

# Development and validation of an acute biotic ligand model (BLM) predicting cobalt toxicity in soil to the potworm *Enchytraeus albidus*

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## Abstract

An acute Biotic Ligand Model (BLM) was developed to predict the effect of cobalt on the survival of the potworm *Enchytraeus albidus*, exposed in nutrient solutions added to acid washed, precombusted sand. The extent to which  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Na}^{+}$  ions and pH independently mitigate cobalt toxicity to *E. albidus* was examined. Higher activities of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{H}^{+}$  linearly increased the 14 d LC50<sub>Co<sup>2+</sup></sub> (LC50 expressed as  $\text{Co}^{2+}$ -activity) whereas  $\text{Na}^{+}$ -activity did not. Stability constants for the binding of  $\text{Co}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{H}^{+}$  to the biotic ligand (BL) were derived, i.e.  $\log K_{\text{CoBL}} \approx 5.13$ ,  $\log K_{\text{CaBL}} \approx 3.83$ ,  $\log K_{\text{MgBL}} \approx 3.95$  and  $\log K_{\text{HBL}} \approx 6.53$ . It was calculated that at Co-concentrations corresponding to the 14d-LC50 value, 32% of the BL sites were occupied by cobalt. An initial validation of the applicability of this BLM in true soil exposure systems was performed by comparing observed and model-predicted 14 d LC50s in a standard artificial soil and a standard field soil. By assuming pore water to be the only route of exposure and assuming equilibrium between pore water  $\text{Co}^{2+}$  and solid phase Co, which is predicted by the geochemical WHAM-Model 6, LC50s (as mg Co kg<sup>-1</sup> dry wt of soil) were predicted within an error of less than a factor two. Further validation in true soil exposures, combined with more detailed knowledge of Co binding to soil solid phases is needed, if this model is to be used as a tool for risk assessment and derivation of soil quality criteria for Co.

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## 1. Introduction

Cobalt is a naturally occurring element and is mainly present in the earth's crust as cobaltite [ $\text{CoAsS}$ ], erythrite [ $\text{Co}_3(\text{AsO}_4)_2$ ] and smaltite [ $\text{CoAs}_2$ ] (Barceloux, 1999). Cobalt is mainly used as a component of very hard, strong and heat-resistant alloys and in permanent magnets. It is also used as drying agent in paintings, as colour pigment in porcelain, as a catalyst in rubber manufacturing and as an additive in fertilizers and fodders (Barceloux, 1999). Elevated cobalt concentrations in the terrestrial environment may, for example, result from deposition from burning of fossil fuels, wear of cobalt-containing alloys and spreading of sewage sludge and manure (Barceloux, 1999).

Metals such as cobalt may present environmental risk when occurring at elevated concentrations and are being managed through the establishment of environmental quality criteria and standards. Recently it has been recognized by regulators, industry and academic scientists that standard procedures for deriving environmental quality criteria are inadequate to accurately assess the potential impact of metals on the ecological quality of ecosystems (Fairbrother et al., 1999; Janssen et al., 2000). This is because current environmental quality criteria and risk-assessment procedures of metals are predominantly based on total metal concentrations. However, there is extensive evidence that total metal concentrations in soils are not good predictors of metal bioavailability and toxicity. The 14 d LC50 of Zn, Cd, Cu and Pb to *Enchytraeus albidus*, for example, varied over more than two orders of magnitude depending on the composition of the soil (Lock et al., 2000; Lock and Janssen, 2001a). These differences in metal toxicity were mainly determined by pH

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and cation exchange capacity of the soil. In addition, metal toxicity is higher in freshly spiked soils compared to historically contaminated field soils (Lock and Janssen, 2001b; Lock and Janssen, 2003).

The dependence of metal toxicity on soil characteristics such as cation exchange capacity, organic matter content and pH indicates the need to develop tools for predicting metal toxicity in soils with distinct properties. Although empirical regression models are promising, the further development of more sophisticated models may provide mechanistic explanations of metal bioavailability in soil. One such approach may be to develop a terrestrial biotic ligand model (BLM). The BLM concept (e.g. Di Toro et al., 2001; De Schamphelaere and Janssen, 2002), originally developed for aquatic metal toxicity, has recently gained increased attention from both academic scientists and regulators and is now considered to be the state-of-the-science metal bioavailability model/concept that might be applicable to regulatory matters concerning metals in the environment.

The main assumption of the BLM is that metal toxicity occurs as the result of free metal ions (or other reactive metal species) reacting with binding sites at the organism–water interface (either physiologically active sites, leading to a direct biological response, or transport sites, leading to metal transport into the cell followed by an indirect biological response), which is represented as the formation of a metal–biotic ligand complexes. The concentration of these metal–biotic ligand complexes directly determines the magnitude of the toxic effect, independent of the chemical characteristics of the test medium.  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$  and  $\text{H}^+$  ions may compete for binding sites at the organism–water interface (Pagenkopf, 1983; Di Toro et al., 2001; Santore et al., 2001), thus reducing the binding of toxic metal species to the BL and eventually reducing the toxicity of the reactive metal species (Pagenkopf, 1983; Di Toro et al., 2001; Santore et al., 2001; De Schamphelaere and Janssen, 2002). Computationally, a BLM makes use of a speciation model (e.g. Model V or Model VI, Tipping, 1998; Lofts and Tipping, 2002) to calculate the free metal ion activity. The binding of metal ions and competing cations to the BL is then calculated in the same way as any other reaction of a cation with an organic or inorganic ligand, i.e. by stability constants.

