2013; Qiu et al., 2013). However, the applicability for other metals is still unknown. In addition, a lack of consistent effects caused by other cations is often found between solution cultures and soil cultures. For example, in hydroponic systems, pH and Na^{+} were reported to alleviate Cu toxicity to *A. caliginosa* (Steenbergen et al., 2005). In a soil matrix, however, only a pH dependence of Cu^{2+} toxicity (no protective effects of other cations) was found for *Lumbricus rubellus*, *Aporrectodea longa* and *E. fetida* (Qiu et al., 2013). More soil toxicity data sets are therefore needed for the purpose of comparison and reliable extrapolation.

The aims of the present study were to investigate the applicability of the Free Ion Approach for predicting Cd and Ni toxicity to *Lumbricus rubellus* and *Aporrectodea longa* in a range of soils of varying compositions, and to examine whether the toxicity-modifying factors are similar for these two earthworm species.

3.2 Materials and methods

Test organisms

The earthworms *Lumbricus rubellus* and *Aporrectodea longa* were used as test organisms. They are natural species and showed to be more sensitive to metals than the artificially cultured *E. fetida* (Qiu et al., 2013; Spurgeon et al., 2000). *L. rubellus* (epigeic) is commonly found throughout Europe and North America. It feeds on surface litter and is found intimately associated with plant roots (Domínguez, 2004). *A. longa* (anecic) is usually present in alkaline soils in open areas such as gardens, grassland and cultivated soils. It draws organic matter from the soil surface into its deep, permanent burrows to feed on (Eisenhauer et al., 2008). Mature *L. rubellus* and *A. longa* were collected from an unexploited grassland in Leiden, The Netherlands. Prior to the toxicity tests the earthworms were cultured in the unspiked soils for at least 1 wk at 15 ± 2 °C to get adapted to the experimental conditions.

Soils and metal spiking

Six different soil samples (coded NL1, NL2, NL3, NL4, NL5, and NL6) were collected from different sites in the Netherlands. The soils were air dried, sieved (< 2mm), and homogenized before use. Several metal concentrations including controls were obtained by spiking the soils with metal acetates (Acros Chemicals; purity > 98%). The spiked Cd concentrations ranged from 25 mg/kg to the maximum of 2500 mg/kg depending on the soils, the spiked Ni concentrations ranged from 50 mg/kg to the maximum of 6600 mg/kg. The spiked soils were left for equilibration for two months before testing, assuring mineralisation of the acetate added (Qiu et al., 2011).

Toxicity tests

The 28-d toxicity tests were performed in a climate chamber at 15 ± 2 °C with a 16h-light: 8h-dark cycle. Adult earthworms with a clearly developed clitellum were used. Earthworms with body weights between 700 and 900 mg for *L. rubellus*, and between 800 and 1000 mg for *A. longa* were selected for testing. Four earthworms were exposed in a plastic jar containing 500 g dry weight of soil. For each treatment, three replicates were used. Soil maximum water-holding capacity was determined by the saturation and gravity drainage method (Pansu and Gautheyrou, 2006). All soils were maintained at 80% maximum water-holding capacity throughout the test by reweighing the test jars periodically and replenishing water loss with deionized water. Earthworms were fed once a week. Approximately 5 g of cow manure was spread on the soil surface of each jar and moistened with 5 mL deionized water. Mortality of the earthworms was scored after 28 d of exposure.

Chemical analysis

Total metal (Cd and Ni) concentrations in soil samples were determined after digestion using a 1:4 v/v mixture of HCl (37% pro analysis, Baker) and HNO_3 (65% pro analysis; Riedel-de-Haen). Soil porewater was collected by suction and subsequent filtration over a 0.45 µm acetate filter of soil samples that were kept at 100% of their maximum water-holding capacity for 1 wk at 15 °C (Qiu et al., 2013). Soil pH (in 0.01M $CaCl_2$ extract) and porewater pH were measured using a pH meter (691, Metrohm AG). Soil properties such as organic matter content (OM), texture, and cation exchange capacity (CEC) were determined following the procedures described by Pansu and Gautheyrou (2006). Dissolved organic carbon (DOC) in soil porewater was measured with a TOC/DOC analyzer (TOC-VCSH, Shimadzu). Concentrations of dissolved metals, Ca, Mg, Na, and K were analysed by flame atomic absorption spectrophotometry (Perkin Elmer AAnalyst 100). The certified reference material ISE 989 (River Clay, Wageningen Evaluating Programs) was used to ensure the accuracy of the analytical procedure for soil digests. Measured metal concentrations were within 15% of the certified reference values.

Data analysis

Metal concentrations inducing 50% mortality (LC50s) of earthworms in each soil were calculated by Probit analysis using SPSS 19.0 (IBM). The soil porewater properties (pH, DOC, and dissolved cations concentrations) corresponding to the total soil metal concentration at the LC50 value (denoted LC50[M]) for each soil were interpolated from the measured values at the tested exposure concentrations. The function TREND in Microsoft Excel 2010 was used for interpolation as generally there were linear relationships between the amount of metal spiked and pH, DOC, and concentrations of dissolved cations in soil porewater. Activities of the free metal ion and of other cations (Ca^{2+}, Mg^{2+}, K^+, and Na^+) in soil porewater were calculated using the speciation model WHAM (VI) (Tipping, 1998). Input parameters included porewater pH, concentrations of dissolved metal and other elements (Ca, Mg, Na, K, Cl^-, and SO_4^{2-}), and colloidal fulvic acid (FA). The latter was estimated from the DOC concentration assuming that 65% DOC was active as colloidal FA and 35% DOC was inert for chemical binding (Tipping et al., 2003). In addition, Cl^- and SO_4^{2-} in a molar ratio of 6:1 were assumed to maintain electroneutrality (Thakali et al., 2006a; 2006b). Previous studies used the same ratio indicating that the calculated free metal ion activity was insensitive to the ratio of Cl^- to SO_4^{2-} and that this approximation is unlikely to induce significant uncertainty since the strong acid anions do not enter into significant complexation reactions with Cd and Ni (Qiu et al., 2013; Tipping et al., 2003). Measured values were used for all data analysis unless otherwise indicated.

