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Ecotoxicology

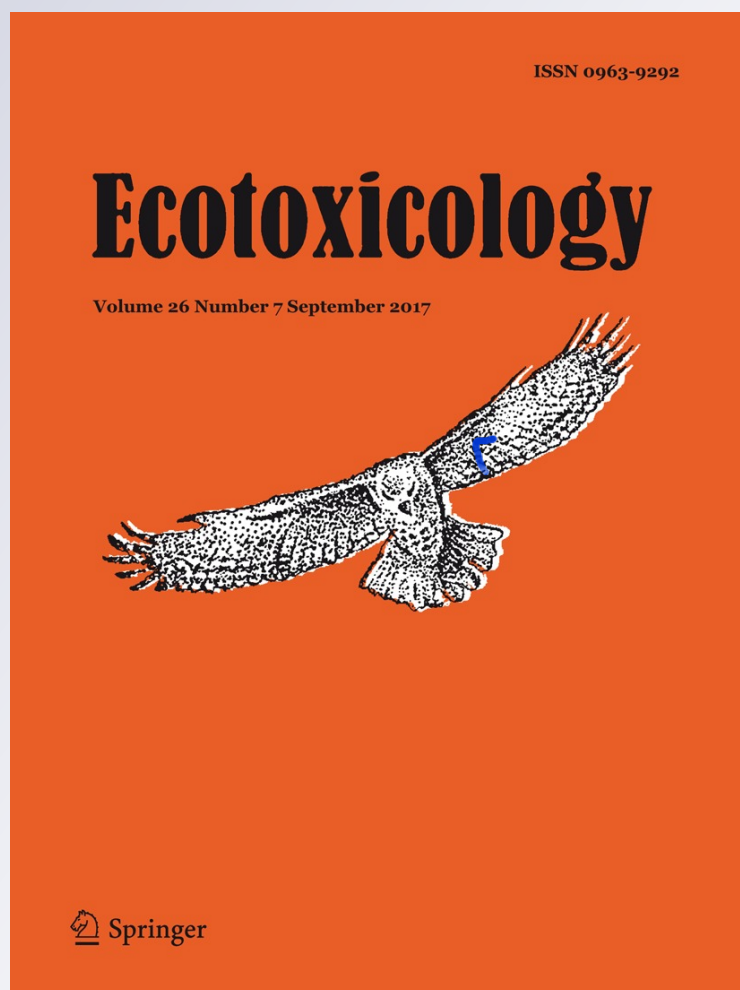
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Nonlinear biotic ligand model for assessing alleviation effects of Ca, Mg, and K on Cd toxicity to soybean roots

Bo-Ching Chen¹ · Pin-Jie Wang² · Pei-Chi Ho² · Kai-Wei Juang²

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Abstract Developing a nonlinear biotic ligand model (BLM) that considers the geometrical constraints for binding of different cations on biotic ligands will provide more reliable details about the hypothetical mechanism governing the alleviation of cadmium (Cd) toxicity by coexistent cations. Soybean seedlings under Cd stress produced by various activities of coexistent cations such as calcium (Ca^{2+}), magnesium (Mg^{2+}), and potassium (K^+) were hydroponically assayed for Cd toxicity to soybean roots. The Cd^{2+} activity resulting in 50% reduction of root elongation (RE), EA_{50} , was used for assessing the Cd toxicity to the soybean seedling. Increasing Ca^{2+} , Mg^{2+} , and K^+ activities resulted in a significant alleviation of Cd toxicity to soybean roots. This alleviation was markedly higher with increasing Ca^{2+} and K^+ levels than with increasing Mg^{2+} level. In addition, EA_{50} increased in nonlinear positive relationships with Ca^{2+} and Mg^{2+} . The real data obtained from the soybean assay were thus used to develop the nonlinear BLM for Cd rhizotoxicity. Two parameters, competition equivalent and stability constant, indicated the profiles of the geometrical constraint and affinity of Ca^{2+} , Mg^{2+} , and K^+ binding on the soybean root surface to alleviate Cd toxicity. Compared with the traditional linear BLM, the nonlinear BLM provided more precise predictions of relative root elongation (RRE) and EA_{50} . Therefore, adopting the nonlinear BLM

approach will successfully improve the monitoring and assessment of heavy metal toxicity to terrestrial plants.

Keywords Free ion activity model · Biotic ligand model · Trace metal · Ion competition · Ligand exchange

Introduction

The total concentration of metals in soil solutions has been used as an indicator for assessing the phytoavailability of soil metals (Jopony and Young 1993; Lai and Chen 2004; Zhang and Young 2006). However, trace metal toxicity in terrestrial plants strongly depends on the chemical speciation of metals in solutions (Kinraide 1991). Free metal ion activity was suggested as an analog of intensity and therefore the major determinant of bioavailability in soil solutions (Campbell 1995; Parker and Pedler 1997). The free ion activity model (FIAM) demonstrates how variations in metal availability can be explained based on metal speciation and interaction with organisms (Paquin et al. 2002). Several studies have reported on the preferential binding of free metal ions with root surface ligands in comparison to other dissolved species (Parker and Pedler 1997; Parker et al. 2001).

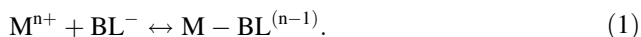
The biotic ligand model (BLM), a refinement of FIAM combined with the gill surface interaction model (GSIM), was developed based on the relation between the chemical behaviors of dissolved metals in an aquatic environment and the stress physiological responses of aquatic biota (Di Toro et al. 2001; Santore et al. 2001). BLM can be used to predict the degree of metal binding on an organism's toxicity action site; the extent by which this degree exceeds a

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threshold is related to the toxicological response. The BLM approach has been verified for different metals and different organisms (Paquin et al. 2002). In the last decade, it has been used for assessing metal toxicity to terrestrial plants (Antunes and Kreager 2009; Wang et al. 2009; Le et al. 2012; Chen et al. 2013; Smith et al. 2015). Thakali et al. (2006a, b) developed a terrestrial BLM for assessing Cu and Ni toxicity to barley seedlings. In the terrestrial BLM, metal ions (M^{n+}) in a soil solution that bind with negatively charged biotic ligands (BL^-) on root surfaces are expressed as a formation reaction of a metal and biotic ligand ($M-BL$) complex on the root surface:



The stability constant (k_M) for the binding of M^{n+} to BL^- is defined as

$$k_M = \frac{[M - BL]}{\{M^{n+}\}[BL^-]}, \quad (2)$$

where $\{M^{n+}\}$ is the activity of free ion M^{n+} in the solution; $[BL^-]$, the density of unoccupied biotic ligands on the root surface; and $[M-BL]$, the density of $M-BL$ complexes. Coexistent cations (e.g., Ca^{2+} , Mg^{2+} , K^+ , Na^+ , H^+) are considered, and the fraction (f_{M-BL}) is derived from the biotic ligands occupied by M^{n+} to form $M-BL$ over the total biotic ligands as (Jho et al. 2011)

$$f_{M-BL} = \frac{k_M \{M^{n+}\}}{1 + k_M \{M^{n+}\} + \sum_{i=1}^l k_i \{Cat_i\}}, \quad (3)$$

where $\{Cat_i\}$ are the activities of coexistent cations in the soil solution and k_i , the stability constants of Cat_i-BL complexes. According to the BLM concept, f_{M-BL} will be a superior determinant of bioavailability in soil solutions. According to Eq. (3), the metal ion activity $\{M^{n+}\}$ can be derived by using a linear function of coexistent cation activities $\{Cat_i\}$:

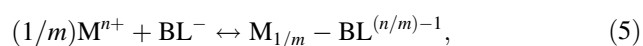
$$\{M^{n+}\} = \frac{f_{M-BL}}{(1 - f_{M-BL}) \cdot k_M} \left(1 + \sum_{i=1}^l k_i \{Cat_i\} \right). \quad (4)$$

Lock et al. (2007a, c) found that the 50% effective concentration (EC50), a toxicity index, of cobalt and nickel ions (Co^{2+} and Ni^{2+}) linearly increased with Mg^{2+} and K^+ activities. The EC50 of Co^{2+} and Ni^{2+} could be expressed as positive linear functions of Mg^{2+} and K^+ activities; these were consistent with the expression of Eq. (4). Therefore, the BLM approach can be used for assessing the alleviation effect of coexistent cations on metal toxicity to terrestrial plants.

However, Lock et al. (2007b) also found that Mg^{2+} activity resulted in a two-fold increase in the EC50 of Cu^{2+} ; however, the relationship between the EC50 of Cu^{2+} and Mg^{2+} activity was nonlinear. Li et al. (2009) found that the EC50 values of Co^{2+} for barley, oilseed rape, and tomatoes

were increasingly nonlinear with a higher level of extractable Ca^{2+} in the soil solution. Owing to the desire for consistency with a linear expression such as Eq. (4), Luo et al. (2008) adopted a linear function to model the positive relationships between the EC50 of Cu^{2+} and Ca^{2+} activities, although this was seemingly nonlinear. A few attempts were also made to use linear regression models to assess the positive relationships between the EC50 of metal ion and coexistent cations, as these relationships were nonlinear (Wang et al. 2010, 2012). Alternatively, the studies emphasized the deviant effect of a high concentration of coexistent cations on metal toxicity, resulting in nonlinear ameliorating effects (Luo et al. 2008; Ho 2012).

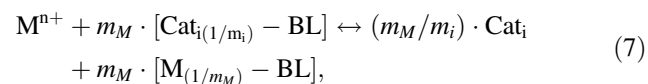
Cation and ligands are held together more or less steadily in a specific structure, as determined by geometry and electrical stability. Furthermore, the ions' opposite charges are not paired-off to achieve neutrality; instead, a cation's positive charge may be divided equally among nearby negatively charged ligands. The number of ligands near each cation that neutralizes positive charges is determined by a geometrical constraint. This is called the cation's coordination number. The coordination number can be addressed in $M-BL$ complex formation as follows:



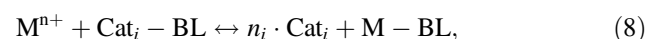
where m is the coordination number. Then, the stability constant, k_M , for M^{n+} binding onto BL^- can be derived as

$$k_M = \frac{[M_{1/m} - BL]}{\{M^{n+}\}^{1/m} [BL^-]}. \quad (6)$$

Equation (2) expresses the case in which the coordination numbers of cations binding onto biotic ligands are the same and equal to one ($m = 1$). Otherwise ($m \neq 1$), the competition between the coexistent cation Cat_i and metal ion M^{n+} in the biotic ligand solution could be expressed as



where m_M and m_i are the coordination numbers of M^{n+} and Cat_i bound by biotic ligands, respectively. When the competition approaches equilibrium, one can simplify the notations as



where n_i (i.e., m_M/m_i) is the competition equivalent of Cat_i for biotic ligands.