Until now, BLMs have mainly been developed to predict metal toxicity to aquatic organisms. Recently, Steenbergen

et al. (2005) successfully developed a terrestrial BLM to predict acute Cu toxicity to the earthworm *Aporrectodea caliginosa*, showing that the BLM concept is also applicable in the terrestrial environment. To our knowledge and according to the review of Niyogi and Wood (2004), no data are available on the effects of competing cations on Co toxicity. However, Richards and Playle (1998) found that increased  $\text{Ca}^{2+}$ ,  $\text{Na}^+$  and  $\text{H}^+$  activities reduced short-term cobalt accumulation in rainbow trout (*Oncorhynchus mykiss*) gills, the primary target of metal toxicity to fish (Paquin et al., 2002).

In this study, it was examined if the BLM concept is applicable to predict acute Co toxicity to the terrestrial invertebrate *E. albidus*. The hypothesis is that exposure only occurs via the pore water and that interactions between  $\text{Co}^{2+}$  and competing cations for the biotic ligand can be described in a similar way as in aquatic BLMs. *E. albidus* was exposed in nutrient solutions added to acid-washed, precombusted sand for the development of a BLM for cobalt. This approach allowed to control all water characteristics affecting cobalt bioavailability. The aims of the present study were three-fold: (1) to investigate the extent to which calcium, magnesium, sodium and hydrogen ions can individually mitigate cobalt ion toxicity in solution to *E. albidus*, (2) to use the obtained toxicity data to derive estimates of the parameters necessary to develop a BLM that can predict cobalt toxicity towards *E. albidus* for a broad range of pore water characteristics and (3) to perform an initial validation of the obtained BLM in a true soil exposure system, using two standard soils spiked with Co.

## 2. Materials and methods

### 2.1. Test design for BLM development

In order to assess the independent effect of different cations on cobalt toxicity, one cation concentration at a time was varied, while keeping all other cation concentrations low and as constant as possible. Four sets of cobalt bioassays were performed: a Ca-set, a Mg-set, a Na-set and a pH-set (Table 1). Each set consisted of a series of test solutions, added to acid-washed and precombusted sand (see further), in which only the cation under consideration was varied. For each test solution, several cobalt concentrations were tested. All toxicity assays of the same set were

Table 1  
Composition of the test media used in the BLM development experiments (values interpolated at LC50)<sup>a</sup>

	Studied parameter	Other parameters
Ca-set	0.20, 2.7, 5.2, 7.7, 10, 13 and 15 mM	0.05 mM Mg, 1.8–1.9 mM Na, pH 5.8–6.4
Mg-set	0.05, 1.1, 2.1, 3.1, 4.2, 5.2 and 6.2 mM	0.20 mM Ca, 1.9–2.2 mM Na, pH 6.3–6.6
Na-set	2.8, 7.1, 11, 15, 20 and 24 mM	0.20 mM Ca, 0.05 mM Mg, pH 5.3–5.8
pH-set	pH 5.4, 5.7, 6.0, 6.1, 6.7 and 7.1	0.20 mM Ca, 0.05 mM Mg, 0.08 – 3.5 mM Na

<sup>a</sup>All test solutions contained 0.078 mM K and 0.05 mM  $\text{SO}_4$ . Cl was between 0.5 and 32 mM.

conducted simultaneously to minimize variability. The selected cation concentrations were based on the ranges occurring in natural pore waters.

## 2.2. Preparation of the test solutions

All chemicals were purchased from VWR (Leuven, Belgium) and were reagent grade. All test media were prepared by adding different volumes of stock solutions of  $\text{CaCl}_2$ ,  $\text{MgSO}_4$ ,  $\text{MgCl}_2$ ,  $\text{NaCl}$  and  $\text{KCl}$  to carbon-filtered, deionized water. Except for the pH-set, these media were adjusted to pH 6.0. pH was controlled using MOPS-buffering (3-[N-morpholino] propane sulfonic acid,  $0.75 \text{ g l}^{-1}$ ) and addition of diluted  $\text{NaOH}$ . MOPS was chosen because it is believed to be non-complexing for metals (Kandegedara and Rorabacher, 1999). It is also recommended by US-EPA (1991) since it does not change the toxicity of effluents and sediment pore waters. Moreover, MOPS-buffering at  $0.75 \text{ g l}^{-1}$  did not affect metal toxicity to *Daphnia magna* and *Pseudokirchneriella subcapitata* (De Schampheleere et al., 2004). At pH 7,  $\text{NaHCO}_3$  (2 mM) was used as a buffer, at pH 6, pH was controlled by MES-buffering (2-[N-morpholino] ethane sulfonic acid,  $0.75 \text{ mg l}^{-1}$ ) and addition of diluted  $\text{NaOH}$ . For each bioassay, the prepared test medium was then used as the dilution water to make logarithmic concentration series of cobalt, added as  $\text{CoCl}_2$ . The test media were added to acid-washed (1.4 N  $\text{HNO}_3$  for 1 d) and precombusted (2 h at 600 °C) sand 1 d prior to test initiation. The chemical characteristics of the different test-media are summarized in Table 1.

