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Modeling of acute cadmium toxicity in solution to barley root elongation using biotic ligand model theory

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ABSTRACT

Protons (H^+) as well as different major and trace elements may inhibit cadmium (Cd) uptake in aquatic organisms and thus alleviate Cd toxicity. However, little is known about such interactions in soil organisms. In this study, the independent effects of the cations calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+), H^+ and zinc (Zn^{2+}) on Cd toxicity were investigated with 5-day long barley root elongation tests in nutrient solutions. The tested concentrations of selected cations and trace metal ions were based on the ranges that occur naturally in soil pore water. The toxicity of Cd decreased with increasing activity of Ca^{2+}, Mg^{2+}, H^+ and Zn^{2+}, but not K^+. Accordingly, conditional binding constants were obtained for the binding of Cd^{2+}, Ca^{2+}, Mg^{2+}, H^+, and Zn^{2+} with the binding ligand: $\log K_{CdBL}$ 5.19, $\log K_{CaBL}$ 2.87, $\log K_{MgBL}$ 2.98, $\log K_{HBL}$ 5.13 and $\log K_{ZnBL}$ 5.42, respectively. Furthermore, it was calculated that on average 29% of the biotic ligand sites needed to be occupied by Cd to induce a 50% decrease in root elongation. Using the estimated constants, a biotic ligand model was successfully developed to predict the Cd toxicity to barley root elongation as a function of solution characteristics. The feasibility and accuracy of its application for predicting Cd toxicity in soils were discussed.

Introduction

Cadmium (Cd), a hazardous heavy metal, can have toxic effects on crop production and on human beings. Cd is widely present in the environment due to human activities, such as mining and smelting, industrial processes and agricultural application of phosphate fertilizers (Carpenè et al., 2006). The bioaccumulation of Cd in edible plants can especially pose risks to human health. Investigations in different plant species have revealed that Cd accumulation in plant tissues can also lead to limitations to the growth and development of plants at a cellular level (Liu et al., 2007). Thus a crucial strategy to alleviate and minimize the adverse biological effects of Cd is to prevent Cd uptake by plant roots (McLaughlin et al., 1999). Over recent years, some researchers have investigated Cd accumulation in the biological environment and the corresponding toxicity in terrestrial biology and related factors. According to a study by Slaveykova et al. (2009) concerning Cd toxicity to the soil bacterium *Sinorhizobium meliloti* in model soil solutions, the Cd uptake by *S. meliloti* was influenced by Cd speciation (free Cd^{2+} activity) and anion competition. Similar results were found in studies on rice (*Oryza sativa* L.) (Kim et al., 2002), durum wheat (*Triticum turgidum*) (Berkelaar and Hale, 2003), maize (*Zea mays*) (Sterckeman et al., 2015), and a bacterium (*Vibrio fischeri*) (An et al., 2012). Therefore, predictive models are required for risk assessment of Cd to estimate and evaluate the speciation and phytotoxicity of Cd under different environmental conditions.

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Recently, a biotic ligand model (BLM) was proposed as a tool to quantitatively evaluate how the speciation and biological availability of metals in aquatic systems is affected by water chemistry (Di Toro et al., 2001). This has attracted increasing attention for the prediction of metal toxicity in terrestrial systems (Antunes et al., 2006; Thakali et al., 2006a,b). In most BLMs developed for metals, major cations are considered as simple competitors for metal binding to uptake sites and may offer some protective effects against potential metal-induced toxicity. Nevertheless, the influence of essential trace elements on metal uptake and toxicity is considered negligible. Hill and Matrone (1970) initially suggested that essential biological interactions can occur among bio-elements and toxic metals with similar physical and chemical properties. Cd has much in common with the bio-element zinc (Zn), with both metals classified in group II B of the post-transition elements of the periodic table. For instance, Cd is commonly found in Zn ores, which are therefore the principal commercial sources of Cd. Numerous experimental results and data show that Zn plays an important role in prevention of Cd toxicity (Kölelia et al., 2004; Rogalska et al., 2011; Li et al., 2014). Therefore, it is important to incorporate the effect of Zn into a Cd-BLM.