Free ion approach

The free ion approach is an empirical expression attributing the variation in the toxic effect of a metal, among soils of varying composition, to the variation in soil porewater pH and the variation in concentrations of other solution cations (e.g., Ca^{2+}, Mg^{2+}, K^+, and Na^+):

$$\log\{M^{2+}\}_{LC50} = \alpha\, pH_{pw} + \sum_1^i \beta_i \log\{X_i^{z+}\} + \gamma \quad (3\text{-}1)$$

In equation 3-1, pH_{pw} is the soil porewater pH, $\{X_i^{z+}\}$ (mol/L) is the activity of protective cation in soil porewater, α, β_i, and γ are constants, $\{M^{2+}\}_{LC50}$ (mol/L) is the activity of the free metal ion in soil porewater inducing 50% mortality of organisms. The free ion approach considers the same parameters as would be considered by the BLM. Instead of complex parameter estimation, this can be regarded as a simplification by replacing the BLM with a linear equation that contains only the most sensitive parameters (Qiu et al., 2013; Verschoor et al., 2012). Stepwise multiple linear regression was performed to decided which parameters (H^+, Ca^{2+}, Mg^{2+}, K^+, and Na^+) need to be included into the model. The parameters were included if they passed stepping criteria ($p < 0.05$ for entry, and $p > 0.1$ for removal) and were significant at the probability level $p < 0.001$.

To extend the free ion approach to consider the entire dose-response curves, equation 3-1 can be rearranged and generalized as follows:

$$\gamma_{effect} = \log\{M^{2+}\}_{effect} - \alpha\, pH_{pw} - \sum_1^i \beta_i \log\{X_i^{z+}\} \quad (3\text{-}2)$$

In equation 3-2, γ_{effect} (dimensionless) can be regarded as the effective dose which considers not only the toxicity-driving factor $\{M^{2+}\}$ (mol/L) but also the toxicity-modifying factors $\{X_i^{z+}\}$ (mol/L). γ_{effect} is constant at a given effect level and varies according to the level of effect being described. $\{M^{2+}\}_{effect}$ is the corresponding $\{M^{2+}\}$ at any given effect level.

When the free ion approach was proposed, ion binding was not explicitly considered. To provide the free ion approach with a theoretical basis, a multicomponent Freundlich model was proposed to relate the effective dose term to metal bound to biotic ligand (Qiu et al., 2013):

$$[MBL] = k\, \{M^{2+}\}^{nM}\, \{H^+\}^{nH} \prod_1^i \{X_i^{z+}\}^{nXi} \quad (3\text{-}3)$$

where [MBL] is the amount of metal bound to the BL, k, n_M, n_H, and n_{Xi} are the Freundlich parameters. Rearranging equation 3-3 after log-transformation yields:

$$\log\{M^{2+}\} = \frac{n_H}{n_M}\, pH + \sum_1^i \frac{n_{Xi}}{n_M} \log\{X_i^{z+}\} + \frac{1}{n_M} \log\left(\frac{[MBL]}{k}\right) \quad (3\text{-}4)$$

If $\frac{n_H}{n_M} = \alpha$, $\frac{n_{Xi}}{n_M} = \beta_i$, and $\frac{1}{n_M}\log\left(\frac{[MBL]}{k}\right) = \gamma_{effect}$ (assuming [MBL] is constant at a given effect level based on BLM theory), equation 3-4 has the same form as equation 3-2. Thus, the effective dose term can be quantitatively linked to metal bound to the biotic ligand of the organism.

A logistic dose-response equation was used to fit the entire toxicity data for each metal:

$$R = \frac{R_0}{1+\left(\frac{X}{X50}\right)^{\beta}} \quad (3\text{-}5)$$

where R is the survival rate, R_o is the control survival rate, β is the slope parameter. When using the free ion approach, $X = \gamma_{effect}$ and X_{50} is the effective dose (dimensionless) causing 50% mortality of the earthworms. The effective dose term can include one or more of the protective cations (H^+, Ca^{2+}, Mg^{2+}, K^+, and Na^+) as well as $\{M^{2+}\}$. The free ion approach is an extension of the free ion activity model by taking the toxicity-modifying factors into account. When no protective effects of cations were found and the γ_{effect} only contained $\{M^{2+}\}$, the free ion approach had the same form as the FIAM. In this case, $X = \{M^{2+}\}$ and X_{50} is the overall $\{M^{2+}\}_{LC50}$. When substituting equation 3-2 into equation 3-5, R was plotted against X to fit the parameters β, X_{50}, and the coefficients for the toxicity-modifying factors. Model parameters were determined by multiple nonlinear regression analysis using SYSTAT 12. Whether the model is significantly improved by the addition of an extra variable is determined by the Akaike information criterion (AIC) (Akaike, 1981). The AIC value deals with the trade-off between the complexity (number of parameters) of the model and the goodness-of-fit of the model. From a statistical viewpoint, the model with the smallest AIC value is preferable.

3.3 Results

Selected properties of soil and porewater

The selected soil and porewater properties of the nonspiked soils are shown in Table 3.1. These soils represented different soil types and varied substantially in pH, OM content, and CEC. Appreciable ranges of DOC and dissolved major cation (Ca, Mg, Na, and K) concentrations in the porewater of the study soils were observed (Table 3.1). The addition of metal salts to the soils resulted in an increase in the Cd or Ni concentration in the soil porewater. The porewater Cd or Ni concentrations correlated linearly with the corresponding total Cd or Ni concentrations in each soil (p < 0.001; Table 3.2-3.3). Generally, adding porewater pH as explanatory variable improved R^2 values and the significance of all the regression equations used for predicting metal partitioning between soil solid phase and porewater (Table 3.2-3.3). This is consistent with the metal partitioning being pH-dependent. Significant correlations between a fair amount of parameters in soil porewater were found (Supporting Information, Table S3.1-S3.2). For instance, dissolved Ca and Mg, as well as Ca and Na concentrations were strongly correlated with each other in both Cd-spiked and Ni-spiked soils.

Dose−response relationships for Cd toxicity

Figure 3.1 shows the effect of Cd on the survival of *L. rubellus* and *A. longa*. Control survival of the two species in the nonspiked soils was more than 90% after 28 d of exposure. The earthworm survival rate decreased with increasing total Cd concentration in soil. When expressed as the total soil concentration, the LC50[Cd] ranged between 37 and 401 mg/kg (a variation of 11-fold) for *L. rubellus*, and between 49 and 1409 mg/kg (a variation of 28-fold) for *A. longa* (Table 3.4). *L. rubellus* was more sensitive to Cd than *A. longa* in all tested soils. When applying the total soil Cd concentration to predict Cd toxicity among different soils, the model fits were poor (R^2 = 0.38 and RMSE = 28.4 for *L. rubellus*; R^2 = 0.24 and RMSE = 28.6 for *A. longa*) (Figure 3.1A and 3.1B). When expressed as $\{Cd^{2+}\}$ in soil porewater, LC50$\{Cd^{2+}\}$ differed by a factor of 2.6 for *L. rubellus* and 2.3 for *A. longa* between the different test soils (Table 3.4). Considerable improvements of the model fits were observed when using $\{Cd^{2+}\}$ as the descriptor for Cd toxicity. The resulting values of R^2 and RMSE were, respectively, 0.94 and 9.5 for *L. rubellus*, and 0.86 and 14.4 for *A. longa* (Figure 3.1C and 3.1D).