The fraction (f_{M-BL}), that is, the ratio of biotic ligands occupied by M^{n+} over total biotic ligands, can thus be derived as

$$f_{M-BL} = \frac{k_M \{M^{n+}\}}{1 + k_M \{M^{n+}\} + \sum_{i=1}^l k_i \{Cat_i\}^{n_i}}. \quad (9)$$

Then metal ion activity $\{M^{n+}\}$ can be expressed as a nonlinear function of the coexistent cation activity $\{Cat_i\}$:

$$\{M^{n+}\} = \frac{f_{MBL}}{(1 - f_{MBL}) \cdot k_M} \left(1 + \sum_{i=1}^l k_i \{Cat_i\}^{n_i} \right). \quad (10)$$

While the relationship between the activity of the coexistent cation $\{Cat_i\}$ and the medium effective activity (EA_{50M}) of metal ion M^{n+} is approximated to a nonlinear form as in Eq. (10), the alleviation effect of coexistent cations on metal toxicity can be modeled reliably by using the derived nonlinear BLM approach.

Nowadays, most BLM approaches are developed for predicting Cu, Ni, and Zn toxicities (Paquin et al. 2002; Niyogi and Wood 2004; Wang et al. 2009; Smith et al. 2015); however, few studies have focused on applying the BLM to Cd toxicity (Borgmann et al. 2004; Di Toro et al. 2005; Jho et al. 2011). Because they are easy to conduct, require less time and space, and have low environmental variability, hydroponic experiments have been extensively employed to investigate the mechanisms of the trace metal toxicity and absorption (Tian et al. 2011; Chen et al. 2012; Farzadfar et al. 2013; Antonkiewicz et al. 2016). A hydroponic study can be regarded as the beginning in assessing metal toxicity by using hypothetical models. In this study, a hydroponic assay was conducted for soybean seedlings under Cd stress produced by various background levels of Ca, Mg, and K (Yang and Juang 2015). The assay results were used to develop a nonlinear BLM for illustrating the effects of major cations (i.e., Ca^{2+} , Mg^{2+} , and K^+) in the soil solution on Cd toxicity to the soybean root. The assessment of the reliability of the predictions of root elongation (RE) and medium Cd^{2+} activity on reduction in RE were validated by using the nonlinear BLM. Furthermore, a comparison with the total concentration model (TCM), FIAM, and linear BLM was performed to emphasize the feasibility of the nonlinear BLM used for modeling the Cd toxicity to the soybean root.

Materials and methods

Hydroponic experiments

Seeds of green manure blue soybean (*Glycine max* L. Merr. cv. Tainan No. 4) were soaked in deionized water for a 36-h germination period in the dark. The germinated seeds were transplanted in 10% modified Hoagland solution for 2 days. A full-strength Hoagland solution contains 15 mM NH_4NO_3 , 5 mM KCl, 5 mM $CaCl_2$, 1 mM KH_2PO_4 , 2 mM $MgSO_4$, 100 μ M Fe(III)-EDTA, 225 μ M $MnSO_4$, 115 μ M H_3BO_3 , 1.19 μ M $ZnSO_4$, 0.8 μ M $CuSO_4$, and 0.277 μ M

$(NH_4)_6Mo_7O_{24}$. Uniformly grown seedlings were selected for Cd treatment in hydroponics.

The background hydroponic solution was 10% modified Hoagland solution. Calcium chloride was applied to the background solution to provide one set of six test solutions of 0.25, 0.5, 1, 10, and 20 mM Ca (Ca-set). Magnesium sulfate was applied to the background solution to provide another set of six test solutions of 0.1, 0.2, 0.4, 1, 2, and 4 mM Mg (Mg-set). Potassium chloride was applied to the background solution to provide a final set of six test solutions of 0.3, 0.6, 1.2, 2.4, 4.8, and 9.6 mM K (K-set). For each set, cadmium chloride was added to each test solution to prepare Cd-spiked solutions with Cd levels of 0, 4, 8, 12, and 16 μ M, respectively. A 50 mL polypropylene bottle was filled with each Cd-spiked solution, and three seedlings were transplanted into each bottle. Three bottles of seedlings were used as replicates for each Cd-spiked solution.

The seedlings were cultivated with the Cd-spiked solutions in a growth chamber with a day/night cycle of 16/8 h at 23 and 27 °C, relative humidity of 65–85%, and photosynthetically active radiation of $150 \mu\text{mol m}^{-2} \text{s}^{-1}$. To prevent the pH effect on Cd toxicity and to keep the main soluble Cd species as a free ion of Cd^{2+} , the solution pH was maintained at 6.0 ± 0.1 with 2-(N-morpholino) ethanesulfonic acid buffer. After 7-day exposure to Cd, the seedlings in each bottle were harvested and thoroughly washed with deionized water before their RE was measured. The detailed assay results have been reported in our previous study (Yang and Juang 2015).

Modeling Cd rhizotoxicity by using FIAM

Following the FIAM approach, Cd rhizotoxicity to soybean seedlings was characterized by the dose-response relationship between the Cd^{2+} activity in solution, $\{Cd^{2+}\}$, and RE , and it was expressed as

$$RE = \frac{a}{1 + (\{Cd^{2+}\}/EA_{50})^b}, \quad (11)$$

where EA_{50} is the effective activity of Cd^{2+} that results in a 50% reduction of RE , and a and b are model parameters that can be obtained by fitting to the outcomes of the hydroponic experiment for Cd toxicity to soybean seedlings. The activities of cations in the solution were assessed using the chemical equilibrium model Visual MINTEQ introduced by Gustafsson (2012). The input data included temperature, pH, and the ion concentrations of Cd^{2+} , Ca^{2+} , Mg^{2+} , K^+ , Na^+ , Cl^- , NO_3^- , $H_2PO_4^-$, and SO_4^{2-} . The pH was set to follow the hydroponic solution pH maintained at 6.0 ± 0.1 . The ion concentrations were thus given ion compositions of 10% modified Hoagland solution at pH 6.0. Inorganic carbon was assumed to be in equilibrium with atmospheric CO_2 at 25 °C.

Modeling Cd rhizotoxicity by using BLM

Based on the M – BL fraction (f_{M-BL}) given in Eqs. (3) and (9), a logistic dose-response relationship for illustrating Cd toxicity to relative root elongation (RRE) was described as a function of f_{Cd-BL} as follows:

$$RRE = \frac{100}{1 + \exp[\beta(f_{Cd-BL} - f_{Cd-BL}^{50})]}, \quad (12)$$

where f_{Cd-BL}^{50} is the Cd–BL fraction at which 50% reduction of RRE occurs, and β is a model parameter obtained by fitting to the outcomes of the soybean assay. RRE was calculated as (RE/a) 100%, where a is the given model fitting of FIAM (Eq. 11).

According to Eq. (4) for linear BLM, the $\{Cd^{2+}\}$ value resulting in 50% reduction of RE was represented as a linear combination of $\{Ca^{2+}\}$, $\{Mg^{2+}\}$, and $\{K^+\}$:

$$EA50_{Cd(l)} = \frac{f_{Cd-BL}^{50}}{(1 - f_{Cd-BL}^{50})k_{Cd}} \cdot (1 + k_{Ca}\{Ca^{2+}\} + k_{Mg}\{Mg^{2+}\} + k_K\{K^+\}). \quad (13)$$

However, according to Eq. (10) for nonlinear BLM, the $\{Cd^{2+}\}$ value resulting in 50% reduction of RE was represented as a nonlinear combination of $\{Ca^{2+}\}$, $\{Mg^{2+}\}$, and $\{K^+\}$:

$$EA50_{Cd(nl)} = \frac{f_{Cd-BL}^{50}}{(1 - f_{Cd-BL}^{50})k_{Cd}} \cdot (1 + k_{Ca}\{Ca^{2+}\}^{n_{Ca}} + k_{Mg}\{Mg^{2+}\}^{n_{Mg}} + k_K\{K^+\}^{n_K}). \quad (14)$$

Nevertheless, Eq. (13) shows the case in which $n_{Ca} = n_{Mg} = n_K = 1$. Then, the linear and nonlinear predictors ($f_{Cd-BL(l)}$ and $f_{Cd-BL(nl)}$, respectively) of the Cd–BL fraction were generated as

$$f_{Cd-BL(l)} = \frac{k_{Cd}\{Cd^{2+}\}}{1 + k_{Cd}\{Cd^{2+}\} + k_{Ca}\{Ca^{2+}\} + k_{Mg}\{Mg^{2+}\} + k_K\{K^+\}} \quad (15)$$

and

$$f_{Cd-BL(nl)} = \frac{k_{Cd}\{Cd^{2+}\}}{1 + k_{Cd}\{Cd^{2+}\} + k_{Ca}\{Ca^{2+}\}^{n_{Ca}} + k_{Mg}\{Mg^{2+}\}^{n_{Mg}} + k_K\{K^+\}^{n_K}}. \quad (16)$$

Estimation of BLM parameters

The data of the soybean assay for assessing Cd toxicity were used to express EA_{50} in the linear and nonlinear functions of $\{Ca^{2+}\}$, $\{Mg^{2+}\}$, and $\{K^+\}$, corresponding to the test solutions of the Ca-set, Mg-set, and K-set. In

Eq. (13), the linear regression model of $EA50_{Cd(l)}$ is expressed by one cation activity (i.e., $\{Ca^{2+}\}$, $\{Mg^{2+}\}$, or $\{K^+\}$) with an intercept and a slope. In Eq. (14), the nonlinear regression model of $EA50_{Cd(nl)}$ is expressed by one cation activity (i.e., $\{Ca^{2+}\}$, $\{Mg^{2+}\}$, or $\{K^+\}$) with an intercept, a slope, and a power. By an arithmetical operation, the slope to intercept ratio can be expressed as a combination of k_{Ca} , k_{Mg} , and k_K . These can be combined with the ratios R_{Ca} , R_{Mg} , and R_K for $\{Ca^{2+}\}$, $\{Mg^{2+}\}$, and $\{K^+\}$, respectively, and be arranged in the following matrix form suggested by De Schamphelaere and Janssen (2002):

$$\begin{pmatrix} R_{Ca} \\ R_{Mg} \\ R_K \end{pmatrix} = \begin{pmatrix} 1 & -R_{Ca}\{Mg^{2+}\}^{n_{Mg}} & -R_{Ca}\{K^+\}^{n_K} \\ -R_{Mg}\{Ca^{2+}\}^{n_{Ca}} & 1 & -R_{Mg}\{K^+\}^{n_K} \\ -R_K\{Ca^{2+}\}^{n_{Ca}} & -R_K\{Mg^{2+}\}^{n_{Mg}} & 1 \end{pmatrix} \cdot \begin{pmatrix} k_{Ca} \\ k_{Mg} \\ k_K \end{pmatrix}, \quad (17)$$

where n_{Ca} , n_{Mg} , and n_K are estimated as the powers in Eq. (4). While this is specified for the linear regression as in Eq. (13), n_{Ca} , n_{Mg} , and n_K all are set as 1. Then, the analytical solution of the matrix results in the estimates of k_{Ca} , k_{Mg} , and k_K .