The present study therefore aimed to investigate the effects of Zn on Cd toxicity to barley root elongation. Furthermore, the effects of other cations, *i.e.*, calcium (Ca^{2+}), magnesium (Mg^{2+}), protons (H^+) and potassium (K^+), on Cd toxicity to barley root elongation were also evaluated across a wide range of ion levels to obtain the conditional binding constants for Cd^{2+} as well as other cations with biotic ligands (BLs). Finally, a BLM to predict Cd toxicity to barley was established for a broad range of solution characteristics.

1. Materials and methods

1.1. Experimental setup

To determine the independent effects of different cations on Cd toxicity, the concentrations of the target cation were varied during each one-set experiment, while the concentrations of all other cations were kept low and constant (Lock et al., 2007a). Five sets of Cd toxicity tests with different target cations were investigated, Ca, Mg, K, pH and Zn-sets (Table 1). Each set consisted of a series of media in which only the activity of the target cations varied. There were six Cd concentrations plus one treatment without Cd supplementation as a control for all series. The test concentrations of Cd in solution were in the range of 0–0.2 mmol/L. The selected cation concentrations of Ca^{2+}, Mg^{2+}, K^+ and H^+ were based on the ranges that occur in natural pore waters (Oorts et al., 2006). The concentrations of Zn^{2+} ranging from 0 to no observed effect concentrations were selected according to the data of Wang et al. (2010).

1.2. Preparation of the test media

Chemicals of analytical reagent or higher grade were used in all tests. Deionized water was used throughout the experiments. Test solution cultures were prepared by adding different volumes of stock solutions of $CaCl_2$, $MgSO_4$, $ZnSO_4$, NaCl and KCl into deionized water. The solutions were buffered with 1 mmol/L MES (2-[N-morpholino] ethane sulfonic acid) for pH < 7.0 treatments and with 3.6 mmol/L MOPS (3-[N-morpholino] propane sulfonic acid) for pH ≥ 7.0 treatments since MES and MOPS do not form complexes with heavy metals (De Schamphelaere et al., 2004). The pH was then adjusted to the desired level with 1 mol/L NaOH or 1 mol/L HCl solution. Except for the pH-sets, the pH values in the media were always adjusted using MES. The test medium prepared for each bioassay was then used to set up a Cd concentration series by adding different amounts of $CdCl_2$ solution. The pH values of the nutrient solutions were tested before and after the bioassay using a pH meter (Delta 320, Mettler, Zurich, Switzerland). To reach near-equilibrium conditions, all media were prepared and stored in the test pots at 20°C for 1 day before the start of the bioassay. The chemical characteristics of different test solution cultures are summarized in Table 1.

1.3. Toxicity assays

The barley root elongation test was performed according to ISO 11269-1 (ISO, 1993). Barley seeds (*Hordeum. vulgare* cv. Pinggu No. 1) were surface-sterilized in 2% NaClO for 30 min, after which they were thoroughly rinsed with deionized water and germinated on filter paper, which was moistened in advance with deionized water, for 36 hr at 20°C in darkness. After the radicle emerged (<2 mm in length), six seeds were transferred to a nylon net fixed on the surface of plastic culture pots that contained 350 mL of the prepared test solution. The test solution was changed every 24 hr to maintain the correct composition. The culture pots were placed randomly in the

Table 1 – Composition of the test media used in various bioassay sets and the observed EC50{Cd^{2+}} for barley root elongation. EC50{Cd^{2+}} is the free Cd^{2+} that results in 50% RE.