Table 3.1 Selected characteristics of the nonspiked soils and porewater

Properties	NL1	NL2	NL3	NL4	NL5	NL6
Soil pH-$CaCl_2$	5.5	6.2	5.8	7.9	7.3	4.5
Total Cd (mg/kg)	0.65	0.17	0.36	0.13	0.64	0.28
Total Ni (mg/kg)	5.0	7.6	10.9	2.16	2.64	6.99
Organic matter (%)	6.4	5.7	21.7	10.2	12.3	2.0
CEC (cmol/kg)[a]	8.3	10.1	38.8	16.5	30.9	1.7
Texture	loamy sand	sandy loam	silt loam	clay loam	loam	sandy
Clay content (%)	5.9	7.0	16.5	33.8	27.1	0.8
Silt content (%)	8.9	22.5	53.2	46.1	40.9	2.9
Sand content (%)	85.2	70.5	30.3	20.1	32.0	96.3
Porewater pH	5.88	6.48	6.25	8.02	7.48	5.00
DOC (mg/L)[b]	149	182	503	192	342	189
Dissolved Na (mg/L)	41.3	66.1	119	97.4	45.0	43.8
Dissolved K (mg/L)	191	68.8	39.0	15.3	31.7	96.8
Dissolved Ca (mg/L)	120	199	509	370	337	101
Dissolved Mg (mg/L)	92.6	71.5	141	56.3	51.5	79.4

[a]Cation exchange capacity; [b]Dissolved organic carbon.

Table 3.2 Linear regression relationships between dissolved Cd concentrations in soil porewater (Cd_{pw}, mg/L), total soil Cd concentrations (Cd_{total}, mg/kg), and pH of the porewater in the six tested soils

Soils	Equations	R^2_{adj} [a]	n	F	p
NL1	$\log Cd_{pw} = 0.99\ (\pm0.11) \log Cd_{total} - 1.49\ (\pm0.28)$	0.89	11	76.3	<0.001
	$\log Cd_{pw} = 1.13\ (\pm0.06) \log Cd_{total} - 0.33\ (\pm0.07)\ pH + 0.11\ (\pm0.36)$	0.97	11	158.2	<0.001
NL2	$\log Cd_{pw} = 0.91\ (\pm0.08) \log Cd_{total} - 1.46\ (\pm0.23)$	0.89	15	110.3	<0.001
	$\log Cd_{pw} = 1.02\ (\pm0.03) \log Cd_{total} - 0.42\ (\pm0.04)\ pH + 1.04\ (\pm0.27)$	0.99	15	494.8	<0.001
NL3	$\log Cd_{pw} = 0.78\ (\pm0.05) \log Cd_{total} -1.32\ (\pm0.17)$	0.92	16	183.7	<0.001
	$\log Cd_{pw} = 0.85\ (\pm0.06) \log Cd_{total} - 0.26\ (\pm0.12)\ pH + 0.11\ (\pm0.72)$	0.94	16	116.7	<0.001
NL4	$\log Cd_{pw} = 1.11\ (\pm0.14) \log Cd_{total} - 2.58\ (\pm0.41)$	0.83	14	62.9	<0.001
	$\log Cd_{pw} = 1.09\ (\pm0.04) \log Cd_{total} - 0.90\ (\pm0.08)\ pH + 4.29\ (\pm0.63)$	0.98	14	413.9	<0.001
NL5	$\log Cd_{pw} = 1.09\ (\pm0.16) \log Cd_{total} - 2.39\ (\pm0.49)$	0.78	14	42.6	<0.001
	$\log Cd_{pw} = 1.17\ (\pm0.11) \log Cd_{total} - 0.43\ (\pm0.10)\ pH + 0.41\ (\pm0.77)$	0.90	14	57.8	<0.001
NL6	$\log Cd_{pw} = 1.28\ (\pm0.24) \log Cd_{total} - 1.47\ (\pm0.43)$	0.85	7	28.3	<0.001
	$\log Cd_{pw} = 1.21\ (\pm0.04) \log Cd_{total} - 0.51\ (\pm0.05)\ pH + 1.25\ (\pm0.28)$	0.99	7	430.4	<0.001
All soils	$\log Cd_{pw} = 0.72\ (\pm0.07) \log Cd_{total} - 1.09\ (\pm0.19)$	0.59	77	106.4	<0.001
	$\log Cd_{pw} = 0.97\ (\pm0.04) \log Cd_{total} - 0.44\ (\pm0.03)\ pH + 1.09\ (\pm0.18)$	0.90	77	308.7	<0.001

[a] R^2_{adj} is the coefficient of determination adjusted for the degrees of freedom; n indicates the number of data points; F is the value of F test; p indicates the statistical significance level; Standard errors are indicated in brackets.

Table 3.3 Linear regression relationships between dissolved Ni concentrations in soil porewater (Ni_{pw}, mg/L), total soil Ni concentrations (Ni_{total}, mg/kg), and pH of the porewater in the six tested soils

Soils	Equations	R^2_{adj} [a]	n	F	p
NL1	$\log Ni_{pw} = 2.14\ (\pm0.32) \log Ni_{total} - 4.36\ (\pm0.86)$	0.87	8	42.9	<0.001
	$\log Ni_{pw} = 2.01\ (\pm0.20) \log Ni_{total} - 1.02\ (\pm0.34)\ pH + 2.49\ (\pm2.34)$	0.95	8	60.4	<0.001
NL2	$\log Ni_{pw} = 1.34\ (\pm0.16) \log Ni_{total} - 2.36\ (\pm0.48)$	0.88	11	69.7	<0.001
	$\log Ni_{pw} = 1.37\ (\pm0.12) \log Ni_{total} - 0.38\ (\pm0.14)\ pH + 0.04\ (\pm1.00)$	0.93	11	63.1	<0.001
NL3	$\log Ni_{pw} = 0.81\ (\pm0.11) \log Ni_{total} - 1.19\ (\pm0.36)$	0.82	14	56.3	<0.001
	$\log Ni_{pw} = 1.13\ (\pm0.09) \log Ni_{total} - 0.36\ (\pm0.08)\ pH + 0.01\ (\pm0.34)$	0.94	14	88.4	<0.001
NL4	$\log Ni_{pw} = 0.91\ (\pm0.11) \log Ni_{total} - 2.88\ (\pm0.39)$	0.82	16	63.4	<0.001
	$\log Ni_{pw} = 0.83\ (\pm0.08) \log Ni_{total} - 0.60\ (\pm0.17)\ pH + 2.08\ (\pm1.49)$	0.90	16	62.9	<0.001
NL5	$\log Ni_{pw} = 1.13\ (\pm0.14) \log Ni_{total} - 2.65\ (\pm0.48)$	0.86	12	67.3	<0.001
	$\log Ni_{pw} = 1.15\ (\pm0.15) \log Ni_{total} - 0.03\ (\pm0.15)\ pH - 2.95\ (\pm1.38)$	0.85	12	30.1	<0.001
NL6	$\log Ni_{pw} = 2.17\ (\pm0.20) \log Ni_{total} - 3.52\ (\pm0.36)$	0.94	7	111.1	<0.001
	$\log Ni_{pw} = 2.33\ (\pm0.14) \log Ni_{total} - 0.89\ (\pm0.32)\ pH + 0.84\ (\pm1.57)$	0.98	7	135.5	<0.001
All soils	$\log Ni_{pw} = 0.70\ (\pm0.14) \log Ni_{total} - 1.15\ (\pm0.43)$	0.29	68	26.2	<0.001
	$\log Ni_{pw} = 1.45\ (\pm0.13) \log Ni_{total} - 0.84\ (\pm0.10)\ pH + 2.27\ (\pm0.52)$	0.66	68	60.6	<0.001