## 2.3. Preparation of test soils

For validation purposes, tests were also carried out in standard artificial soil and a standardized field soil. The artificial soil used as test substrate was composed as prescribed by OECD Guideline 207 (1984): 70% sand, 20% kaolin clay and 10% organic matter (finely ground *Sphagnum* peat), pH was adjusted with  $\text{CaCO}_3$ . The standardized field soil was LUFA 2.2 (Landwirtschaftliche Untersuchungs- und Forschungs- Anstalt, Speyer, Germany), which is a loamy sand. It has an organic carbon content of 2.29% and a clay content of 7.9% (on a dry weight basis). Cation exchange capacity (CEC), as determined with the silver–thiourea method in 0.4 M ammonium acetate at soil pH (Chhabra et al., 1975), was  $15.1 \text{ cmol kg}^{-1}$  for OECD soil and  $11.0 \text{ cmol kg}^{-1}$  for LUFA 2.2 soil. After spiking, soils were equilibrated for 1 week before testing.

## 2.4. Toxicity assays

The acute test with the potworm *E. albidus* was performed according to OECD guideline 220 (OECD, 1999). For each medium, a toxicity assay was conducted, consisting of six treatments (control+5 cobalt concentra-

tions), with a difference of 1 log-unit between the lowest and highest cobalt concentration tested. Each treatment was performed by exposing 10 adult worms in an acid-washed glass vessel, filled with 5 ml of test medium added to 20 g of acid-washed, precombusted sand. Three replicates were tested per cobalt concentration. Tests were carried out at 20 °C with a light cycle of 16h:8h light:dark. The number of surviving enchytraeids was determined after 14 d of exposure. Toxicity assays were performed in two soils by exposing 10 adult worms in 20 g wet weight of soil with a moisture content on a wet weight basis of 25% for LUFA soil and 35% for OECD soil.

## 2.5. Chemical measurements

Chemical analyses were performed on pore water samples collected from the ‘sand plus test solution’ and from the ‘true soil’ exposures. Pore water was obtained by centrifugation (IEC Centra-8 Centrifuge, Needham, Massachusetts, USA) for 25 min at 1300 g over a quartz wool filter and subsequent filtration of the supernatants over an Acrodisc<sup>s</sup> syringe filter with a 0.45 µm Supor<sup>s</sup> membrane (Gelman Sciences, Ann Arbor, Michigan, USA).

Pore water cobalt concentrations in the sand exposures were determined at the end of the test using a graphite furnace atomic absorption spectrophotometer (AAS) (SpectrAA800, Varian, Mulgrave, Australia). Ca, Mg, and Na in the pore water from sand exposures were occasionally determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES, Optima 3300 DV, Perkin-Elmer, Shelton, CT, USA). Measured concentrations of Co, Ca, Mg, and Na were always within 10% of nominal concentrations added to the test solutions. Pore water concentrations of Co, Ca, Mg, Na, K, Fe, Al, Mn, Cu, Zn and Ni in the true soil exposures were measured at the end of the test by ICP-OES. Pore water pH was measured using a pH meter (Consort, P407, Turnhout, Belgium), calibrated with pH 4 and pH 7 buffers. Concentrations of dissolved organic carbon (DOC) were measured with a TOC-analyzer (Shimadzu, type 5050A, Kyoto, Japan).

Soils were digested in  $\text{HNO}_3/\text{HCl}$  at a volumetric ratio of 4:1 prior to measurement of Co. A calcareous loamy soil (CRM 141 R, Community Bureau of Reference, Brussels, Belgium) was used as certified reference material. Measured concentrations were within 10% of the reported certified concentration. Measured concentrations in spiked LUFA and OECD soils were also within 10% of the nominal concentrations. At the end of the exposures, soil pH ( $\text{CaCl}_2$ ) was measured at a 1:2.5 soil:liquid ratio with 0.01 M  $\text{CaCl}_2$ .

## 2.6. Data treatment and statistics

All speciation calculations were conducted using WHAM 6.0.8 Software (Windermere Humic Aqueous Model) (Tipping, 1998; Lofts and Tipping, 2002), with

stability constants for the inorganic cobalt complexes taken from Martell et al. (1997). The chemical activity of the  $\text{Co}^{2+}$ -ion, denoted ( $\text{Co}^{2+}$ ), was calculated for each Co-treatment in each test solution using the chemical composition of the test solution and the Co-concentration as input. Inorganic carbon was assumed to be in equilibrium with atmospheric  $\text{CO}_2$ . LC50s, expressed as  $\text{Co}^{2+}$ -activity, denoted  $\text{LC50}_{\text{Co}^{2+}}$ , were then calculated, based on the activities and the associated mortality after 14 d of exposure, using the trimmed Spearman–Karber method (Hamilton et al., 1977).  $\text{LC50}_{\text{Co}^{2+}}$  was plotted against the activities of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ , and  $\text{H}^+$  and linear regressions were calculated using STATISTICA<sup>s</sup> software (Statsoft, Tulsa, OK, USA) to determine BLM-parameters (see further). The procedure and assumptions for speciation calculations and predictions of LC50s in soil are discussed in the results section.

### 2.7. Short mathematical description of the BLM and derivation of BLM parameters

This section gives a short mathematical description of the BLM, in terms of the equations required to understand the calculations performed in the present study. A more detailed description and the derivation of the equations are given elsewhere (De Schamphelaere and Janssen, 2002).

The toxic effect is, according to the BLM-hypothesis, determined by the fraction of the total number of cobalt binding sites occupied by cobalt ( $f_{\text{CoBL}}$ ), regardless of test solution (or pore water) chemistry. By multiplying this fraction with the Co-binding capacity of the BL, i.e. the total number of BL sites, the concentration of Co bound to the BL can be obtained. The Co-binding capacity was not determined in the present study, but as demonstrated earlier, knowledge of the fraction of BL sites occupied by Co is sufficient for predicting toxic effects (De Schamphelaere and Janssen, 2002). This fraction, i.e.  $f_{\text{CoBL}}$ , equals:

$$f_{\text{CoBL}} = \frac{K_{\text{CoBL}} \delta \text{Co}^{2+}}{1 + K_{\text{CoBL}} \delta \text{Co}^{2+} + K_{\text{CaBL}} \delta \text{Ca}^{2+} + K_{\text{MgBL}} \delta \text{Mg}^{2+} + K_{\text{NaBL}} \delta \text{Na}^+ + K_{\text{HBL}} \delta \text{H}^+} \quad (1)$$

where  $K_{\text{XBL}}$  is the stability constant for binding of cation X to the BL and where ( $\text{X}^{n+}$ ) denotes the chemical activity of cation  $\text{X}^{n+}$ . Stability constants are defined as, for e.g. for Co.

$$K_{\text{CoBL}} = \frac{[\text{CoBL}^+]}{[\text{Co}^{2+}]_{\text{BL}}}, \quad (2)$$

where  $K_{\text{CoBL}}$  is the stability constant for  $\text{Co}^{2+}$  binding to BL sites ( $\text{L mol}^{-1}$ ), ( $\text{Co}^{2+}$ ) the activity of the free cobalt ion ( $\text{mol l}^{-1}$ ),  $[\text{CoBL}^+]$  the concentration of Co bound to

the BL, and  $[\text{BL}^-]$  the concentration of free BL, unoccupied by any cation. Similar equations can be written for the other cations.

According to the BLM-concept,  $f_{\text{CoBL}}$  is constant at 50% effect  $f_{\text{CoBL}}^{50\%}$  (e.g. Meyer et al., 1999) and Eq. (1) can be re-organized to

$$\text{LC50}_{\delta \text{Co}^{2+}} = \frac{f_{\text{CoBL}}^{50\%}}{1 - f_{\text{CoBL}}^{50\%} \frac{K_{\text{CoBL}}}{1 + K_{\text{CaBL}} \delta \text{Ca}^{2+} + K_{\text{MgBL}} \delta \text{Mg}^{2+} + K_{\text{NaBL}} \delta \text{Na}^+ + K_{\text{HBL}} \delta \text{H}^+}} \quad (3)$$

where  $\text{LC50}_{\text{Co}^{2+}}$  is the free cobalt ion activity resulting in 50% mortality in *E. albidus* after 14 d of exposure. With Eq. (3),  $\text{LC50}_{\text{Co}^{2+}}$  can be predicted when ( $\text{Ca}^{2+}$ ), ( $\text{Mg}^{2+}$ ), ( $\text{Na}^+$ ) and ( $\text{H}^+$ ) are known, provided that the values of  $K_{\text{CoBL}}$ ,  $K_{\text{CaBL}}$ , etc. are known (see further). Eq. (3) shows that, if the BLM concept is correct, linear relationships should be observed between  $\text{LC50}_{\text{Co}^{2+}}$  and the activity of one cation when other cation activities are kept constant. The slopes and the intercepts of those relationships can then be used to derive the stability constants of competing cations according to the method described by De Schamphelaere and Janssen (2002). The calculation of  $K_{\text{CoBL}}$  and  $f_{\text{CoBL}}^{50\%}$  is based on the optimization of the logit-transformed effect versus  $f_{\text{CoBL}}$  for varying  $K_{\text{CoBL}}$  (see results section).

### 3. Results

The 14 d- $\text{LC50}_{\text{Co}^{2+}}$  for *E. albidus*, expressed as free cobalt ion activity, ranged from 3.7 to 210  $\text{mM Co}^{2+}$ , which is a 57-fold difference. The 14 d  $\text{LC50}_{\text{Co}^{2+}}$  significantly increased with increasing  $\text{Ca}^{2+}$  activity (9-fold increase,  $R^2 = 0.97$ ,  $p < 0.0001$ ) (Fig. 1A),  $\text{Mg}^{2+}$  activity (11-fold increase,  $R^2 = 0.96$ ,  $p = 0.00012$ ) (Fig. 1B) and  $\text{H}^+$  activity (9-fold increase,  $R^2 = 0.93$ ,  $p = 0.0019$ ) (Fig. 1D). Calculation of the

$\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{H}^+$  stability constants according to De Schamphelaere and Janssen (2002) and using slopes and intercepts of the linear regressions (Fig. 1), resulted in  $\log K_{\text{CaBL}} = 3.83$ ,  $\log K_{\text{MgBL}} = 3.95$  and  $\log K_{\text{HBL}} = 6.53$  (Table 2). Increases in  $\text{Na}^+$  activity did not affect 14 d  $\text{LC50}_{\text{Co}^{2+}}$  in a linear fashion within the tested Na range ( $R^2 = 0.14$ ,  $p = 0.47$ ) (Fig. 1C). At 15  $\text{mM}$  of  $\text{Na}^+$ -activity, the LC50 was higher than those obtained at lower or higher Na-activities. However, even without this outlier, there was no significant relationship between  $\text{Na}^+$ -activity and  $\text{Co}^{2+}$  toxicity and therefore  $\log K_{\text{NaBL}}$  was set to zero.