Bioassay set	Varied concentrations and pH values	Characteristics of background solutions	Observed series of EC50{Cd^{2+}}
Ca	0.2, 1.0, 2.0, 4.0, 7.5, 15.0 mmol/L	0.05 mmol/L Mg, 2.5 mmol/L Na, 0.08 mmol/L K, pH 6.0	1.89, 4.33, 6.39, 9.56, 10.71, 12.46 μmol/L
Mg	0.05, 0.2, 0.5, 1.0, 2.0, 4.0 mmol/L	0.2 mmol/L Ca, 2.5 mmol/L Na, 0.08 mmol/L K, pH 6.0	2.26, 2.40, 3.23, 3.65, 4.43, 5.34 μmol/L
K	0.1, 1.0, 3.0, 5.0, 7.5, 10.0 mmol/L	0.2 mmol/L Ca, 0.05 mmol/L Mg, 2.5 mmol/L Na, pH 6.0	2.83, 1.99, 1.71, 2.03, 3.04, 2.10 μmol/L
Zn	0.1, 1.0, 2.5, 5.0 μmol/L	0.2 mmol/L Ca, 0.05 mmol/L Mg, 0.08 mmol/L K, 2.5 mmol/L Na, pH 6.0	2.31, 2.35, 2.41, 3.63, 4.33 μmol/L
pH	5.0, 5.5, 6.0, 6.5, 7.0, 7.3, 7.7, 8.0	0.2 mmol/L Ca, 0.05 mmol/L Mg, 2.5 mmol/L Na, 0.08 mmol/L K	3.77, 2.34, 2.01, 2.06, 2.08, 2.44, 1.44, 1.54 μmol/L

growth chamber. The temperature was maintained at 20°C during the 16 hr light (22 klux)/8 hr dark cycles. Root length was measured after 5 day and the corresponding elongation (RE, %) was calculated and expressed as a percentage of control:

$$RE = \frac{REt}{REc} \times 100 \quad (1)$$

where REt represents root length in the tested medium and REc root length in the control.

1.4. *Chemical measurements*

Atomic absorption spectrophotometry (Varian AA240FS/GTA120; Melbourne, Australia) was used to determine the concentrations of Cd, Ca, Mg, Zn and K.

1.5. *Speciation of Cd in solutions*

Speciation was calculated by WHAM 6.0 (Windermere Humic Aqueous Model) (Lofts and Tipping, 2002). Input data for WHAM were pH values and the concentrations of Cd, Ca, Mg, Zn, K, Cl and SO_4. As experiments were carried out in an open system, an ambient CO_2 partial pressure of 35.5 Pa was assumed for the WHAM calculation.

1.6. *Mathematical description of the BLM and derivation of parameters*

A more detailed description of the method can be found in De Schamphelaere and Janssen (2002). This approach enables the development of BLMs to predict Cu toxicity to wheat and earthworms (Luo et al., 2008; Steenbergen et al., 2005) as well as cobalt (Co) and nickel (Ni) toxicity to barley (Lock et al., 2007b,c).

The methodology for deriving stability constants, as developed by Pagenkopf (1983) and De Schamphelaere and Janssen (2002), is based on the assumption that f_{CdBL} (the fraction (*f*) of the total biotic ligand sites bound by Cd^{2+}) is constant at 50% effect and independent of the composition of major cations and pH of the test medium:

$$EC50\left\{Cd^{2+}\right\} = \frac{f^{50\%}_{CdBL}}{\left(1-f^{50\%}_{CdBL}\right)K_{CdBL}}\left(1+\sum K_{XBL}\left\{X^{n+}\right\}\right) \quad (2)$$

where K_{CdBL} and K_{XBL} are conditional binding constants for the binding of Cd^{2+} and cation X (*e.g.*, Ca^{2+}, Mg^{2+}, Zn^{2+}, K^+ or H^+) to the BL sites (mol/L), respectively, and curly brackets {} indicates the ion activity, for example $\{X^{n+}\}$ presents the activity of X^{n+} (mol/L). {XBL} is the concentration of the specific cation–BL complex (mol/L). $EC50\{Cd^{2+}\}$ is the free Cd^{2+} that results in 50% RE (50% barley root elongation with respect to the control) and $f^{50\%}_{CdBL}$ is the fraction of the BLs that results in 50% RE when occupied by Cd.