[a] R^2_{adj} is the coefficient of determination adjusted for the degrees of freedom; n indicates the number of data points; F is the value of F test; p indicates the statistical significance level; Standard errors are indicated in brackets.

Table 3.4 Total metal concentrations ([Cd] and [Ni], mg/kg), and free metal ion activity ({Cd^{2+}} and {Ni^{2+}}, M) causing 50% mortality of the earthworms *Lumbricus rubellus* and *Aporrectodea longa* (with the corresponding 95% confidence intervals in parentheses) exposed in different test soils for 28 d.

Soils	*L. rubellus*				*A. longa*			
	LC50[Cd] [a]	pLC50{Cd^{2+}} [b]	LC50[Ni]	pLC50{Ni^{2+}}	LC50[Cd]	pLC50{Cd^{2+}}	LC50[Ni]	pLC50{Ni^{2+}}
NL1	63	5.48	242	4.35	141	5.06	380	3.96
	(48-83)	(5.38-5.59)	(199-295)	(4.08-4.63)	(99-202)	(4.77-5.35)	(285-506)	(3.63-4.28)
NL2	127	5.16	557	3.77	212	4.87	1294	3.63
	(84-192)	(4.92-5.40)	(497-624)	(3.69-3.85)	(181-248)	(4.78-4.96)	(1044-1605)	(3.38-3.88)
NL3	339	5.34	2354	3.71	1231	4.73	4039	3.38
	(232-494)	(5.15-5.52)	(1715-3232)	(3.57-3.85)	(954-1588)	(4.63-4.82)	(3345-4877)	(3.34-3.42)
NL4	410	5.43	3470	4.26	1409	4.80	5266	3.81
	(327-514)	(5.23-5.64)	(3001-4012)	(4.18-4.34)	(1167-1700)	(4.73-4.88)	(4577-6059)	(3.69-3.93)
NL5	319	5.57	2163	4.01	900	4.91	4253	3.65
	(241-421)	(5.35-5.79)	(1889-2476)	(3.88-4.14)	(747-1085)	(4.84-4.98)	(3739-4839)	(3.63-3.68)
NL6	37	5.25	70	4.58	49	5.10	139	4.07
	(31-44)	(5.10-5.40)	(54-90)	(4.16-5.00)	(40-59)	(4.87-5.33)	(105-185)	(3.98-4.16)

[a] LC50 indicates median lethal concentration; [b] pLC50 = −logLC50

In order to tentatively identify the possible protective effects of other cations (H^+, Ca^{2+}, Mg^{2+}, Na^+ and K^+) the effective dose term, consisting of an extra cation as well as Cd^{2+}, was used to fit the data with equation 3-5. The increase in R^2 and decrease in RMSE by using different effective dose terms compared with the model using $\{Cd^{2+}\}$ as the sole descriptor were marginal and the Akaike information criterion favored the latter for both earthworm species tested (Supporting Information, Table S3.3). No significant competing effects of other cations with Cd^{2+} for the biotic ligands were observed.

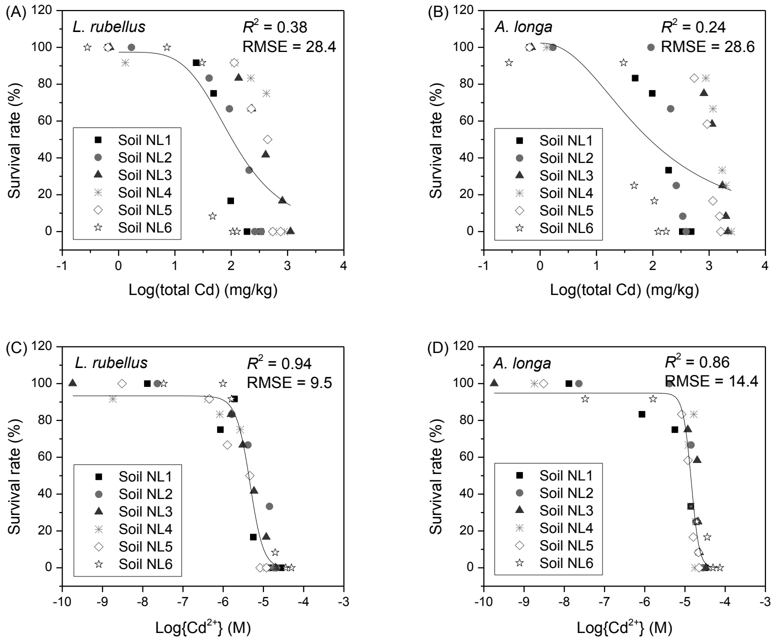

Figure 3.1 Dose-response relationships between survival rate and total Cd concentration in soil (first row), free Cd^{2+} activity in soil porewater (second row) for the earthworms *Lumbricus rubellus* and *Aporrectodea longa* exposed to 6 soils for 28 d. The solid lines represent the logistic model fits. R^2 indicates the determination coefficient of the linear regression between the predicted and observed survival rate. RMSE indicates the root-mean-square error of the predicted survival rate versus the observed values.

Dose−response relationships for Ni toxicity

The relationships between the survival of earthworms and different expressions of exposure of Ni are shown in Figure 3.2. Increasing total Ni concentrations decreased the survival of earthworms. Up to 50- and 38-fold differences in Ni toxicity (LC50[Ni]) occurred for *L. rubellus* and *A. longa*, respectively (Table 3.4). In all soils tested, *L. rubellus* was more sensitive to Ni than *A. longa*. When using total Ni concentration as the expression for Ni toxicity across different soils, poor fits were obtained with R^2 of 0.26 and RMSE of 33.4 for *L. rubellus*, and R^2 of 0.30 and RMSE of 29.4 for *A. longa* (Figure 3.2A and 3.2B). The model fits were considerably improved with $\{Ni^{2+}\}$ as the descriptor for toxicity (Figure 3.2C and 3.2D). The R^2 and RMSE values were 0.77 and 19.7 for *L. rubellus*, and 0.78 and 16.9 for *A. longa*, respectively. When based on $\{Ni^{2+}\}$ in soil porewater, differences in LC50$\{Ni^{2+}\}$ for the different soils were smaller (a variation of 7.4-fold for *L. rubellus* and 4.9-fold for *A. longa*) (Table 3.4).