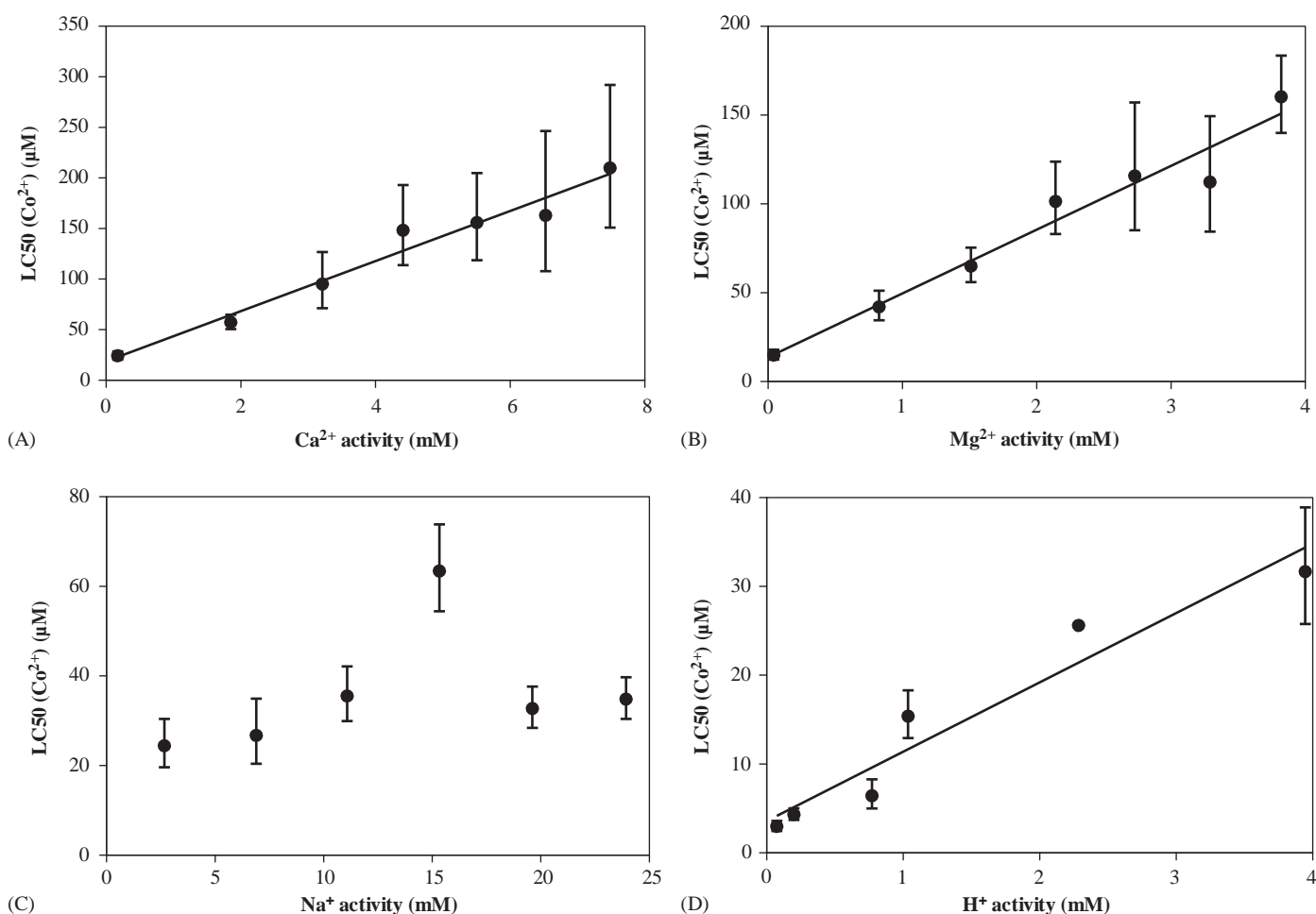


Fig. 1. The 14d LC50<sub>Co<sup>2+</sup></sub> for *Enchytraeus albidus* as a function of the activity of Ca<sup>2+</sup> (A), Mg<sup>2+</sup> (B), Na<sup>+</sup> (C) and H<sup>+</sup> (D). Error bars indicate 95% confidence intervals. Solid lines are the linear regression lines.

Table 2  
Parameters of the Co-BLM for *Enchytraeus albidus* (this study) compared with gill-binding parameters for *Oncorhynchus mykiss* (Richards and Playle, 1998)

	<i>Enchytraeus albidus</i>	<i>Oncorhynchus mykiss</i>
Endpoint	14d LC50	2-3h gill accumulation
Log $K_{CaBL}$	3.83	4.7
Log $K_{MgBL}$	3.95	ND <sup>a</sup>
Log $K_{NaBL}$	0.00	3.2
Log $K_{HBL}$	6.53	6.2
Log $K_{CoBL}$	5.13	5.1
$f_{CoBL}^{50\%}$ <sup>b</sup>	0.32	NA <sup>c</sup>

<sup>a</sup>Not determined.

<sup>b</sup>Fraction of biotic ligand sites occupied by Co at 50% mortality after 14d of exposure.

<sup>c</sup>Not applicable

For the final development of the Co-BLM for *E. albidus*,  $K_{CoBL}$  and  $f_{CoBL}^{50\%}$  were calculated. For every treatment (26 test solutions x 5 Co-concentrations), the fraction of the BL occupied by cobalt was calculated with Eq. (1) for varying log  $K_{CoBL}$ . In analogy with De Schamphelaere and Janssen (2002),  $K_{CoBL}$  was varied until the highest

correlation was obtained between  $f_{CoBL}$  and the logit of the percent mortality of *E. albidus* after 14d of exposure. Values for log  $K_{CoBL}$  ¼ 5.13 and the associated  $f_{CoBL}^{50\%}$  ¼ 0:32 resulted in the best fit ( $R^2$  ¼ 0.67) and were retained.