Eq. (2) shows that linear relationships should be observed between $EC50\{Cd^{2+}\}$ and the activity of one cation when other cation activities are kept constant, if the BLM concept is correct. The slopes and intercepts of these linear relationships can then be used to derive the conditional binding constants of the competing cations. Consequently K_{CdBL} and $f^{50\%}_{CdBL}$ can be calculated based on the optimization of the logit-transformed effect *versus* f_{CdBL} for varying K_{CdBL}.

2. Results

2.1. *Effects of cations on Cd toxicity*

The EC50 for barley root elongation, expressed as free Cd^{2+} activity, was in the range of 1.44–12.46 μmol/L, which showed a nine-fold increase when the concentrations of other cations increased, except for K^+ (Table 1). For instance, the increase of Ca^{2+} and Mg^{2+} concentrations from 0.16 to 7.37 mmol/L and from 0.04 to 2.0 mmol/L, respectively, resulted in corresponding elevations of $EC50\{Cd^{2+}\}$ by factors of 6.59 and 2.36. For both Ca^{2+} and Mg^{2+}, a linear relationship was found between cation concentration and $EC50\{Cd^{2+}\}$ (Ca^{2+}: $p < 0.01$, $R^2 = 0.83$; Mg^{2+}: $p < 0.01$, $R^2 = 0.94$) (Fig. 1 and Table 1). Similarly to Ca and Mg, increase of Zn^{2+} activity affected the $EC50\{Cd^{2+}\}$ in a linear fashion within the tested Zn^{2+} concentration range ($R^2 = 0.94$) (Fig. 1). In the pH test, observed $EC50\{Cd^{2+}\}$ values were in the range of 1.54–3.77 μmol/L as pH decreased from 8.0 to 5.0 (Fig. 1 and Table 1). These observations clearly indicate that the presence of Ca^{2+}, Mg^{2+}, Zn^{2+} and H^+ ions helped alleviate the Cd^{2+} toxicity, which is in agreement with the assumptions of the BLM concept (Eq. (2)). However, there was no significant impact on $EC50\{Cd^{2+}\}$ when K^+ activity varied (Fig. 1). Therefore, competition between K^+ with Cd^{2+} for binding sites on barley roots was neglected when the BLM was developed, and the values of $\log K_{KBL}$ could be set to zero.

2.2. *Estimation of BLM parameters*

Calculation of the stability constants of Ca^{2+}, Mg^{2+}, Zn^{2+} and H^+ using slopes and intercepts of the linear regressions (Fig. 1), according to De Schamphelaere and Janssen (2002), resulted in $\log K_{CaBL} = 2.87$, $\log K_{MgBL} = 2.98$, $\log K_{ZnBL} = 5.42$ and $\log K_{HBL} = 5.13$, respectively (Table 2). For the final development of the Cd-BLM for barley, another two parameters, K_{CdBL} and $f^{50\%}_{CdBL}$, were also introduced. For all treatments (31 test solutions with six Cd concentrations), the fraction of the BL occupied by Cd was calculated for varying $\log K_{CdBL}$. It was assumed that the best approximation of K_{CdBL} would result in the highest correlation between calculated f_{CuBL} and the logit of the percentage of root elongation of barley. Values for $\log K_{CdBL}$ of 5.19 and the associated $f^{50\%}_{CdBL}$ of 0.29 resulted in the best fit ($R^2 = 0.89$) and thus were retained for the BLM. For comparison purpose, values for $\log K_{CdBL}$ with one log unit difference (4.19 and 6.19) from the best fit (5.19) are also presented (Fig. 2).