For the final development of the BLM, two other parameters remain to be determined: k_{Cd} and f_{Cd-BL}^{50} . A simulation procedure to vary k_{Cd} for obtaining $f_{Cd-BL(l)}$ and $f_{Cd-BL(nl)}$ values was then used in Eqs. (15) and (16), respectively. Given the RRE data, the generated values of $f_{Cd-BL(l)}$ and $f_{Cd-BL(nl)}$ were fitted to the logistic dose-response relationship of *Logit* (100– RRE) on f_{Cd-BL} (Eq. 12). The least-square fitting was used to obtain the best fit values of k_{Cd} and f_{Cd-BL}^{50} for the logistic dose-response relationship of *Logit* (100– RRE) on f_{Cd-BL} (Burnham and Anderson 2002). A minimum Akaike's information criterion (AIC) (Murata et al. 1998) was used to determine the best-fit for *Logit* (100– RRE) relative to f_{Cd-BL} , which would be associated with an optimized k_{Cd} . Then, the k_{Cd} value that resulted in the best-fit for *Logit* (100– RRE) relative to f_{Cd-BL} was selected as the estimate of k_{Cd} . The best-fit coefficients of the slope and intercept, associated with the estimate of k_{Cd} , were used to estimate β and f_{Cd-BL}^{50} as in Eq. (12) finally (Lock et al. 2007a, c).

Results

Responses of soybean root to Cd stress under various Ca, Mg, and K levels

Tables 1–3 show the soybean assay results for Cd toxicity in testing solutions of Ca-set, Mg-set, and K-set. RE of

Table 1 Dose-response curves between Cd^{2+} activity, $\{\text{Cd}^{2+}\}$, and root elongation, RE , in testing solutions of Ca-set and fitting parameters, a , b , and EA_{50} , for soybean seedlings

Testing solutions of Ca-set		Root elongation (RE) cm	$RE = a / [1 + (\{\text{Cd}^{2+}\} / EA_{50})^b]$			
$[\text{Ca}^{2+}]$ ($\{\text{Ca}^{2+}\}$) mM	$[\text{Cd}^{2+}]$ ($\{\text{Cd}^{2+}\}$) μM		EA_{50} μM	a	b	r^2
0.25 (0.19)	0.00 (0.00)	6.75 ± 0.12	0.64	6.76	1.00	0.99 ($p = 0.010$)
0.25 (0.19)	4.00 (3.08)	1.52 ± 0.12				
0.25 (0.19)	8.00 (6.17)	0.42 ± 0.22				
0.25 (0.19)	12.0 (9.25)	0.12 ± 0.05				
0.25 (0.19)	16.0 (12.3)	0.09 ± 0.02				
0.50 (0.38)	0.00 (0.00)	7.37 ± 0.63	0.76	7.37	1.00	0.99 ($p = 0.005$)
0.50 (0.38)	4.00 (3.01)	1.65 ± 0.08				
0.50 (0.38)	8.00 (6.03)	0.45 ± 0.12				
0.50 (0.38)	12.0 (9.04)	0.65 ± 0.08				
0.50 (0.38)	16.0 (12.1)	0.57 ± 0.17				
1.00 (0.72)	0.00 (0.00)	8.60 ± 0.17	1.85	8.60	1.00	0.99 ($p = 0.005$)
1.00 (0.72)	4.00 (2.89)	3.25 ± 0.05				
1.00 (0.72)	8.00 (5.78)	2.44 ± 0.24				
1.00 (0.72)	12.0 (8.68)	1.25 ± 0.22				
1.00 (0.72)	16.0 (11.6)	1.15 ± 0.42				
5.00 (2.94)	0.00 (0.00)	6.92 ± 0.16	1.92	6.93	0.68	0.99 ($p = 0.010$)
5.00 (2.94)	4.00 (2.36)	3.37 ± 0.51				
5.00 (2.94)	8.00 (4.72)	2.15 ± 0.09				
5.00 (2.94)	12.0 (7.09)	1.93 ± 0.22				
5.00 (2.94)	16.0 (9.45)	2.00 ± 0.33				
10.0 (5.09)	0.00 (0.00)	7.19 ± 0.09	2.33	7.27	1.00	0.97 ($p = 0.033$)
10.0 (5.09)	4.00 (2.04)	4.37 ± 0.27				
10.0 (5.09)	8.00 (4.08)	2.62 ± 0.18				
10.0 (5.09)	12.0 (6.12)	1.31 ± 0.12				
10.0 (5.09)	16.0 (8.16)	1.76 ± 0.12				
20.0 (8.51)	0.00 (0.00)	6.82 ± 0.42	3.45	7.06	1.00	0.91 ($p = 0.095$)
20.0 (8.51)	4.00 (1.71)	5.25 ± 0.45				
20.0 (8.51)	8.00 (3.41)	4.23 ± 0.60				
20.0 (8.51)	12.0 (5.12)	2.48 ± 0.37				
20.0 (8.51)	16.0 (6.83)	1.48 ± 0.13				

soybean seedlings decreased with higher Cd concentrations in the testing solutions for each set. Based on FIAM, a dose-response curve of RE on $\{\text{Cd}^{2+}\}$, as seen in Eq. (11), was developed for each background level of Ca, Mg, or K. The best-fit values of EA_{50} were calculated. Table 1 shows that for testing solutions of the Ca-set, EA_{50} increased with the Ca level. EA_{50} was $0.64\text{--}3.25 \text{ mg kg}^{-1}$ in the Ca-set. Table 2 shows that for testing solutions of the Mg-set, EA_{50} increased with the Mg level. EA_{50} was $0.07\text{--}1.74 \text{ mg kg}^{-1}$ in the Mg-set. Table 3 shows that for testing solutions of the K-set, EA_{50} increased with the K level. EA_{50} was $0.96\text{--}3.68 \text{ mg kg}^{-1}$ in the K-set.

Delineation of relationships between EA_{50} and consistent cations

The EA_{50} values for the background levels of Ca, Mg, and K are further illustrated in Fig. 1a, b, c, respectively. The EA_{50} patterns with respect to the $\{\text{Ca}^{2+}\}$ and $\{\text{Mg}^{2+}\}$ curves are shown in Fig. 1a, b, respectively. Given the linear expression of $EA_{50\text{Cd}(l)}$ with $\{\text{Ca}^{2+}\}$, $\{\text{Mg}^{2+}\}$, and $\{\text{K}^{+}\}$ as in Eq. (13), regressions for EA_{50} on $\{\text{Ca}^{2+}\}$, $\{\text{Mg}^{2+}\}$, and $\{\text{K}^{+}\}$ were conducted, and the results are shown in Fig. 1a, b, c, respectively. All results were statistically significant ($p < 0.05$). The coefficient of

Table 2 Dose-response curves between Cd^{2+} activity, $\{\text{Cd}^{2+}\}$, and root elongation, RE , in testing solutions of Mg-set and fitting parameters, a , b , and EA_{50} , for soybean seedlings

Testing solutions of Mg-set		Root elongation (RE) cm	$RE = a / [1 + (\{\text{Cd}^{2+}\} / EA_{50})^b]$			
$[\text{Mg}^{2+}]$ ($\{\text{Mg}^{2+}\}$) mM	$[\text{Cd}^{2+}]$ ($\{\text{Cd}^{2+}\}$) μM		EA_{50} μM	a	b	r^2
0.10 (0.08)	0.00 (0.00)	7.42 ± 0.22	0.07	7.42	0.38	1.00
0.10 (0.08)	4.00 (3.05)	1.42 ± 0.21				$(p < 0.001)$
0.10 (0.08)	8.00 (6.10)	1.17 ± 0.04				
0.10 (0.08)	12.0 (9.15)	0.93 ± 0.10				
0.10 (0.08)	16.0 (12.2)	0.95 ± 0.02				
0.20 (0.15)	0.00 (0.00)	6.56 ± 0.52	0.57	6.56	0.36	1.00
0.20 (0.15)	4.00 (3.01)	1.73 ± 0.04				$(p < 0.001)$
0.20 (0.15)	8.00 (6.03)	1.10 ± 0.11				
0.20 (0.15)	12.0 (9.04)	1.00 ± 0.02				
0.20 (0.15)	16.0 (12.1)	0.88 ± 0.05				
0.40 (0.29)	0.00 (0.00)	9.54 ± 0.34	0.59	9.54	1.00	1.00
0.40 (0.29)	4.00 (2.95)	1.64 ± 0.03				$(p = 0.005)$
0.40 (0.29)	8.00 (5.89)	0.72 ± 0.21				
0.40 (0.29)	12.0 (8.84)	0.97 ± 0.17				
0.40 (0.29)	16.0 (11.8)	0.12 ± 0.02				
1.0 (0.69)	0.00 (0.00)	6.41 ± 0.26	1.40	6.43	1.00	0.99
1.0 (0.69)	4.00 (2.78)	2.46 ± 0.20				$(p = 0.009)$
1.0 (0.69)	8.00 (5.56)	1.12 ± 0.16				
1.0 (0.69)	12.0 (8.34)	0.84 ± 0.09				
1.0 (0.69)	16.0 (11.1)	0.42 ± 0.05				
2.0 (1.29)	0.00 (0.00)	6.40 ± 0.27	1.31	6.42	1.00	0.99
2.0 (1.29)	4.00 (2.58)	2.42 ± 0.31				$(p = 0.007)$
2.0 (1.29)	8.00 (5.16)	1.20 ± 0.17				
2.0 (1.29)	12.0 (7.74)	0.82 ± 0.05				
2.0 (1.29)	16.0 (10.3)	0.42 ± 0.02				
4.0 (2.31)	0.00 (0.00)	6.09 ± 0.32	1.74	6.11	1.00	0.99
4.0 (2.31)	4.00 (2.31)	2.75 ± 0.45				$(p = 0.010)$
4.0 (2.31)	8.00 (4.63)	1.87 ± 0.27				
4.0 (2.31)	12.0 (6.94)	0.88 ± 0.05				
4.0 (2.31)	16.0 (9.26)	0.87 ± 0.17				