The Co-BLM, defined by stability constants  $K_{CoBL}$ ,  $K_{CaBL}$ ,  $K_{MgBL}$ ,  $K_{NaBL}$  and by  $f_{CoBL}^{50\%}$ ; was now used to predict 14d LC50<sub>Co<sup>2+</sup></sub> for all bioassays with Eq. (3), using Ca<sup>2+</sup>, Mg<sup>2+</sup> and H<sup>+</sup> activities as input. The predicted

LC50s differed in most cases by less than a factor two from the observed LC50s (Fig. 2), which indicates that the model is well-calibrated to the BLM-development dataset. Three LC50s of the pH test series were predicted somewhat less accurate, but this is probably due to a slightly higher sensitivity of the worms in the whole pH test series, not by a lack of fit of the linear regression of the pH effect (Fig. 1).

To validate the developed BLM in true soil exposures, toxicity tests were also performed in standard artificial OECD soil and LUFA 2.2 soil. The observed 14 d LC50s in OECD soil, i.e. 683 (620–752, 95% confidence interval) mg Co kg<sup>-1</sup> dry wt, was about three times higher than in LUFA 2.2 soil, i.e. 227 (212–243) mg Co kg<sup>-1</sup> dry wt.

The terrestrial BLM for predicting 14 d LC50s (as total Co in soil) in the present study consists of Eq. (3), using the

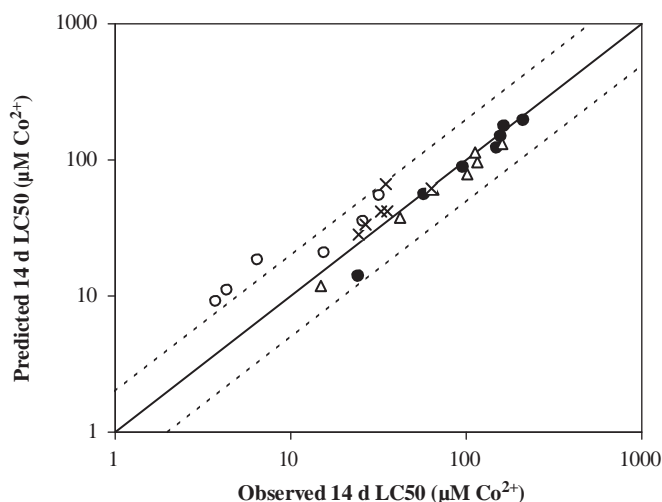


Fig. 2. Relationship between observed and predicted 14 d LC50 for *Enchytraeus albidus* exposed to Co (K, Ca-set; n, Mg-set; x, Na-set; J, pH-set). The full line indicates a perfect match between observed and predicted LC50s, the dashed lines indicate a factor 2 difference from the perfect match.

derived parameters listed in Table 2 linked to the geochemical speciation model WHAM - Model 6 (Lofts and Tipping, 2002). The model was run with the assumptions mentioned in Table 3. Those assumptions are in accordance with the assumptions proposed by Tipping et al. (2003) which have been shown to result in reasonably accurate predictions of metal speciation in English and Welsh soils. The model was run as an aqueous phase problem, i.e. as if the soil particles were suspended in the pore water (Tipping et al., 2003). To that end, a suspended solid concentration of 1.41 and 1.09 kg dry wt l<sup>-1</sup> were used for LUFA 2.2 and OECD soil, respectively (based on the moisture content and an assumed average particle density of 2.65 g cm<sup>-3</sup>). (Brady and Weil, 1996).

Particulate organic carbon and clay concentration required as input in the speciation calculations were obtained by multiplying their fractional contribution to the total dry weight of the soil (see materials and methods) with the suspended solid concentration. Similarly, the conversion of total soil Co (mg Co kg<sup>-1</sup> dry wt) to 'aqueous' Co (in mg Co l<sup>-1</sup>), was achieved by multiplication by the suspended solid concentration.

Success or failure of the speciation part of the developed terrestrial acute BLM can be judged by comparing predicted with measured pore water Co-concentrations. This is achieved by using all inputs mentioned in Table 3, except the dissolved Co-concentration in the pore water. The WHAM 6 speciation model yields as one of the outputs the Co-concentration in the aqueous phase (i.e. the predicted pore water Co). Fig. 3 shows that Co-concentrations in the pore water increased with increasing total Co spiked to the solid phase and that these concentrations are predicted within a factor 2. For both soils, pore water Co was underestimated (Fig. 3).

Important to note is that, as the amount of Co added to the soils increased, cation concentrations clearly increased. Ca and Mg increased by a factor of up to 10.5 and 8.5, respectively, whereas Na and K only increased slightly, i.e. by a factor of 2.4 and 1.7, respectively (Table 3). pH slightly decreased with 0.5–0.8 pH units.

Since Ca, Mg and pH affect Co-toxicity in solution phase (i.e. pore water), we performed predictions of 14 d LC50s for all pore water compositions (from control to highest Co-treatment) to investigate whether this affected predictions of LC50s. Predictions were performed by: (1) running the speciation model without Co, to obtain activities of Ca<sup>2+</sup>, Mg<sup>2+</sup> and H<sup>+</sup>, (2) predicting the LC50<sub>Co2p</sub> using Eq. (3) and (3) running the model again with this Co<sup>2+</sup>-activity as input. The WHAM 6 output yielded a total Co-concentration that equalled the predicted LC50. Interestingly, the predicted 14 d LC50 (as total Co) were only marginally dependent on the pore water composition, i.e. between 281 and 332 mg kg<sup>-1</sup> for LUFA and between 1260 and 1380 mg kg<sup>-1</sup> for OECD soil. This is for both soils higher than the observed values but only by an error of less than a factor two. The rank order of toxicity, i.e. Co being more toxic in LUFA 2.2 soil than in OECD, was also predicted correctly.