2.3. *Validation of BLM*

Finally, the derived Cd-BLM was used in this study to predict the EC50s for the media. The equation of $EC50\{Cd^{2+}\}$ prediction can be expressed as Eq. (3), which is based on Eq. (2):

$$EC50\left\{Cd^{2+}\right\} = \frac{f^{50\%}_{CdBL}\left(1+K_{CaBL}\left\{Ca^{2+}\right\}+K_{MgBL}\left\{Mg^{2+}\right\}+K_{ZnBL}\left\{Zn^{2+}\right\}+K_{HBL}\left\{H^{+}\right\}\right)}{\left(1-f^{50\%}_{CdBL}\right)K_{CdBL}}. \quad (3)$$

In Eq. (3), K^+ was excluded from $EC50\{Cd^{2+}\}$ prediction due to its insignificant effects on $EC50\{Cd^{2+}\}$ values of barley root

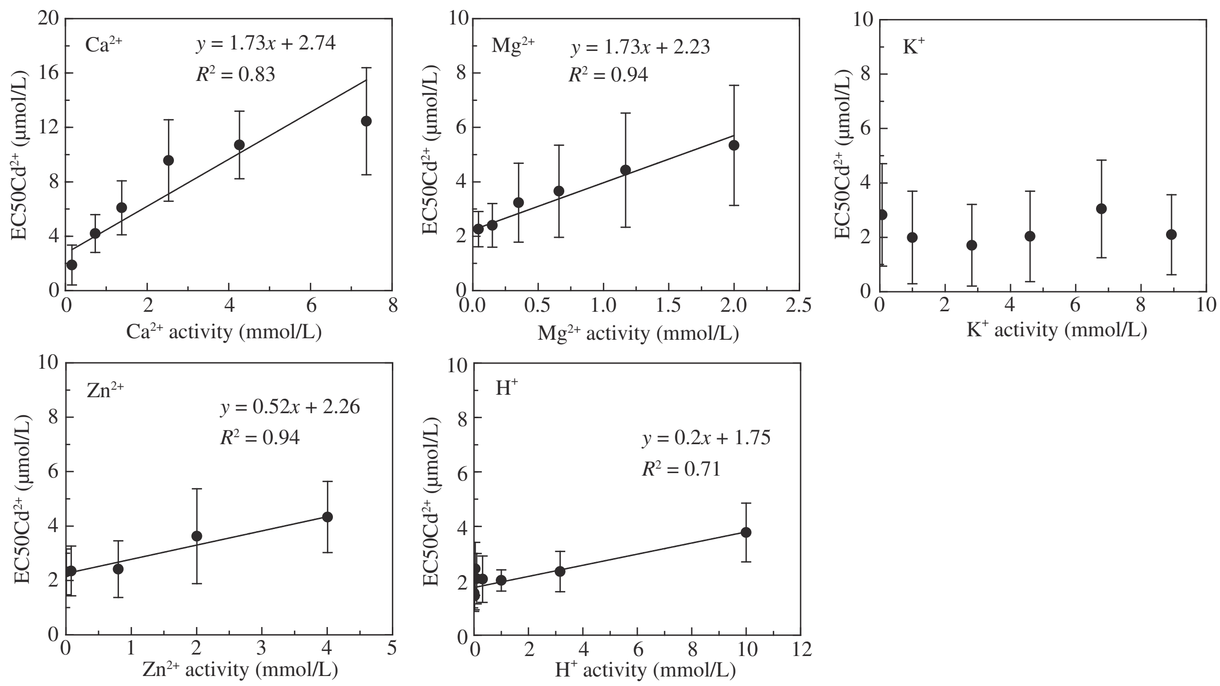

Fig. 1 – The EC50 values expressed as free Cd^{2+} activity (EC50{Cd^{2+}}) for barley root elongation as a function of the free activity of Ca^{2+}, Mg^{2+}, K^+, Zn^{2+}, and H^+. Error bars indicate 95% confidence intervals. Solid lines represent significant correlations.

elongation. The corresponding parameters (K_{CaBL}, K_{MgBL}, K_{ZnBL}, K_{HBL}, K_{CdBL}, and $f^{50\%}_{CdBL}$) are listed in Table 2. Accordingly, EC50{Cd^{2+}} could be predicted when the activities of {Mg^{2+}}, {Ca^{2+}}, {Zn^{2+}}, {H^+} and{Cd^{2+}} were obtained from WHAM. The predicted values of the EC50s by the presented BLM methodology differed from the measured EC50 values by a factor of less than 2.2 (Fig. 3), indicating that the BLM can be used to predict Cd toxicity to barley root elongation.