determination (r^2) for the model of $EA_{50\text{Cd}(l)}$ on $\{K^+\}$ (0.93) is higher than those on $\{\text{Ca}^{2+}\}$ (0.87) and $\{\text{Mg}^{2+}\}$ (0.76). In other words, the relationships between EA_{50} and $\{\text{Ca}^{2+}\}$ and between EA_{50} and $\{\text{Mg}^{2+}\}$ require a lower degree to fit a linear form. Given the nonlinear expression of $EA_{50\text{Cd}(nl)}$ with $\{\text{Ca}^{2+}\}$, $\{\text{Mg}^{2+}\}$, and $\{K^+\}$ as in Eq. (14), the nonlinear regressions for EA_{50} on $\{\text{Ca}^{2+}\}$ and $\{\text{Mg}^{2+}\}$ had higher r^2 values (0.87 and 0.80, respectively) than the linear ones did (see Fig. 1a, b, respectively). Nevertheless, the nonlinear regression for EA_{50} of $\{K^+\}$ did not provide any improvement in r^2 (Fig. 1c).

Validation for RRE predictions using FIAM and BLMs

To compare the RRE predictions by BLMs with those by FIAM, we pooled together the RRE data from the Ca-, Mg-, and K-sets for validation. The RRE prediction by BLMs was given the function of $f_{\text{Cd-BL}}$ (Eq. 12); $f_{\text{Cd-BL}(l)}$ and $f_{\text{Cd-BL}(nl)}$ values were specified for linear and nonlinear BLMs by Eqs. (15) and (16), respectively. For RRE prediction by FIAM, the pooled data were fit

Table 3 Dose-response curves between Cd^{2+} activity, $\{\text{Cd}^{2+}\}$, and root elongation, RE , in testing solutions of K-set and fitting parameters, a , b , and EA_{50} , for soybean seedlings

Testing solutions of K-set		Root elongation (RE) cm	$RE = a / [1 + (\{\text{Cd}^{2+}\} / EA_{50})^b]$			
$[\text{K}^{2+}]$ ($\{\text{K}^{2+}\}$) mM	$[\text{Cd}^{2+}]$ ($\{\text{Cd}^{2+}\}$) μM		EA_{50} μM	a	b	r^2
0.30 (0.28)	0.00 (0.00)	5.41 ± 0.22	0.96	5.41	0.73	1.00
0.30 (0.28)	4.00 (3.04)	1.63 ± 0.10				$(p < 0.001)$
0.30 (0.28)	8.00 (6.08)	1.10 ± 0.13				
0.30 (0.28)	12.0 (9.12)	0.93 ± 0.50				
0.30 (0.28)	16.0 (12.2)	0.70 ± 0.17				
0.60 (0.56)	0.00 (0.00)	6.02 ± 0.29	1.56	6.02	1.00	1.00
0.60 (0.56)	4.00 (3.01)	2.09 ± 0.02				$(p < 0.001)$
0.60 (0.56)	8.00 (6.03)	1.14 ± 0.06				
0.60 (0.56)	12.0 (9.04)	0.90 ± 0.17				
0.60 (0.56)	16.0 (12.1)	0.75 ± 0.02				
1.2 (1.11)	0.00 (0.00)	6.67 ± 0.04	1.49	6.66	1.00	1.00
1.2 (1.11)	4.00 (2.96)	1.73 ± 0.10				$(p = 0.065)$
1.2 (1.11)	8.00 (5.92)	1.27 ± 0.20				
1.2 (1.11)	12.0 (8.88)	1.20 ± 0.17				
1.2 (1.11)	16.0 (11.9)	0.55 ± 0.12				
2.4 (2.21)	0.00 (0.00)	4.20 ± 0.23	2.08	4.21	1.00	1.00
2.4 (2.21)	4.00 (2.87)	1.85 ± 0.32				$(p = 0.002)$
2.4 (2.21)	8.00 (5.74)	1.12 ± 0.05				
2.4 (2.21)	12.0 (8.61)	0.70 ± 0.11				
2.4 (2.21)	16.0 (11.5)	0.63 ± 0.40				
4.8 (4.36)	0.00 (0.00)	5.46 ± 0.10	2.15	5.50	1.00	0.97
4.8 (4.36)	4.00 (2.72)	2.70 ± 0.20				$(p = 0.029)$
4.8 (4.36)	8.00 (5.45)	1.79 ± 0.09				
4.8 (4.36)	12.0 (8.17)	0.93 ± 0.02				
4.8 (4.36)	16.0 (10.9)	0.43 ± 0.10				
9.6 (8.54)	0.00 (0.00)	5.09 ± 0.06	3.68	5.24	1.00	0.90
9.6 (8.54)	4.00 (2.51)	3.55 ± 0.78				$(p = 0.099)$
9.6 (8.54)	8.00 (5.02)	2.67 ± 0.10				
9.6 (8.54)	12.0 (7.53)	1.54 ± 0.04				
9.6 (8.54)	16.0 (10.0)	0.52 ± 0.05				

to the following expression:

$$RRE = \frac{100}{1 + (\{\text{Cd}^{2+}\} / EA_{50\text{Cd}})^\beta}, \quad (18)$$

where the model parameters $EA_{50\text{Cd}}$ and β are given the pooled data of RRE ; however, the different background levels of $\{\text{Ca}^{2+}\}$, $\{\text{Mg}^{2+}\}$, and $\{\text{K}^+\}$ were not considered. Figure 2 shows the validation for the RRE predictions by using FIAM, linear BLM, and nonlinear BLM. The RRE predictions by FIAM ($EA_{50\text{Cd}}$ and β) given in Eq. (18) are

2.36 μM and 1.22, respectively. The scatter patterns for the FIAM prediction relative to the observed RRE are clustered near the 1:1 line; however, some patterns lie beyond the interval of 15% deviation (Fig. 2a). Figure 2b, c shows that the scatter patterns for the BLM prediction relative to the observed RRE are clustered near the 1:1 line and are almost within the interval of 15% deviation. The root mean squared error ($RMSE$) was used for assessing the precision of the RRE predictions (Chai and Draxler 2014). The $RMSE$ for RRE prediction by nonlinear BLM (8.11%) was less than

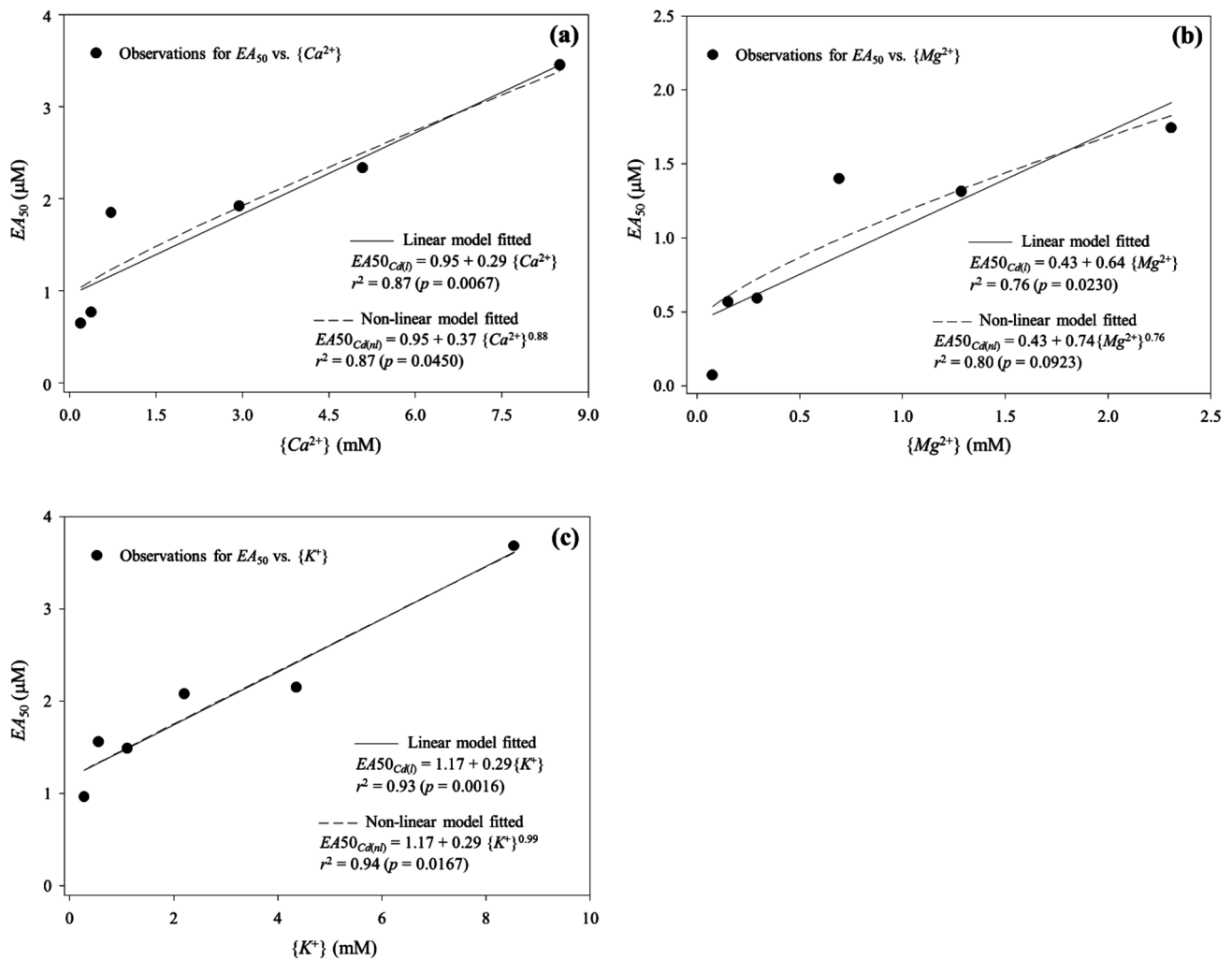


Fig. 1 Effective activity of Cd²⁺ resulting 50% reduction of root elongation (EA_{50}) pronouncedly related to **a** Ca²⁺ activity, $\{Ca^{2+}\}$, **b** Mg²⁺ activity, $\{Mg^{2+}\}$, and **c** K⁺ activity, $\{K^+\}$, respectively