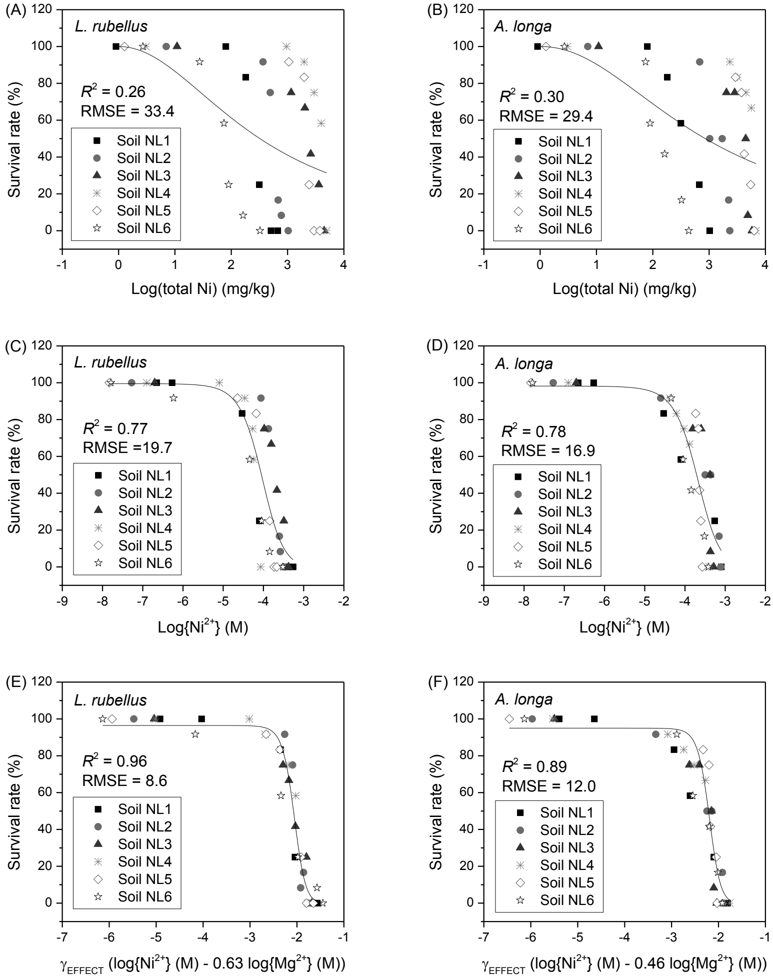

Figure 3.2 Dose-response relationships between the 28-d survival rate of earthworms *Lumbricus rubellus* and *Aporrectodea longa*, and different expressions of Ni exposure in 6 soils (soil total concentration, free Ni^{2+} activity in soil porewater, and effective dose (equation 3-2). The solid lines represent the logistic model fits. R^2 indicates the determination coefficient of the linear regression between the predicted and observed survival rate. RMSE indicates the root-mean-square error of the predicted survival rate versus the observed values.

To gain further insight into the importance of other cations in modifying Ni toxicity, the effective dose term comprising $\{Ni^{2+}\}$ and one of the variables (H^+, Ca^{2+}, Mg^{2+}, Na^+, and K^+)

was substituted into the model (equation 3-5) to fit the entire data set. Adding Mg^{2+} into the model gave the best prediction (R^2 = 0.96 and RMSE = 8.6 for *L. rubellus* (Figure 3.2E); R^2 = 0.89 and RMSE = 12.0 for *A. longa* (Figure 3.2F)) and addition of the other cations slightly improved the model fits (Supporting Information, Table S3.4). The effective dose term was further extended to include Ni^{2+}, Mg^{2+} and one of the other cations (H^+, Ca^{2+}, Na^+, and K^+). There were only marginal improvements in model performance (Supporting Information, Table S3.4). The AIC favored the effective dose terms:

$\gamma_{effect} = \log\{Ni^{2+}\} - 0.63 \log\{Mg^{2+}\}$ for *L. rubellus* (3-6)

$\gamma_{effect} = \log\{Ni^{2+}\} - 0.46 \log\{Mg^{2+}\}$ for *A. longa* (3-7)

Thus, Mg^{2+} was found to be a dominant factor in modifying Ni^{2+} toxicity to the two earthworm species, while the role of H^+, Ca^{2+}, Na^+, and K^+ was not appreciable.

Predictions of LC50s

An overall LC50$\{Cd^{2+}\}$ was obtained by applying the FIAM to the toxicity data for each earthworm species in all soils together. This FIAM predicted that LC50$\{Cd^{2+}\}$ never differed by a factor of more than 2 from the observed LC50$\{Cd^{2+}\}$ (Figure 3.3A), indicating that $\{Cd^{2+}\}$ sufficiently explained variations in Cd toxicity among soils. Thus, there was no further need to develop other predictive models for Cd toxicity.

Similarly, an overall LC50$\{Ni^{2+}\}$ was obtained for each species in all soils together. The FIAM predicted that LC50$\{Ni^{2+}\}$ was more than a factor of 2 of the observed values in some cases (3 out of 6 values for *L. rubellus*, 2 out of 6 values for *A. longa*) (Figure 3.3B). As it was not possible to predict the LC50$\{Ni^{2+}\}$ in all cases within an error factor of 2 using the FIAM, the free ion approach was used to directly link toxicity to the porewater composition. Using the interpolated soil porewater properties corresponding to the total soil metal concentration at LC50[Ni] (Supporting Information, Table S3.5-S3.6) as input parameters, the activities of other cations (H^+, Ca^{2+}, Mg^{2+}, Na^+ and K^+) in soil porewater were then calculated with WHAM VI. The coefficient for each porewater parameter in equation 3-1 was estimated by stepwise multiple linear regression with the observed LC50$\{Ni^{2+}\}$ as the dependent variable and the calculated activities of other cations as independent variables, yielding:

For *L. rubellus*:

$\text{Log LC50}\{Ni^{2+}\} = 1.18\ (\pm 0.19) \log\{Mg^{2+}\} - 0.52\ (\pm 0.15)$

($R^2_{adj} = 0.89$, $n = 6$, $p < 0.01$, $F = 36.6$) (3-8)

For *A. longa*:

$\text{Log LC50}\{Ni^{2+}\} = 0.51\ (\pm 0.11) \log\{Mg^{2+}\} - 2.16\ (\pm 0.34)$

($R^2_{adj} = 0.84$, $n = 6$, $p < 0.01$, $F = 21.5$) (3-9)

Only Mg^{2+} was identified as the explanatory variable and incorporated into the equations for predicting LC50$\{Ni^{2+}\}$. Other cations were the excluded variables as they did not pass stepping criteria. To verify the model parameters, LC50$\{Ni^{2+}\}$ was predicted by filling in the estimated model parameters in the above equations. The resulting values were then compared with the experimentally measured values. Figure 3.3C shows the accuracy of model predictions. Predicted LC50$\{Ni^{2+}\}$ were in good agreement with the observed values, with an error of far less than a factor of 2.