3. Discussion

In the experimental results, the EC50{Cd^{2+}} for barley root elongation was in the range of 1.44–12.46 μmol/L for all treatments — a nine-fold difference. This result clearly showed the limitations of using free ion activity alone for predicting Cd^{2+} toxicity. However, these differences among the cations can be explained to a major extent by the positive linear relationships between EC50{Cd^{2+}} and activities of cations (Ca^{2+}, Mg^{2+}, H^+ and Zn^{2+}). Many researchers have reported that major cations (*i.e.* Ca^{2+}, Mg^{2+} and K^+) and H^+ have a protective effect against the toxicity of several heavy metals. For instance, Wang et al. (2010) declared that Mg^{2+}, Ca^{2+}, K^+ and H^+ could alleviate Zn toxicity to barley. Song et al. (2014) reported that increased Mg^{2+}, Ca^{2+} and H^+ activity could alleviate chromium (III) toxicity to barley. In the case of Copper (Cu) toxicity, the increase of Mg^{2+} and Ca^{2+} activity could alleviate Cu toxicity to both wheat (*T. aestivum*) (Kinraide et al., 2004; Luo et al., 2008) and barley (Wang et al., 2012). For Cd toxicity, Ardestani and Van Gestel (2013) reported that the level of Cd bioaccumulation in *Folsomia candida* (*Collembola*) decreased with pH increase in soil solution. Additionally, An et al. (2012) studied Cd toxicity to a bacterium (*V. fischeri*) and found that the elevation of Ca^{2+} or Mg^{2+} activities resulted in dramatic increases of EC50{Cd^{2+}}. For terrestrial plants, Mg and Ca were found to reduce the bioaccumulated Cd concentrations in rice and durum wheat roots (Kim et al., 2002; Berkelaar and Hale, 2003). In the present study, protective effects of cations on Cd^{2+} toxicity toward barley were found, confirming the results of Berkelaar and Hale (2003) and Kim et al. (2002). One proposed mechanism to explain the reduced level of Cd absorption and toxicity induced by cations that carry the same charge as Cd, is the displacement of cell-surface Cd^{2+}. The increase of cation concentrations makes the electrical potential less negative at the plasma membrane exterior surface. This reduction decreases the Cd^{2+} activity at the membrane surface, and therefore reduces its uptake (Wang

Table 2 – Parameters of Cd-BLM for Barley (*Hordeum vulgare*) in the present study and for *Vibrio fischeri*, *Sinorhizobium Meliloti* and potworm *Enchytraeus albidus*.

Studied organism		$\log K_{CdBL}$	$\log K_{CaBL}$	$\log K_{MgBL}$	$\log K_{KBL}$	$\log K_{NaBl}$	$\log K_{HBL}$	$\log K_{ZnBL}$	Reference
Bacteria	*Vibrio fischeri*	5.02	2.84	2.19	1.56	–	–	–	An et al. (2012)
	Sinorhizobium Meliloti	4.6	2.4	–	–	–	–	5.9	Slaveykova et al. (2009)
		7.4							
Earthworm	*Eisenia fetida*	4.0	3.35	2.82	2.31	1.57	5.41	–	Li et al. (2008)
Barley	*Hordeum vulgare*	5.19	2.87	2.98	–	–	5.13	5.42	Present study