that by linear BLM (9.79%). In addition, the AIC values indicating the goodness of fit for the three models (i.e. FIAM, linear BLM, and nonlinear BLM) fitting to RRE observations were shown in Fig. 2; they were 399.1, 414.9 and 380.9, respectively and in the following order: linear BLM > FIAM > nonlinear BLM. Compared with FIAM and linear BLM, nonlinear BLM was the statistically better for modeling RRE .

Validation for prediction of EA_{50} by using BLMs

To compare the predictions of EA_{50} by linear and nonlinear BLMs, we used EA_{50} data given different background levels of $\{Ca^{2+}\}$, $\{Mg^{2+}\}$, and $\{K^+\}$ as obtained from Tables 1–3, respectively, for validation. The predictions of EA_{50} by linear and nonlinear BLMs were obtained from Eqs. (13) and (14) and denoted as $EA50_{Cd(l)}$ and $EA50_{Cd(nl)}$,

respectively. In Fig. 3, the $EA50_{Cd(nl)}$ values obtained by nonlinear BLM were clustered closer to the 1:1 line compared with the $EA50_{Cd(l)}$ values obtained by linear BLM. In Fig. 3, among the $EA50_{Cd(l)}$ values obtained by linear BLM, there were more patterns that lay beyond the 15% deviation. The $RMSE$ for $EA50_{Cd(l)}$ prediction by linear BLM (0.73 μM) was higher than that for $EA50_{Cd(nl)}$ prediction by nonlinear BLM (0.68 μM). In addition, the Mg-set showed more extreme deviations in the EA_{50} predictions by linear and nonlinear BLMs than the Ca- and K-sets. Additionally, the AIC values indicating the goodness of fit for linear BLM and nonlinear BLM fitting to EA_{50} were -5.5 and -8.0 , respectively (Fig. 3). This result was consistent with the comparison above of the $RMSE$ values and revealed that nonlinear BLM provided the superior goodness of fit for modeling EA_{50} .

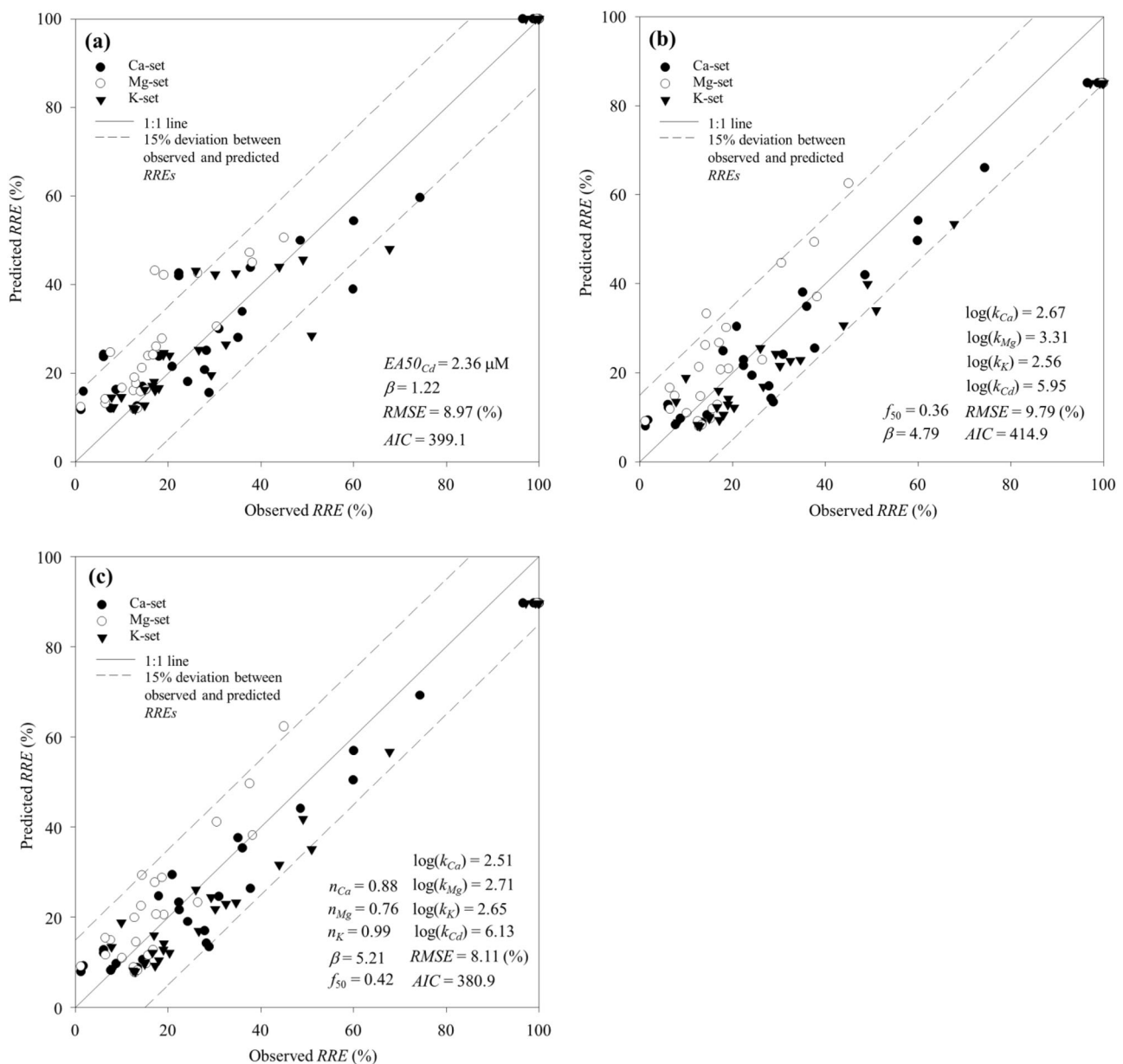


Fig. 2 Validation with observed ones for predicted values of relative root elongation (*RRE*) by using **a** free ion activity model (FIAM), **b** linear biotic ligand model (BLM), and **c** non-linear BLM, respectively.

Akaike's information criterion (*AIC*) indicates the goodness of fit for identification of the best model fitting to *RRE*

Discussion

Alleviation effects of Ca, Mg, and K on Cd toxicity to soybean root

Tables 1–3 showed that Cd toxicity to soybean root decreased with increasing Ca, Mg, and K levels in the solution. According to the EA_{50} ranges in the three sets, the alleviation of Cd toxicity was distinctly higher with increasing Ca and K levels than with increasing Mg levels. According to the ranges of EA_{50} in the three sets, the values

EA_{50} were distinctly elevated by raising Ca and K levels than increasing Mg levels. That is, the effects of Ca and K on the alleviation of Cd toxicity were more evident than that of Mg. Owing to uses of Ca, Mg, and K sources of $CaCl_2$, $MgSO_4$, and KCl , respectively in the study, the alleviations of Cd toxicity by adding Ca, Mg, and K would be related to the influences of chloride and sulfate on the Cd toxicity to soybean root. Zhao et al. (2003) ever showed that there was no significant difference in Cd uptake by spring wheat between applications of KCl and K_2SO_4 . Benaissa and Benguella (2004) found that the less Cd sorption by chitin

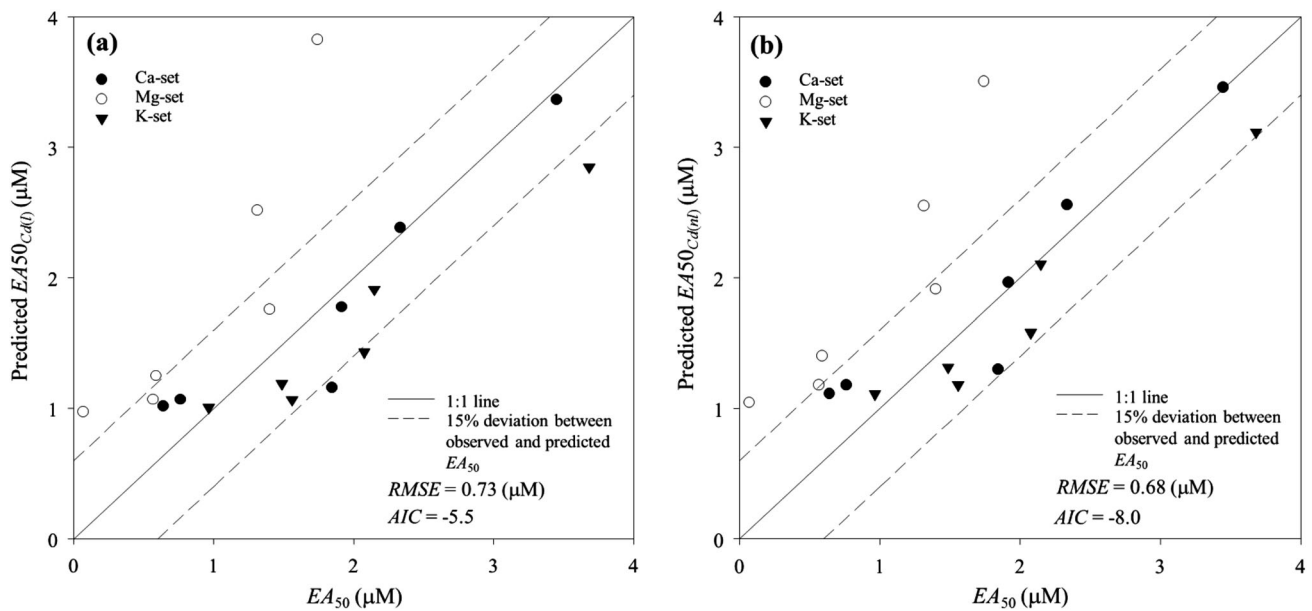


Fig. 3 Validation for the predictions of effective activity of Cd^{2+} resulting 50% reduction of root elongation (EA_{50}) by using **a** linear biotic ligand model (BLM) and **b** non-linear BLM, respectively.

