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# Development and validation of a terrestrial biotic ligand model for Ni toxicity to barley root elongation for non-calcareous soils

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ABSTRACT

A Terrestrial Biotic Ligand Model (TBLM) for Ni toxicity to barley root elongation (RE) developed from experiments conducted in sand culture was used to predict toxicity in non-calcareous soils. $Ca^{2+}$ and $Mg^{2+}$ concentrations and pH in sand solution were varied individually and TBLM parameters were computed. EC50 increased as $Mg^{2+}$ increased, whereas the effect of $Ca^{2+}$ was insignificant. TBLM parameters developed from sand culture were validated by toxicity tests in eight Ni-amended, non-calcareous soils. Additional to $Ni^{2+}$ toxicity, toxicity from all solution ions was modelled independently as an osmotic effect and needed to be included for soil culture results. The EC50s and EC10s in soil culture were predicted within twofold of measured results. These are close to the results obtained using parameters estimated from the soil culture data itself.



## 1. Introduction

Understanding nickel toxicity in soils is necessary to properly address environmental quality criteria and standards (Janssen et al., 2000). Total metal concentration does not account for metal bioavailability and toxicity to the soil ecosystem (Allen, 2002). Metal bioavailability and toxicity in aquatic and sediment systems have been predicted based on its speciation and competition from other cations in solution (Di Toro et al., 2001, 2005). The terrestrial biotic ligand model (TBLM) similarly addressed soil (Steenbergen et al., 2005; Thakali et al., 2006), in which partitioning of nickel and its toxicity in the soil system are considered (Supplementary data, Fig. SD1).

Several studies have been conducted to develop the TBLM for Ni toxicity in controlled hydroponic water solution culture systems (Antunes and Kreager, 2009; Li et al., 2009; Lock et al., 2007c). However, these were not used to model Ni toxicity in soil systems for validation. Lock et al. (2006; 2007b) were able to predict Co toxicity to potworm survival in an artificial soil and a field soil within a factor of 2, but only within a factor of 4 for Ni toxicity to barley root elongation. Wang et al. (2011b) were able to predict EC50 for $Ni^{2+}$ toxicity to barley root elongation for non-calcareous soils within a factor of 2 based on an electrostatic toxicity model.

Thakali et al. (2006) predicted Ni toxicity to barley root elongation (RE) in $NiCl_2$ or $CuCl_2$ amended soils, using TBLM parameters developed from the soil culture itself. Adding $Ni^{2+}$ to soil causes release of protons and major cations to the soil solution (Ponizovsky et al., 2006b). Therefore, it is difficult to control soil solution composition to study the effect of cations individually and independently. Constant composition can be achieved in hydroponic systems with water only or with inert sand, but the solution (hydroponic sand solution or hydroponic water solution, which we refer to as sand solution or water solution) must be frequently renewed as these systems lack soil's buffering capacity.

This study was conducted to develop TBLM for Ni toxicity in an inert sand culture system, in which the cations and pH were varied individually and independently, and to use those values to predict the Ni toxicity in non-calcareous soil cultures independent of the systems used to develop the model's constants. The soil toxicity data used for validation was taken from previous work (Rooney et al., 2007; Thakali et al., 2006). Competitive effects of $H^+$, $Ca^{2+}$, and $Mg^{2+}$ on Ni toxicity to barley root elongation in the sand culture were studied individually. Prediction of soil culture data by the parameters obtained from the sand system was compared with the prediction using model parameters estimated from soil culture data itself. The present study also provided a basis for additional work

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(Lin, 2014) including comparison of the predictions obtained by including the activity at the root plasma membrane surface (Kinraide, 2006) and by using TBLM parameters obtained from water solution culture.

## 2. Materials and methods

### 2.1. Toxicity bioassays

Toxicity testing of barley, *Hordeum vulgare* (Doyce), was conducted according to the ISO 11269-1 (ISO, 1993) method for the measurement of inhibition of root growth. Barley plants were grown for four days in acid washed quartz sand that containing defined composition solution. Then root length was determined, and % root elongation (%RE, root elongation = root length after harvest − root length before planting) relative to the control bioassay was calculated for each plant harvested (see Supplementary data for experimental procedures).

Each experiment contained 10 bioassays, including 8 exposures which, based on parameters from Thakali et al. (2006), were predicted to result in 5%, 10%, 25%, 50%, 75%, 90%, 95%, and 100% of RE relative to control, and 2 additional system controls (discussed in Supplementary data). Each bioassay had 12 barley plants, except for the exposure control run with no added $Ni^{2+}$ (%RE = 100) which had 24 plants. Barley RE was measured after harvest (on day 4) and the average and standard deviation were calculated. RE values that exceeded 2 standard deviations of average root elongation were excluded. On average, 5.6 of the 132 measurements were excluded from each of the total 15 experiments.

### 2.2. Physical and chemical measurements

To compensate for evaporative water loss in sand culture the solutions were renewed daily. An Orion 370 pH meter with an accupHast combination pH electrode was used to determine pH. Ni, Ca, and Mg were measured using a PerkinElmer Optima 5300 ICP − OES. Measured pH was within 0.2 units and measured metal concentrations were within 15% of nominal values.