#### 4. Discussion

The 14 d LC50<sub>Co2p</sub> varied about 57-fold, which clearly demonstrates the limitations of using free ion activity for predicting cobalt toxicity. However, a large part of these differences could be explained by positive linear relations between LC50<sub>Co2p</sub> and the activity of Ca<sup>2+</sup>, Mg<sup>2+</sup> and H<sup>+</sup>. This supports the assumptions of the BLM concept (Eq. (3)) and has often been associated with competitive reactions at biological surfaces of a variety of species (Pagenkopf, 1983; Santore et al., 2001; De Schamphelaere and Janssen, 2002), including the oligochaetes *Lumbriculus variegatus* (Meyer et al., 1999) and *A. caliginosa* (Steenbergen et al., 2005).

Stability constants were derived for Ca<sup>2+</sup>, Mg<sup>2+</sup> and H<sup>+</sup> (Table 2). It is noted that the derived stability constants should not be regarded as conventional stability constants used to describe a chemical reaction equilibrium, but rather as parameters that reflect the observed relations between the Ca<sup>2+</sup>, Mg<sup>2+</sup> and H<sup>+</sup> activity and the toxicity of Co<sup>2+</sup>. These relations may be the result of true competitive reactions at the organisms surface (e.g. at transport sites), but also of shifts in membrane permeability due to differences in external cation concentrations (Hille et al., 1975).

The binding constants derived for *E. albidus*, can be compared with those reported for short-term (2–3 h) Co accumulation on the gills of rainbow trout (*O. mykiss*) (Table 2) (Richards and Playle, 1998). In the latter study cobalt accumulation decreased with increasing Ca<sup>2+</sup>, Na<sup>+</sup> and H<sup>+</sup> activity. The effect of Mg<sup>2+</sup> was not investigated. A Co-binding constant (log *K*<sub>CoBL</sub>) was derived of 5.1,

Table 3  
Measured soil characteristics (input values for speciation calculations)<sup>a</sup>

Total soil Co (mg kg <sup>-1</sup> dry wt)	pH CaCl <sub>2</sub>	pH PW	DOC (mg l <sup>-1</sup> )	Na (M)	Mg (M)	K (M)	Ca (M)	Co (M)
<i>LUFA 2.2</i>								
0	5.91	5.77	650	1.61E-03	1.05E-03	5.03E-04	7.45E-03	1.15E-06
56	5.83	5.67	682	1.77E-03	1.45E-03	5.95E-04	1.02E-02	7.04E-05
100	5.49	5.67	537	1.68E-03	1.48E-03	6.15E-04	1.04E-02	1.60E-04
180	5.45	5.36	564	1.95E-03	2.42E-03	7.93E-04	1.76E-02	6.69E-04
320	5.36	5.06	505	1.89E-03	2.82E-03	8.37E-04	2.13E-02	1.74E-03
560	5.40	4.99	892	2.25E-03	4.57E-03	1.16E-03	3.60E-02	6.28E-03
<i>OECD</i>								
0	7.32	7.21	324	4.63E-03	6.65E-04	6.19E-04	5.17E-03	5.65E-07
180	7.31	7.11	316	5.54E-03	1.21E-03	8.75E-04	9.11E-03	2.55E-05
320	7.30	7.12	263	5.64E-03	1.58E-03	9.67E-04	1.18E-02	7.30E-05
560	7.18	6.93	269	6.79E-03	2.63E-03	1.19E-03	1.99E-02	2.88E-04
1000	7.14	6.92	277	6.62E-03	3.58E-03	1.25E-03	2.95E-02	1.06E-03
1800	7.08	6.68	304	7.82E-03	5.63E-03	1.48E-03	5.45E-02	4.27E-03

Na, Mg, K, Ca, Co, DOC and pH refer to values measured in filtered (0.45 mm) pore water.

<sup>a</sup>Following assumptions were made for all calculations, in accordance with Tipping et al. (2003): (1) pH of the pore water is used; (2) inorganic carbon is in equilibrium with atmospheric CO<sub>2</sub>; (3) organic matter in the solid phase was assumed to consist of 84% humic acid and 16% fulvic acid; (4) dissolved organic matter in solution phase consists of 65% active fulvic acid; (5) humic and fulvic acid have a C-content of 50% on a weight basis; (6) Fe and Al are in equilibrium with their hydroxide-precipitates (using solubility constants mentioned in Tipping et al. (2003)); (7) the system was charge balanced by adding Cl<sup>-</sup> and SO<sub>4</sub><sup>2-</sup> to the input in a 3:1 molar ratio; (8) trace metal concentrations of Cu, Zn, Ni, etc. were too low to affect Co<sup>2+</sup>-activity to a significant extent and were not used (data not shown).