Akaike's information criterion (AIC) indicates the goodness of fit for identification of the best model fitting to EA_{50}

was observed in the presence of sulfate than of chloride; however the result was not significant. Rao et al. (2010) also emphasized that generally the presence of chloride and sulfate would inhibit Cd sorption, but there was little information in detail to show the different influences between chloride and sulfate on Cd sorption. The anion effect of chloride and sulfate on the alleviation of Cd toxicity to soybean root was concerned and thereby suggested to be examined in further studies.

In addition, the rates of EA_{50} values increased with comparatively low levels of $\{\text{Ca}^{2+}\}$ and $\{\text{Mg}^{2+}\}$ and were obviously higher than those with high levels of $\{\text{Ca}^{2+}\}$ and $\{\text{Mg}^{2+}\}$ (Fig. 1a, b, respectively). In other words, there were two stages of alleviation of Cd toxicity with elevated $\{\text{Ca}^{2+}\}$ and $\{\text{Mg}^{2+}\}$. The deviant alleviation of Cd toxicity with two apparent stages was 0.72 mM for $\{\text{Ca}^{2+}\}$ and 0.69 mM for $\{\text{Mg}^{2+}\}$ (Tables 1 and 2, respectively). The deviant effects of increasing the concentrations of competitive cations on the alleviation of metal toxicity replicate the findings of previous studies (Luo et al. 2008; Antunes and Kreager 2009; Wang et al. 2010; Ho 2012). However, there was no deviant alleviation of Cd toxicity with increasing K^+ (Fig. 1c). Overall the results in Fig. 1 indicated that increases of the concentrations of Ca^{2+} , Mg^{2+} , and K^+ which competed with Cd^{2+} for sorption sites of root solid phase would result in desorption of Cd and reduction of its phytotoxicity (Du Laing et al. 2009). Nazar et al. (2012) summarized the current understandings of mineral nutrients in the alleviation of Cd phytotoxicity and showed that Ca reduces Cd toxicity mainly by reducing its uptake

and competing at the transport site. The protective effect of Mg against Cd toxicity was referred to the enhanced antioxidant status (Chou et al. 2011) and the inhibition of Cd uptake (Kashem and Kawai 2007). Application of K would be effective against Cd toxicity by improving activity of antioxidant enzymes and then enhance plant growth (Umar et al. 2008; Siddiqui et al. 2012). In contrast, Benaissa and Benguella (2004) suggested the competitor effect of Ca^{2+} and Mg^{2+} towards the sorption of Cd^{2+} was far more important than that of mono-valent alkaline cations (e.g. Na^+ and K^+). Mei et al. (2014) emphasized Cd was absorbed by the amaranth roots through Ca^{2+} ion channels but not through Na^+ and K^+ ion channels. Thus, the finding of deviant alleviation of Cd toxicity associated with increasing Ca^{2+} and Mg^{2+} but not increasing K^+ would be relative to their ionic radius and valence.

According to the definitions in Eqs. (13) and (14), EA_{50} could be expressed as a function of $\{\text{Ca}^{2+}\}$, $\{\text{Mg}^{2+}\}$, or $\{\text{K}^+\}$ in a linear $EA_{50\text{Cd}(t)}$ or nonlinear $EA_{50\text{Cd}(nl)}$ form. The relationships between EA_{50} and $\{\text{Ca}^{2+}\}$ and between EA_{50} and $\{\text{Mg}^{2+}\}$ were almost nonlinear; however, that between EA_{50} and $\{\text{K}^+\}$ was linear. Based on the concept of nonlinear BLM (Eq. 8), the competition equivalents of Ca^{2+} and Mg^{2+} (n_{Ca} and n_{Mg}) with Cd^{2+} for the binding sites on the root surface could be assigned to the powers of the nonlinear forms (0.88 and 0.76) for the relationships between EA_{50} and $\{\text{Ca}^{2+}\}$ and between EA_{50} and $\{\text{Mg}^{2+}\}$, respectively (Fig. 1a, b). This could be because the coordination number of Cd^{2+} binding onto the biotic ligand was significantly different from those of Ca^{2+} and Mg^{2+} . The

competition equivalent of K^+ with Cd^{2+} was $n_K = 0.99$ (Fig. 1c). Therefore, the coordination numbers of K^+ and Cd^{2+} binding onto the biotic ligand would be equal to each other ($m_K = m_{Cd}$). Efforts to implement BLM are seldom concerned with the reliability of linear competition for biotic ligands between toxic metal ions and coexistent cations (Luo et al. 2008; Ho 2012). The results in the present study suggest that the competition of K^+ with Cd^{2+} for the binding sites on the root surface was consistent with a linear relationship (Eq. 13); however, those of Ca^{2+} and Mg^{2+} with Cd^{2+} were not. Therefore, the nonlinear approach (Eq. 14) may be more promising for illustrating the competition of Ca^{2+} and Mg^{2+} with Cd^{2+} . The nonlinear effects of Ca and Mg have been found on nickel and zinc toxicity to barley root; nevertheless, these were not considered to improve the BLM development (Li et al. 2009; Wang et al. 2010).

BLMs used for assessing alleviation of Cd toxicity with Ca, Mg, and K

In linear BLM, the logarithmic values of the stability constants for Ca^{2+} , Mg^{2+} , K^+ , and Cd^{2+} binding on biotic ligands ($\log k_{Ca}$, $\log k_{Mg}$, $\log k_K$, and $\log k_{Cd}$) were 2.67, 3.31, 2.56, and 5.95, respectively (Fig. 2b). The stability constants may indicate the affinities of Ca^{2+} , Mg^{2+} , K^+ , and Cd^{2+} absorbed on biotic ligands. The larger stability constant of $\log k_{Mg}$ suggested that Mg^{2+} is superior to Ca^{2+} and K^+ for competition with Cd^{2+} for binding on biotic ligands. However, very few studies on the linear BLM approach for Cd toxicity to terrestrial plant suggested any finding about $\log k_{Ca}$, $\log k_{Mg}$, and $\log k_K$ (Niyogi and Wood 2004). Lock et al. (2007a) reported that cobalt toxicity to barley with $\log k_{Mg} = 3.86$ and $\log k_K = 2.50$. Study of copper toxicity to wheat reported $\log k_{Ca} = 2.43$ and $\log k_{Mg} = 3.34$ (Luo et al. 2008). Antunes and Kreager (2009) developed a linear BLM for predicting nickel toxicity to barley with $\log k_{Ca} = 3.30$ and $\log k_{Mg} = 4.60$. Wang et al. (2010) suggested $\log k_{Mg} = 3.72$ and $\log k_K = 2.62$ for zinc toxicity to barley root elongation. According to the selected results from those studies, the stability constants for Ca^{2+} , Mg^{2+} , and K^+ were in the order $\log k_{Mg} > \log k_{Ca} > \log k_K$, that is similar to that in the present study. Thus, the expected potentials of coexistent cations to reduce Cd rhizotoxicity were in the order $Mg^{2+} > Ca^{2+} > K^+$. However, the above implications of linear BLM conflicted with the results shown in Tables 1–3.

In nonlinear BLM, the competition equivalents of Ca^{2+} , Mg^{2+} , and K^+ with Cd^{2+} (n_{Ca} , n_{Mg} , and n_K) were 0.88, 0.76, and 0.99, respectively (Fig. 2c). The competition equivalent is related to the number of ligands near each cation, which is determined by the geometrical constraint. Compared with Mg^{2+} , Ca^{2+} and K^+ binding on biotic

ligands were thus under a lower geometrical constraint. Ca^{2+} and K^+ were also more capable than Mg^{2+} of competing with Cd^{2+} . The logarithmic values of the stability constants ($\log k_{Ca}$, $\log k_{Mg}$, $\log k_K$, and $\log k_{Cd}$) obtained in the nonlinear BLM were 2.51, 2.71, 2.65, and 6.13, respectively (Fig. 2c). This suggested that the affinity of Mg^{2+} absorbed on the biotic ligand was higher than those of Ca^{2+} and K^+ , as in the case of linear BLM. Although Mg^{2+} had superior affinity for biotic ligands, the higher geometrical constraint for Mg^{2+} binding on biotic ligands would result in less alleviation effect on Cd toxicity to the soybean root. The above deduction using the non-linear BLM approach with the two parameters, competition equivalent (e.g. n_{Ca} , n_{Mg} , and n_K) and stability constant (e.g. $\log k_{Ca}$, $\log k_{Mg}$, and $\log k_K$), was consistent with the results shown in Tables 1–3. Therefore, the nonlinear BLM provided more reliable details about the hypothetical mechanism for the alleviation of Cd toxicity with Ca^{2+} , Mg^{2+} , and K^+ . Nevertheless, the hypothetical mechanism for developing non-linear BLM promised well in the present study (Eqs. 7–9) and should need further study cases to be verified.

Reliability of nonlinear BLMs used for RRE and EA_{50} predictions

Such as Figs. 2 and 3, the validations for reliability of using BLM approaches were frequently conducted and promised for estimation of model parameters (Di Toro et al. 2005; Thakali et al. 2006a; Jho et al. 2011). Figure 2 shows a comparison of the RRE predictions using FIAM, linear BLM, and nonlinear BLM. There were some prediction errors in the RRE that exceeded the 15% interval, as shown in Fig. 2a. Compared with the RRE prediction by FIAM, those by linear and nonlinear BLMs more closely matched the observed RRE. In other words, the accuracy of the RRE predictions by linear and nonlinear BLMs was higher than that by FIAM. Moreover, the RMSE values shown in Fig. 2 revealed that the RRE prediction by nonlinear BLM was more precise than that by linear BLM.