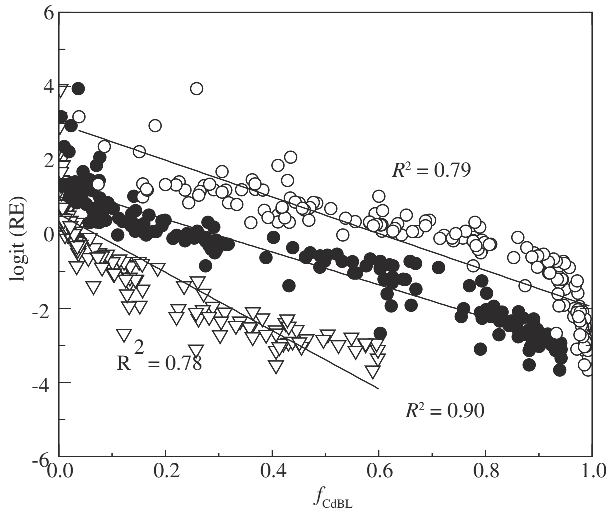

Fig. 2 – Relationship between the logit of the observed percent root growth of *Hordeum vulgare* after 5 days of exposure and the calculated fraction of the biotic ligand sites occupied by Cd (f_{CdBL}) for logK_{CdBL} of 4.19, 5.19 and 6.19, respectively. logit(RE) = ln(RE / (100 − RE)), RE: root elongation.

et al., 2011). Another possible mechanism is that several cations with similar ionic radii to Cd^{2+} may compete with Cd^{2+} for uptake. For instance, the crystal ionic radii of the cations Cd^{2+}, Ca^{2+}, Mg^{2+} and Zn^{2+} are 0.97, 0.99, 0.77 and 0.72 Å, respectively. Although effects of sodium (Na) on Cu toxicity were observed for both *Daphnia magna* (De Schamphelaere and Janssen, 2002) and *fathead minnow* (Erickson et al., 1996) in aquatic ecosystems, Na^+ activity has minor effects on toxicities of most metals to terrestrial plants (Lock et al., 2007a,b,c; Wang et al., 2010; Luo et al., 2008). However, since K^+ activity did not affect the EC50 for barley exposed to Ni^{2+} (Li et al., 2009) and Cu^{2+} (Lock et al., 2007a), it was assumed that K^+ would not affect metal toxicity in the development of most other BLMs. In the present study, Na^+ and K^+ did not affect Cd^{2+} toxicity to barley root elongation.

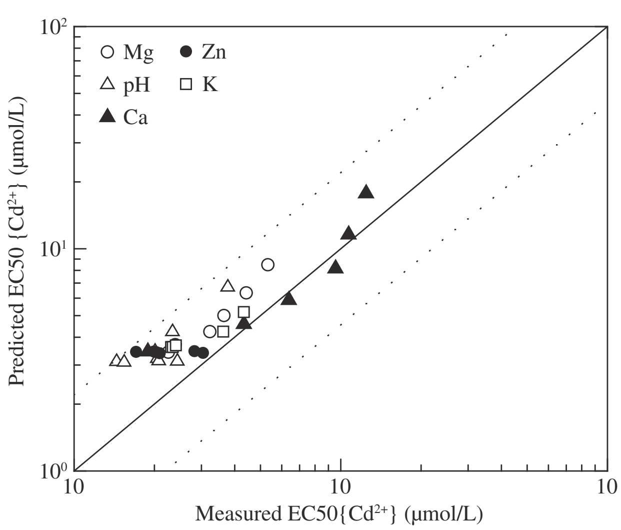

Fig. 3 – Relationship between the measured and predicted values of the EC50{Cd^{2+}} based on the BLM developed in the present study. The solid line indicates a perfect match between measured and predicted values of the EC50{Cd^{2+}}, and the dashed lines indicate the range of the difference, by a factor of 2.2, between the observed and predicted values of the EC50{Cd^{2+}}. Cadmium: Cd; BLM: biotic ligand model.