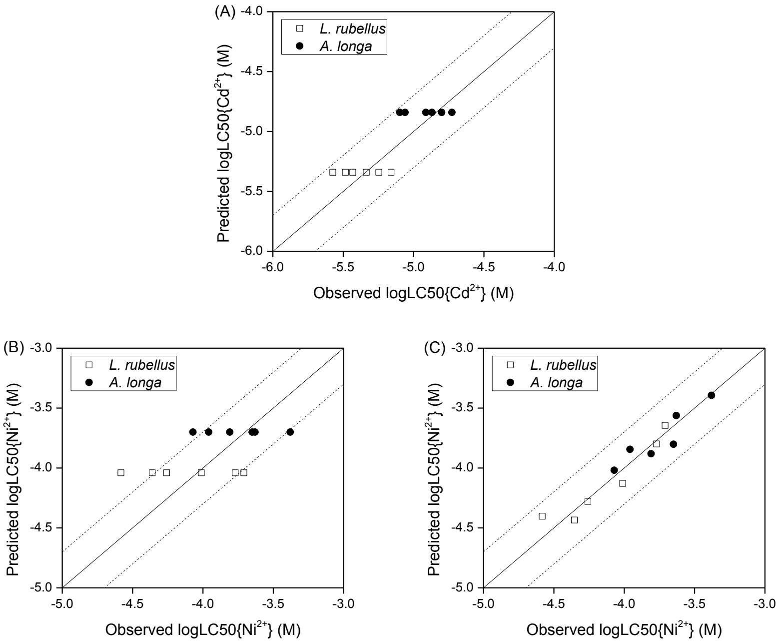

Figure 3.3 Relationship between the predicted and observed logLC50 expressed as free Cd^{2+} or Ni^{2+} activity in soil porewater for the earthworms *Lumbricus rubellus* and *Aporrectodea longa* in all 6 tested soils after 28 d of exposure. The predictions for logLC50{Cd^{2+}} were based on the free ion activity model (A). The predictions for logLC50{Ni^{2+}} were based on the free ion activity model (B) and the free ion approach (C) using the equations 8 and 9. The solid line represents the 1:1 line, and the dashed lines are a factor of 2 above and below the 1:1 line.

3.4 Discussion

A wide variation of Cd and Ni toxicity to earthworms among different soils stressed the importance of taking bioavailability and toxicity-modifying factors into account. In developing models for predicting metal toxicity to earthworms, dermal uptake of free metal ions present in soil porewater is assumed as the main route of exposure (Steenbergen et al., 2005; Vijver et al., 2003). According to the basic assumption of the BLM, solution cations (e.g., H^+, Ca^{2+}, Mg^{2+}, Na^+, and K^+) may alleviate metal toxicity through site-specific competition with metals for binding to the same active sites (transport sites or ion channels) of the organisms (De Schamphelaere and Janssen, 2002). Previously, it has been reported that all the above-mentioned cations alleviated Cd toxicity to *E. fetida* (Li et al., 2008), and that adding Mg^{2+} reduced Ni toxicity to plants (Lock et al., 2007). Therefore, in the present study, these cations in soil porewater were selected to determine if they exert a significant protective role on Cd and Ni toxicity to the two earthworm species tested.

Cd toxicity predictions

In the present study, {Cd^{2+}} sufficiently explained variations in Cd toxicity across soils. There were no significant improvements in the predictive ability of the model by accounting for competition of other cations (H^+, Ca^{2+}, Mg^{2+}, Na^+ and K^+). This finding provided direct evidence that the organisms respond to free Cd^{2+}. It was expected that Ca^{2+} would alleviate Cd^{2+} toxicity according to the hypothesis that Ca^{2+} and Cd^{2+} compete for the same binding sites (transport sites) (Niyogi and Wood, 2004). However, no significant effect of Ca^{2+} on Cd^{2+} toxicity was observed in the present study. This suggests that Ca^{2+} was not necessary to disrupt Cd^{2+} uptake by earthworms. The lack of improvement upon correction for Ca^{2+} might be attributed to the fact that Ca^{2+} positively or negatively correlated with other porewater parameters (see Supporting Information, Table S3.1). Besides, it should be noted that differences in porewater cation concentrations between the tested soils were generally small (approximately a factor of 5 for dissolved Ca concentrations), which may hinder the cations from manifesting their protective role.

Many publications have shown that the overall ability of the free metal ion to reflect toxicity of metals for aquatic and terrestrial organisms is limited (De Schamphelaere and Janssen, 2002; Steenbergen et al., 2005; Van Gestel and Koolhaas, 2004). The FIAM is a special case of the free ion approach with no impact of cations. It is remarkable that the overall LC50{Cd^{2+}} predicted with FIAM was within a factor of 2 of the individual LC50{Cd^{2+}} for the two earthworm species in such diverse soils. The presence of protons and other cations reacting with biological binding sites was supposed to affect Cd toxicity according to the BLM theory. A Cd-BLM was previously developed for the earthworm *E. fetida* exposed in a hydroponic system (Li et al., 2008). Competing effects of H^+, Ca^{2+}, Mg^{2+}, Na^+ and K^+ were observed and all integrated into the developed BLM. There was up to 1.7-fold difference between the predicted and observed LC50{Cd^{2+}} even though the difference in the observed LC50{Cd^{2+}} was always less than a factor of 2.2 for all cation sets. It therefore seems reasonably to use {Cd^{2+}} only to reflect the toxicity of Cd without considering the presence of protons and other cations. Ardestani et al. (2013) studied the impact of Ca and pH on the toxicity of Cd to *Folsomia candida* exposed to a simplified soil solution. They found that increasing Ca^{2+} activities had inconsistent effects on Cd toxicity and that toxicity was slightly lower at pH 7 and 6 than at pH 5. This finding does not seem to be in agreement with the BLM assumptions of cation (proton) competition, which should alleviate metal toxicity.