Figure 3 shows a comparison of EA_{50} predictions by linear and nonlinear BLMs. The RMSE values suggested that nonlinear BLM provided a more precise EA_{50} prediction than linear BLM. Furthermore, some of the patterns in the Mg-set, namely, the $EA_{50Cd(l)}$ and $EA_{50Cd(nl)}$ predictions, were under the 1:1 line and exceeded a positive deviation of 15%. The dramatically positive deviations in the EA_{50} prediction, especially in the Mg-set, could be attributable to the computational framework given by Eq. (17). The computational framework for the estimation of k_{Ca} , k_{Mg} , and k_K was dominantly governed by the outcomes of the Ca- and K-sets, because the EA_{50} values of the

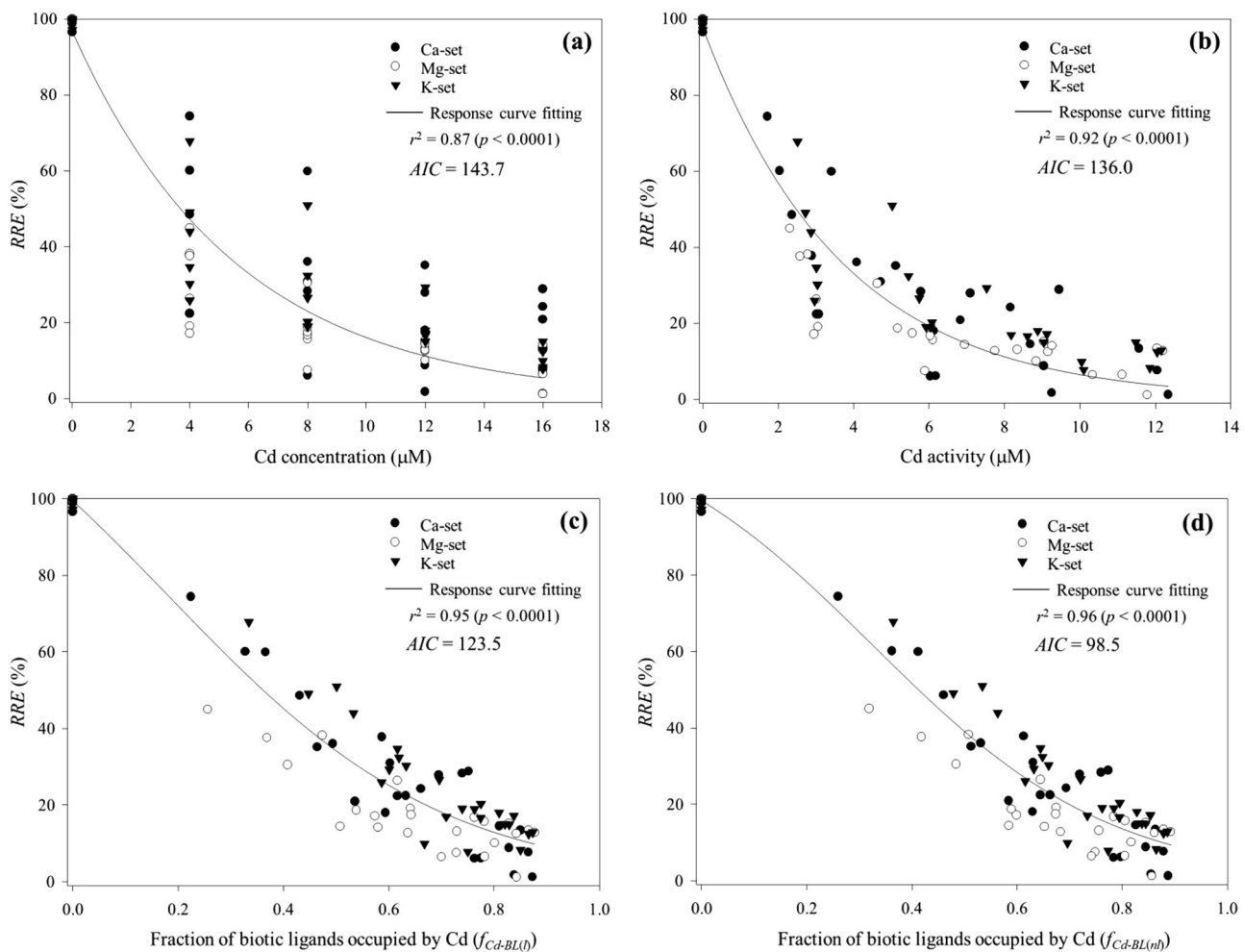


Fig. 4 Dose-response curves of relative root elongation (*RRE*) of soybean seedling vs. **a** Cd^{2+} concentration, $[\text{Cd}^{2+}]$, **b** Cd^{2+} activity, $\{\text{Cd}^{2+}\}$, and **c** and **d** fractions of biotic ligands occupied by Cd^{2+} , $f_{\text{Cd}-\text{BL}(l)}$ and $f_{\text{Cd}-\text{BL}(nl)}$, based on linear and non-linear biotic ligand

models (BLM), respectively. Akaike's information criterion (*AIC*) indicates the goodness of fit for identification of the best response curve fitting

Ca- and K-sets (Tables 1 and 2, respectively) ranged much more widely than did that of the Mg-set (Table 3).

Feasibility of nonlinear BLM used for modeling Cd toxicity to soybean root

To highlight the relative improvement of the nonlinear BLM approach used for modeling the Cd toxicity to the soybean root, an additional comparison of TCM, FIAM, and linear and nonlinear BLMs was conducted. Figure 4 shows the *RREs* of soybean seedlings vs. (a) Cd^{2+} concentration, (b) Cd^{2+} activity, and (c) and (d) fractions of biotic ligands binding Cd^{2+} ($f_{\text{Cd}-\text{BL}(l)}$ and $f_{\text{Cd}-\text{BL}(nl)}$, respectively). In contrast, *RRE* could be expressed reliably as a function of the fraction of biotic ligands binding Cd^{2+} rather than as functions of Cd^{2+} concentration and activity. Linear and nonlinear BLMs were undoubtedly superior

compared to TCM and FIAM for the modeling of the Cd toxicity to the soybean root. In other words, the fraction of biotic ligands binding Cd^{2+} is the factor governing Cd toxicity but not the Cd^{2+} concentration or activity. Moreover, the coefficients of determination (r^2) for *RRE* expressed as functions of $f_{\text{Cd}-\text{BL}(l)}$ and $f_{\text{Cd}-\text{BL}(nl)}$, which are based on linear and nonlinear BLMs, were 0.95 and 0.96, respectively (Fig. 4c, d). The soybean root responses to Cd toxicity were fitted to both linear and nonlinear BLM. However, there were few improvements when using nonlinear BLM based $f_{\text{Cd}-\text{BL}(nl)}$ for modeling the *RRE* with Cd toxicity. Compared with the post patterns of $f_{\text{Cd}-\text{BL}(l)}$, those of $f_{\text{Cd}-\text{BL}(nl)}$ clustered more closely to the fitting line. As shown in Fig. 2, nonlinear BLM provided more precise predictions of *RRE* than did linear BLM. Thus, nonlinear BLM shows good feasibility for modeling the Cd toxicity to soybean root.

Conclusions

Cadmium toxicity to soybean roots was alleviated significantly with increased Ca^{2+} , Mg^{2+} , and K^{+} activities. The amelioration of Cd rhizotoxicity was more intensified by increased Ca^{2+} and K^{+} levels than by increased Mg^{2+} level. The EA_{50} values for Cd toxicity to soybean roots increased positively with Ca^{2+} and Mg^{2+} with a nonlinear relationship and with K^{+} with a linear relationship. Nevertheless, the nonlinear BLM approach, which takes into account the geometrical constraints for binding on biotic ligands, should be considered for assessing the alleviation of Cd rhizotoxicity by the addition of different coexistent cations. Compared with linear BLM, nonlinear BLM showed greater precision for RRE and EA_{50} predictions. In the nonlinear BLM approach, two parameters (competition equivalent and stability constant) could be used to assess the contributions of Ca^{2+} , Mg^{2+} , and K^{+} to the alleviation of Cd rhizotoxicity. It was confirmed that nonlinear BLM improved the modeling of the deviant alleviation of Cd toxicity by Ca^{2+} and Mg^{2+} . Nonlinear BLM thus provides more details about the hypothetical mechanism of the alleviation of metal toxicity by coexistent cations. The nonlinear BLM approach can be used to improve the prediction of metal toxicity to terrestrial plants in specific soil solutions irrespective of the combinations of coexistent cations. Nonlinear BLM can be recommended in priority for prediction of trace metal phytotoxicity in solution given different levels of coexistent Ca^{2+} and Mg^{2+} . Nevertheless, BLM approach is based on the relation between chemical behaviors of dissolved trace metals in a solution phase and stress physiological responses of biota. Trace metal toxicity response of different plant species should be more intensively examined in further studies to investigate the mechanisms involved in the interactions between metal ion and biotic ligand. This type of investigation enables verification of the universality of BLM approach for various organisms on trace metals toxicity.