The conditional binding constants derived in this study for barley root elongation (5-d EC50) of *H. vulgare* were thus compared with those reported for bioluminescence inhibition of a bioluminescent bacterium (*V. fischeri*) (An et al., 2012), for mortality of potworms (*E. fetida*) (Li et al., 2008), and for Cd uptake by a soil bacterium (*S. meliloti*) (Slaveykova et al., 2009) (Table 2). The value of log K_{CdBL} (5.19) obtained in the present study was higher than that reported by Li et al. (2008) in simulated soil solution (log K_{CdBL} = 4.0), but was similar to that reported by An et al. (2012) in culture solutions (log K_{CdBL} = 5.02). Slaveykova et al. (2009) studied the effects of different major and trace elements on Cd uptake by the soil bacterium *S. meliloti*. The results demonstrated that there were two Cd uptake sites with conditional binding constants of log $K_{CdBL,1}$ = 7.4 and log $K_{CdBL,2}$ = 4.6 for *S. meliloti*. Thus the value of log K_{CdBL} (5.19) in the present study was between these two values. The binding constants logK_{CaBL} (2.87) and logK_{MgBL} (2.98) in the present study were also similar to results reported by Slaveykova et al. (2009) (logK_{CaBL} = 2.84, logK_{MgBL} = 2.19) and by Luo et al. (2008) (logK_{CaBL} = 3.35, logK_{MgBL} = 2.82). Many researchers have demonstrated that both Cd and Zn have a high affinity to biological structures compared to other major cations such as Ca and Mg. Moreover, the affinity of Cd should be greater than that of Zn. However, contradictory results were observed in the present study, and the affinity of Zn^{2+} to the BL (logK_{ZnBL} = 5.42) was slightly higher than that of Cd^{2+} (logK_{CdBL} = 5.19). Slaveykova et al. (2009) found that the value of logK_{ZnBL} was about 5.9, which was similar to the result in the present study. Differences in binding constants may, for example, result from different exposure durations, endpoints, target tissues or BLs, or mechanisms of Cd uptake and/or toxicity (Lock et al., 2006). In addition, the nature and dynamic properties of the BL need to be taken into consideration, as the BL (biological membrane) is an important part of a living organism that is very likely to change in response to environmental disturbances such as ionic strength and pH. Therefore further research on Cd is necessary to determine and explain both the differences and similarities across organisms, endpoints and exposure durations.

By using the BLM developed in this study, the EC50s could be predicted relatively accurately within a difference factor of 2.2, which indicates the possibility of using it to predict metal toxicity to terrestrial plants. Generally speaking, Cd contamination is of concern because it tends to accumulate within plant tissues to levels that are toxic to animals (including humans) but not to the plant (Dudka and Miller, 1999). Therefore it is important to study the role of plants in Cd-contaminated systems and their subsequent impacts on the health of animals (including humans). In addition, this study was based only on nutrient solutions, and did not include other factors, such as metal solid–liquid distribution, microbial activity and root exudates, which can also accumulate in the rhizosphere and affect Cd toxicity to plants (Antunes et al., 2006). Consequently, the potential applications of the presented methodology and results are limited to soil solutions. Thus, further research is required to refine such a semi-mechanistic model for application in soil environments.

4. Conclusions

In this study, a BLM was developed for predicting the toxicity of Cd to barley (*H. vulgare*) in nutrient solutions. It was first demonstrated that Cd^{2+} was a toxic species and that its competition with Mg^{2+} Ca^{2+}, Zn^{2+} and H^{+} for binding sites of the BL should be incorporated into the BLM. Accordingly the BLM parameters were derived and validated and the developed BLM demonstrated good performance in predicting acute Cd toxicity toward barley root elongation. The difference between the predicted and measured toxicities was by a factor of less than 2.2. The BLM, therefore, may be a promising tool for improving the ecological relevancy of risk assessment procedures for divalent metals in water and soil environments. However, further research on the refinement of such a tool is necessary for potential applications on various natural/field soils with broad ranges of properties, before BLMs can be used for risk assessment of metal-contaminated soils in the field.

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