Ni toxicity predictions

A mitigating effect was shown for Mg^{2+} on Ni^{2+} toxicity to both *L. rubellus* and *A. longa*. The interaction of Mg^{2+} and Ni^{2+} has been widely reported (Lock et al., 2007; Thakali et al., 2006b; Antunes and Kreager, 2009). Similar ionic radii are found in Mg^{2+} and Ni^{2+} (0.066 nm for Mg^{2+} and 0.069 nm for Ni^{2+}), which enables Mg^{2+} to compete with Ni^{2+} for the Mg^{2+} channel or with carriers (Antunes and Kreager, 2009). Except for Mg^{2+}, the protective effects of H^+, Ca^{2+}, Na^+, and K^+ on Ni^{2+} toxicity to earthworms were negligible. Actually, when compared with the model using {Ni^{2+}} solely as the variable, the addition of Ca^{2+} or Na^+ did add some value to model performance, but this might be a result of the significant correlation between dissolved Ca and Mg concentrations (r = 0.74), and between dissolved Na and Mg concentrations (r = 0.81) (Supporting Information, Table S3.2). In addition to Ni^{2+} and Mg^{2+}, the contribution of an extra parameter (Ca^{2+} or Na^{+}) to accuracy of model prediction was marginal as indicated by the AIC values. This led to the conclusion that only Mg^{2+} significantly alleviated Ni^{2+} toxicity. In practice, the concentration of major cations in soil porewater often tends to be negatively correlated with the porewater pH (Wang et al., 2011a). Therefore, porewater pH was expected to exert a significant role in modifying metal toxicity as it can not only represent the role of H^{+}, but also the overall role of other cations (Lofts et al., 2004). The lack of protective effects of pH in the present study might be a result of the significant protective effects of Mg^{2+}; that is, Mg^{2+} suppressed or masked the competitive effect of H^{+}.

A lack of consistent effects from other cations was found in the literature. The alleviative effects of H^{+}, Ca^{2+} and Mg^{2+} were found for Ni toxicity to *Hordeum vulgare* root elongation and *Lycopersicon esculentum* shoot yield. Both H^{+} and Mg^{2+} competitions were observed for *E. fetida* cocoon production and *F. candida* reproduction (Thakali et al., 2006b). Only Mg^{2+} had an effect on Ni toxicity to *H. vulgare* root elongation (Lock et al., 2007). This might be attributed to several causes, such as covariance between H^{+} and other cations, species-specific ion binding ability, the use of different endpoints and differences in exposure medium.

The interaction of Mg^{2+} with Ni^{2+} for the biotic ligands, as considered by the free ion approach, accurately predicted the LC50$\{Ni^{2+}\}$. This shows the applicability of the free ion approach for predicting Ni toxicity to earthworms in a range of soils. The coefficients for log$\{Mg^{2+}\}$ (i.e., n_{Mg}/n_{Ni}) in equations 3-8 and 3-9 quantified the competition of Mg^{2+} with Ni^{2+} at the biotic ligands. These values were different for the two earthworm species, suggesting that the protective effects of Mg^{2+} on Ni^{2+} toxicity were species-dependent. In applying BLM theory to the soil system, parameterization of the BLM faces a variety of challenges and uncertainties (Erickson, 2013; Thakali et al., 2006b). The free ion approach avoids the need to fit separate affinity parameters for the toxic metal and potentially competing ions as the coefficients for each cation express the binding affinities of protective cations relative to metal ions (Lofts et al., 2013; Qiu et al., 2013). When developing a predictive model, it would be highly desirable to make the model as simple as possible, and to avoid over-parameterization as that suggests details on processes that cannot be justified (Van Zelm and Huijbregts, 2013). The ultimate goal for modellers should be, to meet the law of parsimony (Occam's Razor), to look for simple explanation of the observed phenomena rather postulating complex processes without empirical evidence (Hauschild et al., 2008). The free ion approach, therefore, is the preferred one when compared with the BLM or the complicated electrostatic model (Wang et al., 2011b; 2013) in predicting soil metal toxicity.

In the present study, Cd toxicity to earthworms was found to be driven by the $\{Cd^{2+}\}$ alone. In case of Ni, alleviation of Ni^{2+} toxicity by Mg^{2+} was observed. Previously, a pH dependence of Cu^{2+} toxicity to different earthworm species was reported (Qiu et al., 2013). These findings clearly showed that there is a lack of consistent effects from the presence of possible competing cations for different metals. Therefore, care should be taken when extrapolating the free ion approach across metals. We therefore strongly suggest that metal toxicity to earthworms needs to be evaluated on a metal-specific basis.

3.5 Conclusions

The free ion activity was a sufficient descriptor for Cd toxicity across the different soils, while it was in itself unsuccessful in describing the variability in Ni toxicity. The free ion approach, in line with the basic assumptions of the BLM, seemed to be a feasible way of modelling both Cd and Ni toxicity to different earthworms. As validity is proven, it may replace BLM or other complicated approaches and increase the applicability for site-specific risk assessment.

Supporting Information

Table S3.1 Pearson's correlation between porewater parameters (pH, dissolved Ca, Mg, Na, K, and Cd) for all unspiked and Cd-spiked soils

	Range	Correlation						
		pH	DOC	Ca	Mg	Na	K	Cd
pH	4.5-7.9	1	-0.11	0.39**	-0.27*	0.17	-0.61**	-0.01
DOC	92-569 mg/L		1	0.42**	0.42**	0.30*	-0.20	0.21
Ca	110-572 mg/L			1	0.60**	0.72**	-0.47**	-0.12
Mg	33-182 mg/L				1	0.68**	0.21	0.06
Na	28-136 mg/L					1	-0.19	0.03
K	11-229 mg/L						1	-0.10
Cd	0-31 mg/L							1

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

Table S3.2 Pearson's correlation between porewater parameters (pH, dissolved Ca, Mg, Na, K, and Ni) for all unspiked and Ni-spiked soils

	Range	Correlation						
		pH	DOC	Ca	Mg	Na	K	Ni
pH	4.6-7.9	1	-0.12	0.15	-0.27*	0.00	-0.39**	-0.07
DOC	95-561 mg/L		1	0.56**	0.39**	0.24	-0.24	-0.04
Ca	57-600 mg/L			1	0.74**	0.79**	-0.17	0.04
Mg	13-186 mg/L				1	0.81**	0.44**	0.29*
Na	14-133 mg/L					1	0.14	0.15
K	10-157 mg/L						1	0.29*
Ni	0-132 mg/L							1

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

Table S3.3 Fitting statistics for models (equation 5) using different effective dose terms for predicting the effect of Cd on the survival of earthworms *Lumbricus rubellus* and *Aporrectodea longa* in all six tested soils after 28 days of exposure. The parameters were estimated by multiple nonlinear regression (n = 36).

species	Effective dose	Model fit		
		R^2_{adj} [a]	RMSE [b]	AIC [c]
L. rubellus	$\log\{Cd^{2+}\}$ [d]	0.943	9.5	170
	$\log\{Cd^{2+}\}$ - 0.02 pH	0.947	9.5	172
	$\log\{Cd^{2+}\}$ + 0.008 $\log\{Ca^{2+}\}$	0.946	9.5	172
	$\log\{Cd^{2+}\}$ + 0.16 $\log\{Mg^{2+}\}$	0.947	9.5	172
	$\log\{Cd^{2+}\}$ - 0.14 $\log\{Na^{+}\}$	0.947	9.4	172
	$\log\{Cd^{2+}\}$ + 0.07 $\log\{K^{+}\}$	0.947	9.5	172
A. longa	$\log\{Cd^{2+}\}$ [d]	0.862	14.4	200
	$\log\{Cd^{2+}\}$ + 0.006 pH	0.862	14.3	202
	$\log\{Cd^{2+}\}$- 0.232 $\log\{Ca^{2+}\}$	0.870	13.9	200
	$\log\{Cd^{2+}\}$ - 0.102 $\log\{Mg^{2+}\}$	0.864	14.3	201
	$\log\{Cd^{2+}\}$ - 0.214 $\log\{Na^{+}\}$	0.871	13.9	200
	$\log\{Cd^{2+}\}$ - 0.004 $\log\{K^{+}\}$	0.862	14.4	202

[a] R^2_{adj} is the explained variance; [b] RMSE = Root mean squared error; [c] Akaike information criterion; [d] Model favoured.

Table S3.4 Fitting statistics for models (equation 5) using different effective dose terms for predicting the effect of Ni on the survival of earthworms *Lumbricus rubellus* and *Aporrectodea longa* in all six tested soils after 28 days of exposure. The parameters were estimated by multiple-nonlinear-regression (n = 36).

Species	Effective dose term	Model fit		
		R^2_{adj} [a]	RMSE [b]	AIC [c]
L.rubellus	$\log\{Ni^{2+}\}$	0.768	19.4	221
	$\log\{Ni^{2+}\}$ - 0.628 $\log\{Mg^{2+}\}$ [d]	0.955	8.6	164
	$\log\{Ni^{2+}\}$ - 0.001 pH	0.768	19.4	223
	$\log\{Ni^{2+}\}$ - 0.504 $\log\{Ca^{2+}\}$	0.862	15.0	205
	$\log\{Ni^{2+}\}$ - 0.943 $\log\{Na^{+}\}$	0.867	14.7	203
	$\log\{Ni^{2+}\}$ - 0.219 $\log\{K^{+}\}$	0.785	18.7	221
	$\log\{Ni^{2+}\}$ - 0.723 $\log\{Mg^{2+}\}$ - 0.083 pH	0.960	8.0	162
	$\log\{Ni^{2+}\}$ - 0.515 $\log\{Mg^{2+}\}$ - 0.180 $\log\{Ca^{2+}\}$	0.959	8.2	163
	$\log\{Ni^{2+}\}$ - 0.513 $\log\{Mg^{2+}\}$ - 0.333 $\log\{Na^{+}\}$	0.956	8.4	166
	$\log\{Ni^{2+}\}$ - 0.712 $\log\{Mg^{2+}\}$ + 0.114 $\log\{K^{+}\}$	0.957	8.3	165
A. longa	$\log\{Ni^{2+}\}$	0.784	16.9	212
	$\log\{Ni^{2+}\}$ - 0.456 $\log\{Mg^{2+}\}$ [d]	0.892	12.0	189
	$\log\{Ni^{2+}\}$ - 0.02 pH	0.785	16.9	214
	$\log\{Ni^{2+}\}$ - 0.462 $\log\{Ca^{2+}\}$	0.839	14.4	202
	$\log\{Ni^{2+}\}$ - 0.426 $\log\{Na^{+}\}$	0.821	15.5	207
	$\log\{Ni^{2+}\}$ - 0.136 $\log\{K^{+}\}$	0.791	16.7	213
	$\log\{Ni^{2+}\}$ - 0.455 $\log\{Mg^{2+}\}$ - 0.013 pH	0.893	11.9	191
	$\log\{Ni^{2+}\}$ - 0.305 $\log\{Mg^{2+}\}$ - 0.240 $\log\{Ca^{2+}\}$	0.902	11.5	188
	$\log\{Ni^{2+}\}$ - 0.524 $\log\{Mg^{2+}\}$ + 0.116 $\log\{Na^{+}\}$	0.897	11.7	189
	$\log\{Ni^{2+}\}$ - 0.511 $\log\{Mg^{2+}\}$ + 0.081 $\log\{K^{+}\}$	0.893	12.0	191

[a] R^2_{adj} is the explained variance; [b] RMSE = Root mean squared error; [c] Akaike information criterion; [d] Model favoured.

Table S3.5 The soil porewater properties (pH, dissolved organic carbon (DOC), and dissolved cations concentrations) interpolated at LC50 values of Ni (expressed as total soil concentration) for *Lumbricus rubellus* in the six soils tested. The function TREND in Microsoft Excel 2010 was used for interpolation based on the measured values at the tested exposure concentrations.

Soil	LC50 of Ni (mg kg^{-1})	Porewater pH	DOC (mg L^{-1})	Dissolved cations in soil porewater (mg L^{-1})				
				Ca	Mg	Na	K	Ni
NL1	242	6.42	244	126	86.6	57.5	201	18.8
NL2	557	6.52	158	232	88.0	78.4	99.0	25.0
NL3	2354	6.26	445	496	138	105	39.1	34.7
NL4	3470	7.89	198	204	24.9	64.2	8.70	24.0
NL5	2163	7.45	349	280	44.1	30.4	26.8	18.8
NL6	70	5.14	221	83.0	47.6	42.1	106	6.35

Table S3.6 The soil porewater properties (pH, dissolved organic carbon (DOC), and dissolved cations concentrations) interpolated at LC50 values of Ni (expressed as total soil concentration) for *Aporrectodea longa* in the six soils tested. The function TREND in Microsoft Excel 2010 was used for interpolation based on the measured values at the tested exposure concentrations.

Soil	LC50 of Ni (mg kg^{-1})	Porewater pH	DOC (mg L^{-1})	Dissolved cations in soil porewater (mg L^{-1})				
				Ca	Mg	Na	K	Ni
NL1	380	6.42	235	130	93.1	59.0	206	34.5
NL2	1294	6.49	167	251	99.3	85.5	126	68.7
NL3	3046	6.37	479	507	135	109	38.8	45.7
NL4	5266	7.62	248	201	21.6	65.1	8.46	33.2
NL5	4253	7.42	363	217	29.0	25.6	19.8	33.9
NL6	139	5.04	225	70.9	40.6	39.2	94.5	15.8