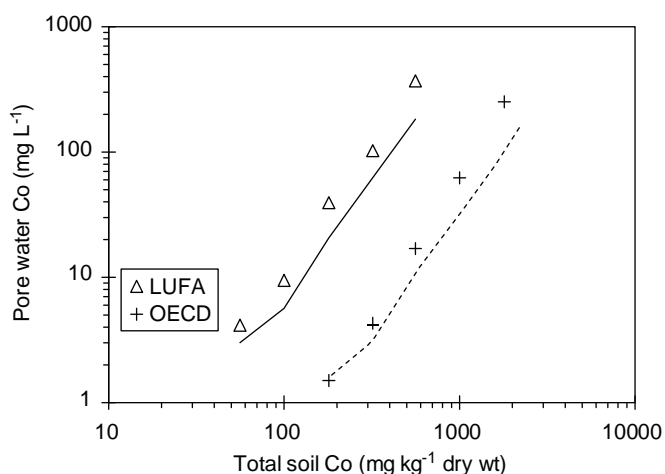


Fig. 3. Observed pore water Co-concentrations in OECD (+) and LUFA (Δ) soils and predicted concentrations (bold line for LUFA, dashed line for OECD). Predictions are performed as explained in text, using input data mentioned in Table 3.

which is the same as observed for *E. albidus* in the present study. The constant for proton competition was also very similar for both species. Log  $K_{HBL}$  was 6.5 for *E. albidus* vs. 6.2 for *O. mykiss*. The effects of Ca and Na were clearly different between *E. albidus* and *O. mykiss*. A log  $K_{CABL}$  of 3.8 was found for *E. albidus* vs. 4.7 for *O. mykiss*. Na affected Co-accumulation on fish gills, as indicated by a log  $K_{NABL}$  of 3.2, whereas no Na-constant was derived in our study as this would not correctly reflect the Na-effect over the whole studied Na-range. Differences in binding constants may, for example, result from different exposure duration (2–3 h vs. 14 d), endpoint (accumulation vs. survival), target tissue or biotic ligand (gill for *O. mykiss*

vs. unknown for *E. albidus*), or mechanisms of Co uptake and/or toxicity. Clearly more research with Co is necessary to determine and explain differences and similarities across organisms, endpoints and exposure duration.

To validate the use of the developed BLM in true soils, toxicity tests were also performed in standard artificial soil and LUFA 2.2 soil. The 14 d LC50 for *E. albidus* was 683 (620–752) mg Co kg<sup>-1</sup> dry wt in OECD soil and 227 (212–243) mg Co kg<sup>-1</sup> dry wt in LUFA 2.2 soil. The difference in toxicity between these two soils can be explained by the higher pH, higher cation exchange capacity and higher organic matter content of OECD soil. Increases in pH, organic matter content and cation exchange capacity have previously been shown to reduce metal toxicity in spiked soils (Lock et al., 2000; Lock and Janssen, 2001a).

The developed terrestrial BLM accounts for speciation and interactions at the BL for predicting LC50s of Co in soil. It thus integrates the combined effects of those physico-chemical characteristics which are fed as an input into the model, including organic matter content, clay content and pore-water chemistry. The speciation part of the BLM, i.e. WHAM-Model 6, can accurately predict dissolved pore-water concentrations of Co. According to Tipping et al. (2003) such analysis can be used to judge the model's success or failure. The average ratio of predicted to observed dissolved Co was 0.64 (range 0.53–0.96), indicating an underestimation of pore water Co. Tipping et al. (2003) also found ratios close to unity for Zn (0.7) and Cu (1.4), but much higher ratios for Cd (2.8) and Pb (8.9). These authors suggested that WHAM-Model 6 could be easily optimized by modest adjustments of selected binding constants. Alternatively, it might be worthwhile to investigate



and/or improve the assumptions made for speciation calculations (see Table 3).

14 d LC50s (as total Co, mg kg<sup>-1</sup> dry wt) were also accurately predicted, i.e. by an error of less than a factor two. Co toxicity was also correctly predicted to be higher in the LUF 2.2 soil than in OECD soil. Interestingly, the predicted LC50s were only slightly dependent on Ca, Mg or pH in the pore water, whereas Ca, Mg, and pH clearly affected Co toxicity in the experiments in sand (BLM-development). Increased Ca and Mg concentrations and reduced pH levels in the pore water were observed at higher spiked Co concentrations (Table 3). Most likely, Co has displaced Ca<sup>2+</sup>, Mg<sup>2+</sup> and H<sup>+</sup> from binding sites on the soil solid phase (e.g. organic matter). Apparently, the relative binding strengths of Co<sup>2+</sup> vs. Ca<sup>2+</sup>, Mg<sup>2+</sup> and H<sup>+</sup> are on average similar for the soil particles and for the BL. As such, the competition between Co<sup>2+</sup> and Ca<sup>2+</sup>, Mg<sup>2+</sup> and H<sup>+</sup> for binding sites on soil particles cancels out the competition at the biotic ligand. This is an advantage for the applicability of the model as it means that detailed pore water chemistry may not be needed to predict LC50s. Further validation with soils with a broader range of soil characteristics is needed to test this hypothesis.

The fact that LC50s were slightly overestimated, i.e. toxicity is underestimated, may result from the overestimation of Co-binding to the soil or from the fact that in the true-soil exposures *E. albidus* may be slightly more sensitive than in the 'precombusted-sand' exposures. Because in the latter case, pore water is the only exposure route, it may be suggested that exposure via the dietary route may also be important (e.g. via ingestion of Co bound to organic matter).

Further research with natural soils with a broader range of properties is needed to test and/or to improve the capacity of WHAM-Model 6 (and the assumptions) to predict Co speciation in soil solution, to test if dietary Co may invoke toxicity and to test and/or to improve the capacity of the BLM to predict 14 d LC50s to *E. albidus*. The initial results obtained in the present study are promising and suggest that the terrestrial BLM may be a useful tool in risk assessment and the derivation of soil quality criteria for Co.

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