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Compliance with ethical standards

Conflict of interest Corresponding author, Kai-Wei Juang, has received research grants from the Ministry of Science and Technology, Taiwan. The other authors, declare that they have no competing interests.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

- Antonkiewicz J, Jasiewicz C, Konciewicz-Baran M, Sendor R (2016) Nickel bioaccumulation by the chosen plant species. *Acta Physiol Plant* 38:40
- Antunes PMC, Kreager NJ (2009) Development of the terrestrial biotic ligand model for predicting nickel toxicity to barley (*Hordeum vulgare*): ion effects at low pH. *Environ Toxicol Chem* 28:1704–1710
- Benaissa H, Benguella B (2004) Effect of anions and cations on cadmium sorption kinetics from aqueous solutions by chitin: experimental studies and modeling. *Environ Pollut* 130:157–163
- Borgmann U, Norwood WP, Dixon DG (2004) Re-evaluation of metal bioaccumulation and chronic toxicity in *Hyalella azteca* using saturation curves and the biotic ligand model. *Environ Pollut* 131:469–484
- Burnham KP, Anderson DR (2002) Model selection and inference: a practical information-theoretic approach, (2nd edn). Springer-Verlag, New York, NY
- Campbell PGC (1995) Interactions between trace metals and aquatic organisms: a critique of the free-ion activity model. In: Tessier A, Turner DR (eds) Metal speciation and bioavailability in aquatic systems. John Wiley, New York, NY, p 45–102
- Chai T, Draxler RR (2014) Root mean square error (RMSE) or mean absolute error (MAE)? – Arguments against avoiding RMSE in the literature. *Geosci Model Dev* 7:1247–1250
- Chen BC, Ho PC, Juang KW (2013) Alleviation effects of magnesium on copper toxicity and accumulation in grapevine roots evaluated with biotic ligand models. *Ecotoxicology* 22:174–183
- Chen BC, Lai HY, Juang KW (2012) Model evaluation of plant metal content and biomass yield for the phytoextraction of heavy metals by switchgrass. *Ecotoxicol Environ Saf* 80:393–400
- Chou TS, Chao YY, Huang WD, Hong CY, Kao CH (2011) Effect of magnesium deficiency on antioxidant status and cadmium toxicity in rice seedlings. *J Plant Physiol* 168:1021–1030
- De Schamphelaere KAC, Janssen CR (2002) A biotic ligand model predicting acute copper toxicity for *Daphnia magna*: the effects of calcium, magnesium, sodium, potassium, and pH. *Environ Sci Technol* 36:48–54
- Di Toro DM, Allen HE, Bergman HL, Meyer JS, Paquin PR, Santore RC (2001) Biotic ligand model of the acute toxicity of metals. I. Technical basis. *Environ Toxicol Chem* 20:2383–2396
- Di Toro DM, McGrath JA, Hansen DJ, Berry WJ, Paquin PR, Mathew R, Wu KB, Santore RC (2005) Predicting sediment metal toxicity using a sediment biotic ligand model: methodology and initial application. *Environ Toxicol Chem* 24:2410–2427
- Du Laing G, Rinklebe J, Vandecasteele B, Meers E, Tack FMG (2009) Trace metal behavior in estuarine and riverine floodplain soils and sediments: a review. *Sci Total Environ* 407:3972–3985
- Farzadfar S, Zarinkamar F, Modarres-Sanavy SAM, Hojati M (2013) Exogenously applied calcium alleviates cadmium toxicity in *Matricaria chamomilla* L. plants. *Environ Sci Pollut Res* 20:1413–1422
- Gustafsson JP (2012) Visual MINTEQ ver. 3.0 (<http://vminteq.lwr.kth.se/>)
- Ho PC (2012) Effects of calcium, magnesium and potassium on alleviation of cadmium toxicity to soybean evaluated with bioligand model (BLM). Master thesis, National Chiayi University, Chiayi City, Taiwan
- Jho EH, An J, Nam K (2011) Extended biotic ligand model for prediction of mixture toxicity of Cd and Pb using single metal toxicity data. *Environ Toxicol Chem* 30:1697–1703
- Jopony M, Young S (1993) Assessment of lead availability in soils contaminated by mine spoil. *Plant Soil* 151:273–278

- Kashem MDA, Kawai S (2007) Alleviation of cadmium phytotoxicity by magnesium in Japanese mustard spinach. *Soil Sci Plant Nutr* 53:246–251
- Kinraide TB (1991) Identity of rhizotoxic aluminum species. *Plant Soil* 134:167–178
- Lai HY, Chen ZS (2004) Effects of EDTA on solubility of cadmium, zinc, and lead and their uptake by rainbow pink and vetiver grass. *Chemosphere* 55:421–430
- Le TTY, Peijnenburg WJGM, Hendriks AJ, Vijver MG (2012) Predicting effects of cations on copper toxicity to lettuce (*Lactuca sativa*) by the biotic ligand model. *Environ Toxicol Chem* 31:355–359
- Li HF, Gray C, Mico C, Zhao FJ, McGrath SP (2009) Phytotoxicity and bioavailability of cobalt to plants in a range of soils. *Chemosphere* 75:979–986
- Lock K, De Schamphelaere KAC, Because S, Criel P, Van Eeckhout H, Janssen CR (2007a) Development and validation of a terrestrial biotic ligand model predicting the effect of cobalt on root growth of barley (*Hordeum vulgare*). *Environ Pollut* 147:626–633
- Lock K, Criel P, De Schamphelaere KAC, Van Eeckhout H, Janssen CR (2007b) Influence of calcium, magnesium, sodium, potassium and pH on copper toxicity to barley (*Hordeum vulgare*). *Ecotoxicol Environ Saf* 68:299–304
- Lock K, Van Eeckhout H, De Schamphelaere KAC, Criel P, Janssen CR (2007c) Development of a biotic ligand model (BLM) predicting nickel toxicity to barley (*Hordeum vulgare*). *Chemosphere* 66:1346–1352
- Luo XS, Li LZ, Zhou DM (2008) Effect of cations on copper toxicity to wheat root: implications for the biotic ligand model. *Chemosphere* 73:401–406
- Mei XQ, Li SS, Li QS, Yang YF, Luo X, He BY, Li H, Xu ZM (2014) Sodium chloride salinity reduces Cd uptake by edible amaranth (*Amaranthus mangostanus* L.) via competition for Ca channels. *Ecotoxicol Environ Saf* 105:59–64
- Murata Y, Fujita M, Nakatani T, Obi I, Kakutani T (1998) Effect of Na⁺ on Ca²⁺-binding on the plasma membrane of barley mesophyll cells: an electrophoretic study. *Plant Cell Physiol* 39:452–457
- Nazar R, Iqbal N, Masood A, Khan MIR, Syeed S, Khan NA (2012) Cadmium toxicity in plants and role of mineral nutrients in its alleviation. *Am J Plant Sci* 3:1476–1489
- Niyogi S, Wood CM (2004) Biotic ligand model, a flexible tool for developing site-specific water quality guidelines for metals. *Environ Sci Technol* 38:6177–6192
- Paquin PP, Gorsuch JW, Apte S, Batley GE, Bowles KC, Campbell PGC, Delos CG, Di Toro DM, Dwyer RL, Galvez F, Gensemer RW, Goss GG, Hogstrand C, Janssen CR, McGeer JC, Naddy RB, Playle RC, Santore RC, Schneider U, Stubblefield WA, Wood CM, Wu KB (2002) The biotic ligand model: a historical overview. *Comp Biochem Physiol C* 133:3–35
- Parker DR, Pedler JF (1997) Reevaluating the free-ion activity model of trace metal availability to higher plants. In: Ando T, Fujita K, Mae T, Matsumoto H, Mori S, Sekiya J (eds) *Plant nutrition for sustainable food production and environment*. Kluwer Academic Publ, Dordrecht, p 107–112
- Parker DR, Pedler JF, Ahnstrom ZAS, Resketo M (2001) Reevaluating the free-ion activity model of trace metal toxicity toward higher plants: experimental evidence with copper and zinc. *Environ Toxicol Chem* 20:899–906
- Rao KS, Anand S, Venkateswarlu P (2010) Equilibrium and kinetic studies for Cd(II) adsorption from aqueous solution on *Terminalia catappa* Linn leaf powder biosorbent. *Indian J Chem Technol* 17:329–336
- Santore RC, Di Toro DM, Paquin PR, Allen HE, Meyer JS (2001) Biotic ligand model of the acute toxicity of metals. 2. Application to acute copper toxicity in freshwater fish and Daphnia. *Environ Toxicol Chem* 20:2397–2402
- Siddiqui MH, Al-Whaibi MH, Sakran AM, Basalah MO, Ali HM (2012) Effect of calcium and potassium on antioxidant system of *Vicia faba* L. under cadmium stress. *Int J Mol Sci* 13:6604–6619
- Smith KS, Balistrieri LS, Todd AS (2015) Using biotic ligand models to predict metal toxicity in mineralized. *Appl Geochem* 57:55–72
- Thakali S, Allen HE, Di Toro DM, Ponizovsky AA, Rooney CP, Zhao F-J, McGrath SP (2006a) A terrestrial biotic ligand model. 1. Development and application to Cu and Ni toxicities to barley root elongation in soils. *Environ Sci Technol* 40:7085–7093
- Thakali S, Allen HE, Di Toro DM, Ponizovsky AA, Rooney CP, Zhao F-J, McGrath SP, Criel P, Van Eeckhout H, Janssen CR, Oorts K, Smolders E (2006b) Terrestrial biotic ligand model. 2. Application to Ni and Cu toxicities to plants, invertebrates, and microbes in soil. *Environ Sci Technol* 40:7094–7100
- Tian S, Lu L, Zhang J, Wang K, Brown P, He Z, Liang J, Yang X (2011) Calcium protects roots of *Sedum alfredii* H. against cadmium induced oxidative stress. *Chemosphere* 84:63–69
- Umar S, Diva I, Anjum NA, Iqbal M (2008) Potassium nutrition reduces cadmium accumulation and oxidative burst in mustard (*Brassica campestris* L.). e-*ifc* No. 16
- Wang X, Hua L, Ma Y (2012) A biotic ligand model predicting acute copper toxicity for barley (*Hordeum vulgare*): influence of calcium, magnesium, sodium, potassium and pH. *Chemosphere* 89:89–95
- Wang X, Li B, Ma Y, Hua L (2010) Development of a biotic ligand model for acute zinc toxicity to barley root elongation. *Ecotoxicol Environ Saf* 73:1272–1278
- Wang X, Ma Y, Hua L, McLaughlin MJ (2009) Identification of hydroxyl copper toxicity to barley (*Hordeum vulgare*) root elongation in solution culture. *Environ Toxicol Chem* 28:662–667
- Yang CM, Juang KW (2015) Alleviation effects of calcium and potassium on cadmium rhizotoxicity and absorption by soybean and wheat roots. *J Plant Nutr Soil Sci* 178:748–754
- Zhang H, Young SD (2006) Characterizing the availability of metals in contaminated soils. II. The soil solution. *Soil Use Manag* 21:459–467
- Zhao Z-Q, Zhu Y-G, Li H-Y, Smith SE, Smith FA (2003) Effects of forms and rates of potassium fertilizers on cadmium uptake by two cultivars of spring wheat (*Triticum aestivum*, L.). *Environ Int* 29:973–978