### 2.3. Soils for validation

The TBLM developed in this study was validated by prediction of RE for barley grown in the soils reported by Thakali et al. (2006) and Rooney et al. (2007). These soils were amended with different amounts of $NiCl_2$ required to bracket their total metal EC50 concentration (Oorts et al., 2006). Following Thakali et al. (2006), the eight non-calcareous soils having OC content > 1% were selected because of the unavailability of a speciation model able to accurately predict nickel partitioning for calcareous soils or those with low soil organic matter (SOM, characterized by OC). Activities of $Ni^{2+}$ and other cations in soil solution for the eight soils were predicted with the soil properties using the WHAM computation (Thakali et al., 2006), assuming that Ni was mainly bound to SOM. For high pH soils metal precipitation with $CO_3^{2-}$ must be considered, and for low OM soils (OC < 1%) it is likely that Ni is not bound only to SOM. These factors will not allow prediction of Ni partitioning for the calcareous or low OM soils (Ponizovsky et al., 2006a). The Ni concentrations of the low SOM soils for which OC < 1% cannot be predicted within a half log unit using WHAM VI, whereas the selected soils were predicted with that accuracy (Ponizovsky et al., 2008).

The other reason for choosing non-calcareous soils was that soils with low pH are more prone to exhibiting metal toxicity than are soils with higher pH. Metal toxicity is generally reduced after soil pH increases (Lexmond, 1980; Smith, 1994).

The eight soils had a wide range of properties, with soil solution pH from 3.5 to 7.2, and organic carbon content from 1.1% to 33.1%. The soils were not leached with water after $NiCl_2$ had been added and prior to testing although Ni toxicity would have been reduced if the Ni-amended soil had been leached (Li et al., 2011).

### 2.4. TBLM equations

The TBLM assumes that toxicity is related to $Ni^{2+}$ binding with the biotic ligand (BL) sites, and competition of $H^+$, $Ca^{2+}$, and $Mg^{2+}$ for the BL alleviates toxicity. Toxicity is determined by the fraction ($f$) of total BL bound by $Ni^{2+}$ (Di Toro et al., 2001; Thakali et al., 2006):

$$f = \frac{[\mathrm{NiBL}]}{[\mathrm{TBL}]} = \frac{K_{\mathrm{NiBL}}\{\mathrm{Ni}^{2+}\}}{1 + K_{\mathrm{NiBL}}\{\mathrm{Ni}^{2+}\} + K_{\mathrm{HBL}}\{\mathrm{H}^{+}\} + K_{\mathrm{CaBL}}\{\mathrm{Ca}^{2+}\} + K_{\mathrm{MgBL}}\{\mathrm{Mg}^{2+}\}} \quad (1)$$

where [NiBL] is the concentration (moles $g^{-1}$) of BL sites bound by Ni, [TBL] is total BL sites (moles $g^{-1}$), and $K_{\mathrm{XiBL}}$ (L $mol^{-1}$) is the conditional binding constant for specific cation−BL interaction for an endpoint (representing the affinity of cation $X_i$ for BL), and {} represents cation activity.

It was found that $NiHCO_3^+$ was toxic (Li et al., 2009), and $NiOH^+$ was possibly toxic as well (Antunes et al., 2012; De Schamphelaere and Janssen, 2002). In our system with inert sand, the only carbonate source was from the atmosphere, so $\{NiHCO_3^+\}$ was low compared to $\{Ni^{2+}\}$. Using MINEQL+ (Environmental Research Software, Hallowell, ME) (Schecher and McAvoy, 1992) we found the $\{Ni^{2+}\}$ is more than $10^5$ times that of $\{NiHCO_3^+\}$ for pH 4 and about 140 times for pH 7. For our sand solutions at pH 4–7 the $\{NiOH^+\}$ was even lower and was correlated to $\{NiHCO_3^+\}$, with the $\{NiOH^+\}$ about 21% of the $\{NiHCO_3^+\}$, which was similar to the results of Li et al. (2009) in hydroponic water solution. If we consider the toxicity from the predominant free $Ni^{2+}$ only, the toxic response that correlates to the fraction of the total BL bound by the free $Ni^{2+}$ using the log−logistic dose response function is:

$$R = \frac{100}{1 + \left(\frac{f}{f_{50}}\right)^{\beta}} = \frac{100}{1 + \left(\frac{K_{\mathrm{NiBL}}\{\mathrm{Ni}^{2+}\}}{f_{50}\left(1 + K_{\mathrm{NiBL}}\{\mathrm{Ni}^{2+}\} + K_{\mathrm{HBL}}\{\mathrm{H}^{+}\} + K_{\mathrm{CaBL}}\{\mathrm{Ca}^{2+}\} + K_{\mathrm{MgBL}}\{\mathrm{Mg}^{2+}\}\right)}\right)^{\beta}} \quad (2)$$

where $R$ = biological response as % of the control and for the control bioassay $R = 100$, $f_{50}$ is the fraction of the total BL sites occupied by $Ni^{2+}$ for a 50% response, and $\beta$ is the dose−response shape parameter.

The EC50 is derived from Eq. (2) with $R = 50\%$:

$$\mathrm{EC50}\left\{\mathrm{Ni}^{2+}\right\} = \frac{f_{50}}{(1 - f_{50})K_{\mathrm{NiBL}}}\left(1 + K_{\mathrm{HBL}}\left\{\mathrm{H}^{+}\right\}_{50} + K_{\mathrm{CaBL}}\left\{\mathrm{Ca}^{2+}\right\}_{50} + K_{\mathrm{MgBL}}\left\{\mathrm{Mg}^{2+}\right\}_{50}\right) \quad (3)$$

where the subscript "50" represents the activity of a cation at the 50% effect level. Eq. (2) can be used to calculate the toxic response as percent of the control, and Eq. (3) can be used to calculate the EC50.

If the toxicity of $NiHCO_3^+$ is also considered, Eq. (2) can be modified to include binding of $NiHCO_3^+$ to the BL: