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Prediction of Cd and Pb toxicity to *Vibrio fischeri* using biotic ligand-based models in soil

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ABSTRACT

Biotic ligand-based models to predict site-specific toxicity of Cd and Pb contaminated soil were developed by using a *Vibrio fischeri* toxicity test. Firstly, competition effect by cations (i.e., Ca, Mg, K) commonly found in soil solution was incorporated into the models. For this purpose, biotic ligand-based model parameters including conditional binding constants of cations and metal ions to binding sites (i.e., biotic ligands) and the fractions of binding sites occupied by the metal ions were determined. Data from aqueous phase toxicity test showed that the difference between model-predicted EC_{50} values of Cd and Pb and experimentally determined EC_{50} values ranged within a factor of two, suggesting that the developed model parameters were reliable. Secondly, the use of soil solution to predict soil toxicity of Cd and Pb was experimentally verified with freshly spiked and field-aged soils. The results showed linear relationships in both soils, meaning that toxicity of soil solution can be representative of toxicity of soil. Finally, applicability of the developed models in Cd- or Pb-spiked soils was investigated by comparing predicted toxic effects (i.e., % bioluminescence inhibition at given cations and metal activities in soil solution) and experimentally obtained toxic effects determined by Microtox® solid phase toxicity test. Our data demonstrate that toxicity of Cd- or Pb-contaminated soil can be predicted by using the developed biotic ligand-based model with the chemical analysis data of soil solution as input data.

1. Introduction

It is widely recognized that total metal concentration in soil is not commensurate with the available fractions to soil biota [1,2]. This phenomenon occurs mainly due to the complex interactions of metals with soil constituents (e.g., organic carbon, clay) and soil solution (e.g., dissolved organic carbon, pH, cations/anions), which commonly result in altered bioavailability. Such altered bioavailability may require site-specific soil quality criteria that take into account soil physic-chemical properties and pollution history of a site of interest. Therefore, determination of the site-specific bioavailability and toxicity of metals-contaminated soil is crucial for realistic risk assessment.

An appropriate assessment of metal toxicity in soil has received increasing attention, and some readily available tools exist. Measure of metal toxicity in soil may be accomplished by indirect chemical means or direct biological means [3]. Biological methods (e.g., bioaccumulation or toxicity tests of plants and soil invertebrates) are reliable and realistic in determining metals toxicity in soil, but they are time-consuming and costly. As an alternative, chemical methods mimicking biological response were proposed, including solvent extraction [3,4], solid phase extraction [5], and solid-phase microextraction fibers [6]. For these methods to be valid, however, a strong correlation between the chemically recovered concentration and toxicity should be established, which is a very difficult goal to attain [3].

Biotic ligand model was developed and widely used to predict the metal toxicity in water bodies [7,8]. Recently, terrestrial biotic ligand model (TBLM) [9,10] modified from biotic ligand model [7] has been proposed as a promising tool for assessing metal toxicity in soil. It is a semi-mathematical model that predicts soil toxicity of metals by using porewater chemistry (i.e., pH, dissolved organic carbon, concentrations of cations and anions) [10,11]. TBLM supposes that metal ions adsorb to soil matrix and those present in porewater exist in an equilibrium state, and free metal ions in porewater bind to the active sites (i.e., biotic ligands; BL) of organisms, causing toxic effect. In addition, it is assumed that major cations (e.g., Ca, Mg, K) present in porewater compete with free metal ions for the BL sites and such competition mitigates the toxicity of free metal ions.

In the present study, biotic ligand-based models to predict the toxicity of Cd and Pb in soil were developed. Although Cu- and Ni-TBLMs have been thoroughly studied [10,12–14], to our knowledge, few biotic ligand-based models are available for Cd and Pb.

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Specifically, parameters (i.e., K_{CaBL}, K_{MgBL}, K_{KBL}, K_{MBL} and $f_{MBL}^{50\%}$) of biotic ligand-based models were determined by using aqueous phase toxicity test over a wide range of cations using the bioluminescent bacterium *Vibrio fischeri*. In order to apply the developed models to soil, metal toxicity in soil and soil solution was compared, which enables this prediction process fast and cost-effective.

2. Materials and methods

2.1. Toxicity test

The toxicity of Cd and Pb was evaluated using the bioluminescence bacterium *V. fischeri*. It was purchased as a freeze-dried form (SDI, Carlsbad, CA) and activated with reconstitution solution. Because *V. fischeri* is a marine organism, osmotic pressure of the samples had to be adjusted to 2.0% salinity using a concentrated salt solution throughout the aqueous phase experiments. The bioluminescence intensities emitted by *V. fischeri* in the control and the test samples after 5 min of exposure to Cd and Pb were detected by Microtox® 500 Analyzer (SDI, Carlsbad, CA), and the differences were used to calculate the toxicity of the metals under the given metal concentration.

$$\text{Inhibition } (\%) = \left(1 - \frac{I_t}{I_0 R_t}\right) \times 100 \quad (1)$$

where R_t is the correction factor obtained when the bioluminescence intensity of the control remaining after time t is divided by the initial intensity of the control, I_t is the bioluminescence intensity of individual samples after time t and I_0 is the initial bioluminescence intensity of samples. The percent of inhibition from Eq. (1) was calculated using the Microtox Omni software.

The changes of bioluminescence inhibition with the changes in the concentrations of Cd and Pb were fitted to the sigmoidal concentration–response curve [15] using Sigma Plot 8.02 to calculate EC_{50} values (i.e., effective concentration causing 50% inhibition on the bioluminescence of *V. fischeri*).

$$y = y_0 + \frac{a}{1 + e^{-(x - x_0)/b}} \quad (2)$$

where y is the observed inhibition of bioluminescence, x is the natural logarithm of an exposed metal concentration, x_0 is the logarithm of the concentration at which the bioluminescence is half the uninhibited level, y_0 is the stochastic error term, a is the uninhibited bioluminescence level, and b is the slope parameter related to inhibition rate.

2.2. Effect of cations competition on metal toxicity

In order to investigate the individual effect of cations (i.e., Ca, Mg, K) existing in solution on Cd and Pb toxicity, various concentrations of each cation were tested while the concentrations of the other cations were kept constant [13,16,19]. Both Cd and Pb toxicity tests contained three experimental sets of Ca-set, Mg-set, and K-set and each set was composed of a series of media having four different cation concentrations (i.e., 0.250, 2.50, 12.5, and 25.0 mM). Each test was conducted following the Extended 9 Dilution Test Method [20] with some modifications to maintain the concentrations of cations in solution the same during the serial dilution step. Stock solutions of 10 mM Cd (pH 5.63) and 0.1 mM Pb (pH 5.81) were prepared by dissolving cadmium chloride, 2.5-hydrate ($CdCl_2 \cdot 2.5H_2O$) and lead chloride ($PbCl_2$) (Wako Pure Chemical Industries, Osaka, Japan), respectively. All chemicals used in this study were reagent grades. The initial concentrations of Cd and Pb in test samples were continuously diluted following the Extended 9 Dilution Test Method, to have the final concentrations of 2.25, 1.13, 0.560, 0.280, 0.140, 0.070, 0.040, 0.020, 0.010 and 0 mM for Cd and 22.5, 11.3, 5.60, 2.80, 1.40, 0.700, 0.350, 0.180, 0.090 and 0 μM for Pb. Chemical composition of each set is presented in Table 1. Chemical speciation calculations were conducted by WinhumicV software [17,18]. Input data included temperature, pH, and the concentrations of Cd or Pb, Mg, Ca, K, Na and Cl. An equilibrium state between inorganic carbon concentration in the aqueous phase and atmospheric CO_2 was assumed. The activities of free Cd and Pb ions were calculated by using the input data for each test solution, and the previously obtained EC_{50} values were recalculated to express as free metal ion activities.

Analysis of variance (ANOVA) was performed using Microsoft Excel 2007 Analysis ToolPak to evaluate whether or not cations competition significantly influenced the toxicity of Cd and Pb.

2.3. Mathematical description and derivation of parameters

Metals and cations present in solution react with BL sites (i.e., active binding sites located in negative charged cell surface membrane) of an organism. The overall process can be expressed with the conditional binding constant as follows.

$$K_{MBL} = \frac{[MBL^+]}{\{M^{2+}\}[BL^-]} \quad (3)$$

where $[MBL^+]$ is the concentration of metal-*BL* complex (mol/L), $\{M^{2+}\}$ is the activity of free metal ion (mol/L), $[BL^-]$ is the concentration of unoccupied *BL* sites (mol/L), and K_{MBL} is the conditional binding constant for metal bound to *BL* sites (L/mol). The BL sites can be occupied by competing cations (i.e., Ca^{2+}, Mg^{2+}, K^+), and the complexation reaction is expressed in Eq. (3). The concentration of the total BL sites ($[TBL]$) can be expressed as Eq. (4), and substituting Eq. (4) with Eq. (3) yields Eq. (5).

$$[TBL] = [BL^-] + [CaBL^+] + [MgBL^+] + [KBL] + [MBL^+] \quad (4)$$

$$[TBL] = [BL^-](1 + K_{CaBL}\{Ca^{2+}\} + K_{MgBL}\{Mg^{2+}\} + K_{KBL}\{K^+\} + K_{MBL}\{M^{2+}\}) \quad (5)$$

The fraction of *BL* sites occupied by metals (f) is proportional to the toxicity imposed on an organism followed by the main hypothesis of the BLM (Eq. (6)) [21]. The EC_{50} values can be expressed as follows.

$$f = \frac{[MBL^+]}{[TBL]} = \frac{K_{MBL}\{M^{2+}\}}{1 + K_{CaBL}\{Ca^{2+}\} + K_{MgBL}\{Mg^{2+}\} + K_{KBL}\{K^+\} + K_{MBL}\{M^{2+}\}} \quad (6)$$

$$EC_{50}\{M^{2+}\} = \frac{f_{MBL}^{50\%}(1 + K_{CaBL}\{Ca^{2+}\} + K_{MgBL}\{Mg^{2+}\} + K_{KBL}\{K^+\})}{(1 - f_{MBL}^{50\%})K_{MBL}} \quad (7)$$

where $EC_{50}\{M^{2+}\}$ is the free metal ion activity resulting in 50% bioluminescence inhibition of *V. fischeri* and $f_{MBL}^{50\%}$ is the *BL* sites needed to be occupied by a free metal ion to cause 50% bioluminescence inhibition. Conditional binding constants of competing cations (i.e., K_{CaBL}, K_{MgBL}, K_{KBL}) were derived from the slopes and intercepts of the linear relationships using Eq. (7). The K_{MBL} and $f_{MBL}^{50\%}$ were determined by optimizing the linear relationship between the logit-transformed toxic effect (i.e., bioluminescence inhibition) and the fraction of *BL* sites occupied by metals while changing K_{MBL} values [13,19].

Table 1
Chemical composition of the test media used for toxicity test and experimentally determined 5-min EC_{50} values[a] for *Vibrio fischeri*.

Bioassay set	Ca^{2+} [mM]	Mg^{2+} [mM]	K^+ [mM]	EC_{50} as $[Cd_T]$[b] [μM]	EC_{50} as $[Pb_T]$[b] [μM]
Control	0.025	0.025	0.025	259 (±34.0)	6.78 (±0.686)
Ca	0.25	0.025	0.025	370 (±108)	4.59 (±1.19)
	2.50	0.025	0.025	575 (±97.0)	7.13 (±0.922)
	12.5	0.025	0.025	1160 (±323)	8.73 (±3.83)
	25.0	0.025	0.025	2160 (±511)	15.6 (±0.950)
Mg	0.025	0.25	0.025	659 (±145)	8.36 (±2.29)
	0.025	2.50	0.025	835 (±54.0)	10.8 (±2.33)
	0.025	12.5	0.025	1190 (±61.0)	13.9 (±1.27)
	0.025	25.0	0.025	1230 (±185)	17.5 (±4.23)
K	0.025	0.025	0.25	414 (±92.0)	3.94 (±0.613)
	0.025	0.025	2.50	351 (±52.0)	2.61 (±0.322)
	0.025	0.025	12.5	420 (±59.0)	3.39 (±1.10)
	0.025	0.025	25.0	578 (±80.0)	3.90 (±0.481)

[a] The EC_{50} values were expressed as means ± standard deviations ($n = 3$).
[b] $[Cd_T]$ and $[Pb_T]$ represent total dissolved concentrations in solution.

Table 2
Physicochemical properties and metal concentrations of the soil samples used.

Soils	pH (1:5)	CEC (cmol/kg)	Organic matter (%)	Soil texture	Metal concentration (mg/kg)[a]			
					Cd	Pb	As	Cu
Cd-spiked soil	5.12	10.9	1.08	Sandy loam	2810	NA[b]	NA	NA
Pb-spiked soil	4.57	11.6	1.16	Sandy loam	NA	175	NA	NA
Field-aged soil A	5.71	3.12	2.24	Sand	3.3	44.6	121.2	35.1
Field-aged soil B	5.23	2.96	1.42	Sand	2.8	20.0	81.7	51.2

[a] Soils were digested by aqua regia ($HCl:HNO_3 = 3:1$, v/v).
[b] NA = not applicable.

2.4. Comparison of toxic effect between soil and soil solution

Solid and aqueous phase toxicity tests were conducted with field soil samples contaminated with various metals for many decades (i.e., field-aged) and model soil samples freshly spiked with either Cd or Pb for one week. Soils were dried at room temperature and sieved to remove gravels and debris larger than 2 mm in diameter. Total concentrations of metals in soils were determined by flame atomic absorbance spectrophotometer (SpectrAA880, Varian, Australia), following digestion with aqua regia (i.e., soil samples in 15 mL concentrated HCl and 5 mL concentrated HNO_3 were heated at 90 °C until near-dry and diluted with 20 mL of deionized water). Soil pH was determined in a mixture of 1:5 soil:water (w/v). Soil organic matter content and cation exchange capacity (CEC) were determined with the loss-on-ignition and the compulsive exchange method, respectively [22]. The properties and metal concentrations of soils are presented in Table 2.

Each soil was stirred in a 2.0% NaCl solution for 1 h maintaining 1:5 solid to liquid ratio (g/mL). The 2 mL of suspension was used as an initial exposure medium to determine soil toxicity (i.e., solid phase toxicity test) of Cd or Pb after 5 min of exposure. Then, the remaining suspension was settled down for 5 min and the supernatant was filtered through a 0.45-μm GHP syringe filter (Pall Corporation, Port Washington, NY). This solution defined as 'soil solution' in the present study was also used as an initial exposure medium for the aqueous phase toxicity test. The toxicities between the two exposure media were compared, and a correlation analysis and Student's *t*-test were performed using Microsoft Excel 2007 Analysis ToolPak.

2.5. Applicability of developed models to soil

Using the parameters derived using Eq. (7), the toxic effect (R) of Pb or Cd to *V. fischeri* (i.e., % bioluminescence inhibition at given cations and metal activities) was calculated with Eq. (8).

$$R = \frac{100}{1 + (f/f_{50})^{\beta}} \tag{8}$$

where f is fraction of BL sites occupied by metal ions defined in Eq. (6), f_{50} is identical to previously described $f_{MBL}^{50\%}$, and β is the fitting parameter determining the shape of the dose–response curve.

To determine the f value, the conditional binding constants of the competing cations and the metal of interest (i.e., K_{CaBL}, K_{MgBL}, K_{KBL}, K_{CdBL} or K_{PbBL}) determined as above and the activities of the competing cations and the free metal ions in solution estimated from the aqueous phase toxicity tests were substituted in Eq. (6).

$$R = \frac{100}{1 + (K_{MBL}\{M^{2+}\}/(f_{50}(1 + K_{MBL}\{M^{2+}\} + K_{MgBL}\{Mg^{2+}\} + K_{CaBL}\{Ca^{2+}\} + K_{KBL}\{K^{+}\})))^{\beta}} \tag{9}$$

By substituting the experimentally determined toxic effect ($R_{determined}$) and the corresponding activities of the free metal ions and the competing cations in Eq. (9), the parameters β and f_{50} were derived. From the established Eq. (9), toxic effects of another soils can then be calculated by measuring the activities of the competing cations and the metal of interest in the soils. The toxic effect value (R) calculated from Eq. (9) is more meaningful and site-specific in that it represents both the activity of free metal ions and the activities of competing cations whereas Eq. (7) considers only the activities of the competing cations. The experimentally determined toxic effects of Pb- or Cd-spiked soils by the solid phase toxicity test and the predicted toxic effects using the chemical composition of the soil solution (Table 3) were compared to validate the developed models.

The metal and cations concentrations in soil solutions were determined by ICP-AES (Optima 4300DV, Perkin-Elmer, USA). The concentration of dissolved organic carbon was determined using the TOC Analyzer (V-CPH, Shimadzu, Japan). The activities of metal and cations in soil solutions calculated using the WinhumicV speciation model were substituted into Eq. (9). The concentration of

Table 3
Chemical composition of the soil solutions obtained from freshly spiked soil samples used.

Soils	pH	DOC (mg/L)	Concentration of metal and cations (mg/L)				
			Cd	Pb	Ca	Mg	K
Cd-spiked soil	6.55	14.5	536	ND[a]	221	70.0	24.5
Pb-spiked soil	6.17	2.14	ND	34.3	885	439	24.3

[a] ND = not detected.

dissolved organic carbon in soil solutions was used as an input data when the activities of free metal ions were calculated with the WinhumicV model.

3. Results and discussion

3.1. Effect of cations competition on Cd and Pb toxicity to V. fischeri

Many researchers have reported protective effect of major cations (i.e., Ca, Mg, K) on the toxicity of Cu, Ni, Ag and Co [13,16,19,23,24], and hence the concentration of the cations in background solutions is a well known factor reducing toxicity of metals in soil. Such toxicity-diminishing effect by some common cations was also evident for Cd and Pb in this study. The total dissolved concentration of a metal causing 50% inhibition on the bioluminescence of *V. fischeri* after 5 min of exposure (i.e., $EC_{50}[M_T]$) was determined and recalculated as $EC_{50}\{M^{2+}\}$ to express as the free Cd or Pb ion activity instead of the concentration. Strong linear relationships between Ca^{2+} or Mg^{2+} activities and EC_{50} values were found (Fig. 1), indicating the decreasing Cd or Pb toxicity at higher Ca^{2+} or Mg^{2+} activities. The elevation of Ca^{2+} or Mg^{2+} activities resulted in drastic increases of $EC_{50}\{Cd^{2+}\}$ and $EC_{50}\{Pb^{2+}\}$. $EC_{50}\{Cd^{2+}\}$ varied from 4.64 to 33.2 μM or 18.8 μM as Ca^{2+} or Mg^{2+} activity increased from 0.007 to 6.63 mM, respectively. Meanwhile, $EC_{50}\{Pb^{2+}\}$ varied from 0.339 to 0.679 μM or 0.762 μM when Ca^{2+} or Mg^{2+} activity increased from 0.007 to 6.63 mM, respectively (Fig. 1). A slight increase in $EC_{50}\{Cd^{2+}\}$ was observed as K^+ activity increases, however, no significant change was evident between the Pb toxicity and the K^+ activity. Similarly, Kim et al. [25] observed that the increases in the Ca and Mg concentrations in solution decreased the bioaccumulated concentrations of Cd and Pb in rice root, but K did not diminish the Cd and Pb bioaccumulation.

ANOVA was performed to evaluate whether or not the toxicity-protective effect of the competing cations was the strongest factor controlling the change in the observed $EC_{50}\{M^{2+}\}$. When p value of the ANOVA test with a cation set data was larger than 0.05, the protective effect by the cation was disregarded. The ANOVA data showed that all the cations studied (i.e., Ca, Mg, and K) had competing effects for Cd while only Ca and Mg showed toxicity inhibition for Pb. Therefore, K was excluded from Eq. (7) for $EC_{50}\{Pb^{2+}\}$ calculation.

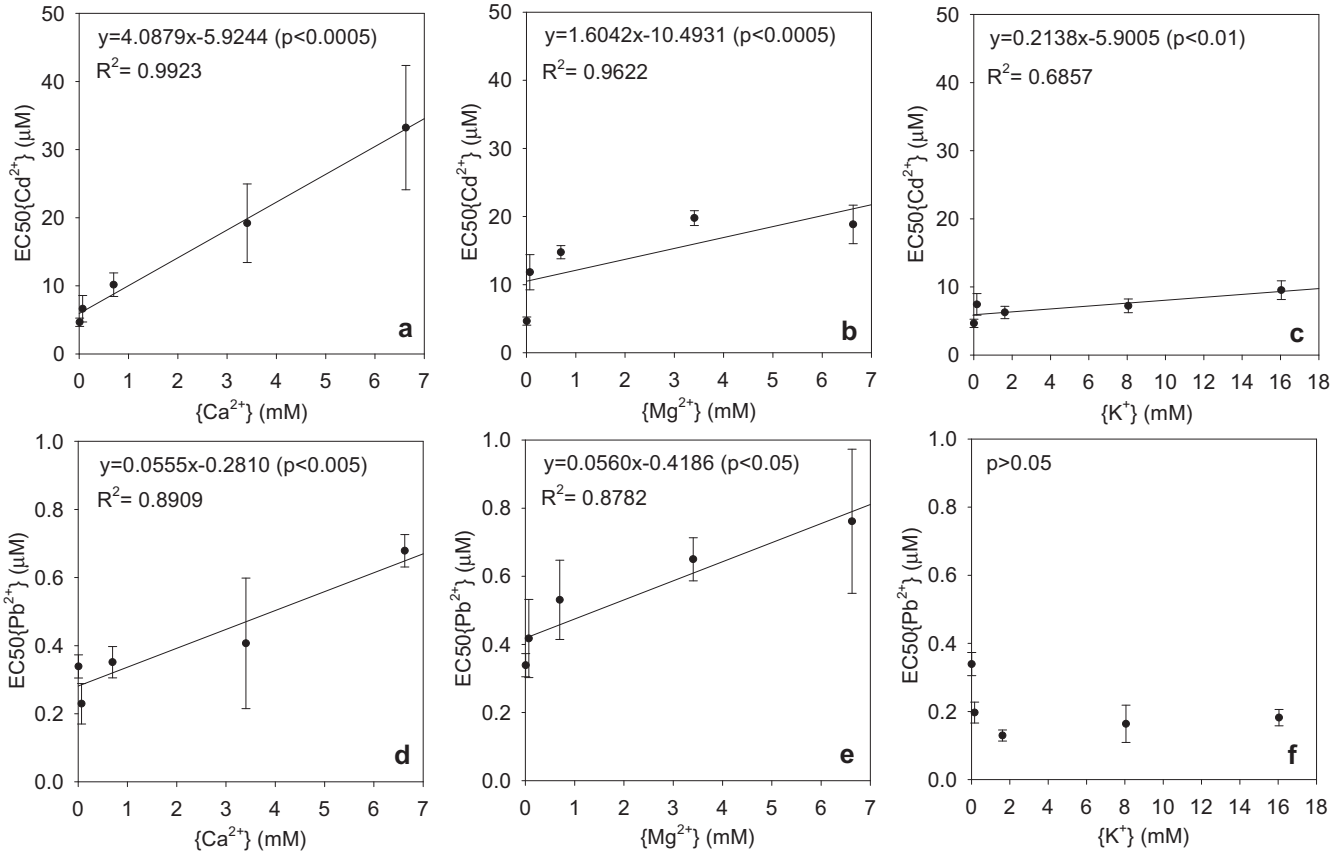

Fig. 1. Effects of competing cations activities on $EC_{50}\{Cd^{2+}\}$ (panels a–c) and $EC_{50}\{Pb^{2+}\}$ (panels d–f). Error bars indicate standard deviations (n = 3) and solid lines represent linear regression curves passed through ANOVA.

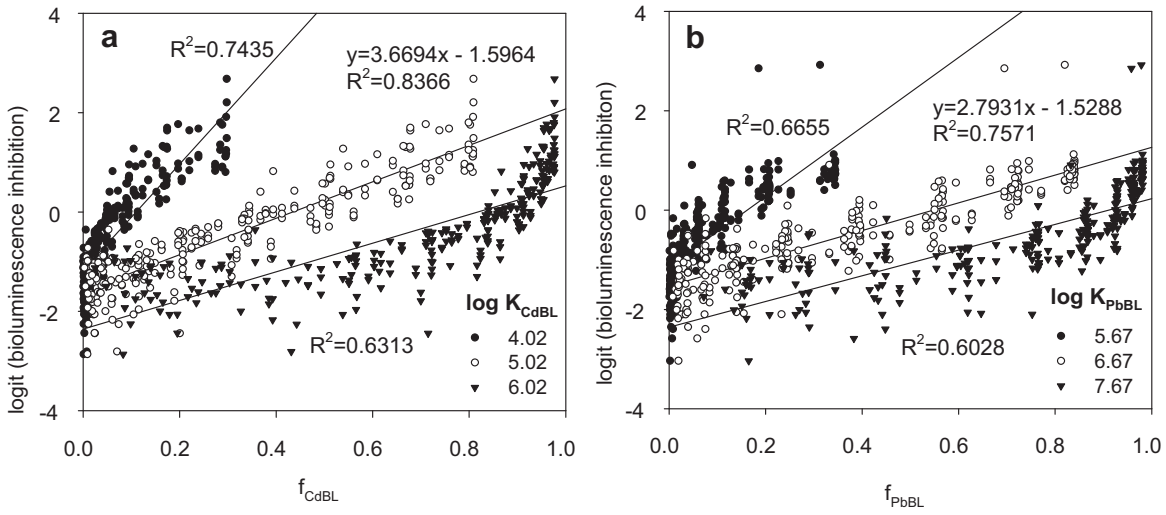

Fig. 2. Linear relationships between the logit of the measured inhibition of bioluminescence of *V. fischeri* after 5-min exposure and the calculated fraction of *BL* sites occupied by Cd (f_{CdBL}) and Pb (f_{PbBL}). Open circles in each panel show the best approximation results: (a) For Cd, $\log K_{CdBL}$ is determined to be 5.02 and the corresponding f_{CdBL} is 0.435 and (b) For Pb, $\log K_{PbBL}$ is determined to be 6.67 and the corresponding f_{PbBL} is 0.547.

3.2. Determination of model parameters

In order to derive the conditional binding constants (i.e., K_{CaBL}, K_{MgBL}, K_{KBL}) of competing cations, the slopes and the intercepts of regression curves were used [19]. The determined values were $\log K_{CaBL}$ of 2.84, $\log K_{MgBL}$ of 2.19, and $\log K_{KBL}$ of 1.56 for Cd. For Pb, they were $\log K_{CaBL}$ of 2.30 and $\log K_{MgBL}$ of 2.13. Since the constants indicate the binding affinity of metals to active binding sites (i.e., *BL* sites) in the membrane of an organism, the results suggest that Ca exhibits greater competing effects than Mg for Cd. However, a significant competing effect on Pb toxicity by the ions was not evident. The values of $f_{CdBL}^{50\%}$ and $f_{PbBL}^{50\%}$ were also calculated with the best approximated conditional binding constants (i.e., K_{CdBL} and K_{PbBL}) as described elsewhere [13,16,19], which resulted in the highest correlation between the calculated f_{CdBL} and f_{PbBL} from Eq. (6) and the logit transformation of the experimentally determined inhibition of *V. fischeri* bioluminescence (Fig. 2). The $\log K_{CdBL}$ of 5.02 and $f_{CdBL}^{50\%}$ of 0.435 were determined, meaning that 43.5% of the *BL* sites needs to be occupied by Cd to cause a 50% inhibition effect after 5 min exposure. The $\log K_{PbBL}$ of 6.67 and $f_{PbBL}^{50\%}$ of 0.547 were derived for Pb.

Our results showed that the conditional binding constants of metals were larger than those of competing cations, and similar tendency was also observed by other researchers. The conditional binding constants of Cd ($\log K_{CdBL}$ = 4.0), Ca ($\log K_{CaBL}$ = 3.35), Mg ($\log K_{MgBL}$ = 2.82), and K ($\log K_{KBL}$ = 2.31) for earthworm *Eisenia fetida* were reported [26]. In their study with barley *Hordeum vulgare*, Wang et al. [27] reported the conditional binding constants of Zn ($\log K_{ZnBL}$ = 5.02), Ca ($\log K_{CaBL}$ = 1.99), Mg ($\log K_{MgBL}$ = 3.72), and K ($\log K_{KBL}$ = 2.62).

The EC_{50} prediction models for Cd and Pb developed as described above were shown in Eqs. (10) and (11). In order to verify the model parameters, the experimentally determined EC_{50} values and model-predicted EC_{50} values were compared. The differences between the two sets of EC_{50} values for Cd and Pb were within a factor of two (Fig. 3), suggesting that the developed model parameters were reliable for site-specific toxicity prediction.

$$EC_{50}\{Cd^{2+}\} = 7.35 \times 10^{-6} + 5.08 \times 10^{-3}\{Ca^{2+}\} + 1.13 \times 10^{-3}\{Mg^{2+}\} + 2.68 \times 10^{-4}\{K^{+}\} \quad (10)$$

$$EC_{50}\{Pb^{2+}\} = 2.58 \times 10^{-7} + 5.10 \times 10^{-5}\{Ca^{2+}\} + 3.46 \times 10^{-5}\{Mg^{2+}\} \quad (11)$$

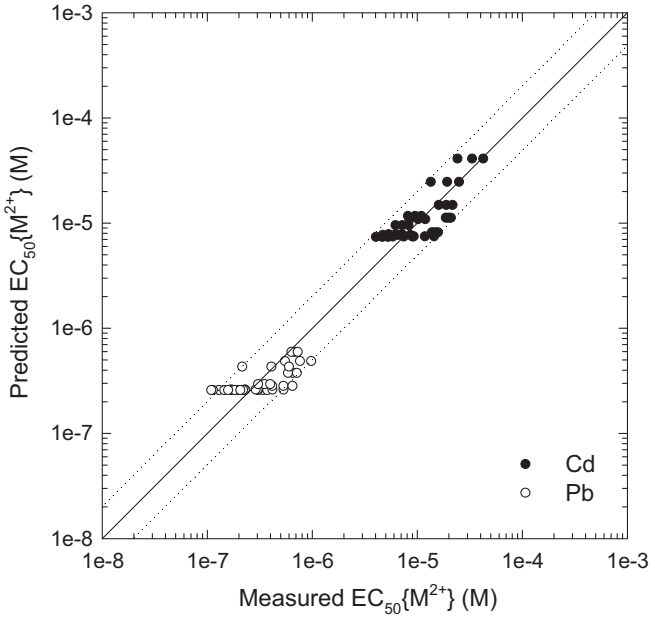

Fig. 3. Linear relationship between the measured EC_{50} using *V. fischeri* toxicity test expressed as free ion activities of Cd or Pb and the predicted EC_{50} from the developed models. The solid line indicates a perfect match between the measured and predicted EC_{50} values, and the dashed lines indicate a factor of two differences between the measured and predicted values.

3.3. Toxicological relationship between soil and soil solution

The toxicities in the soil and the soil solution were compared to confirm whether the biotic ligand-based model determined from the aqueous phase toxicity tests was able to be applied to predict toxicities of Cd and Pb in soil to *V. fischeri*.

Because the TBLM has a hypothesis that the only dissolved fraction of metals in the soil might exhibit toxic effect to the biota such as microorganisms, porewater recovery from the soil and its analysis are required to apply the TBLM to predict metals toxicity with consideration of the soil properties in a contaminated site. However, obtaining porewater from soil is a tedious process, and the

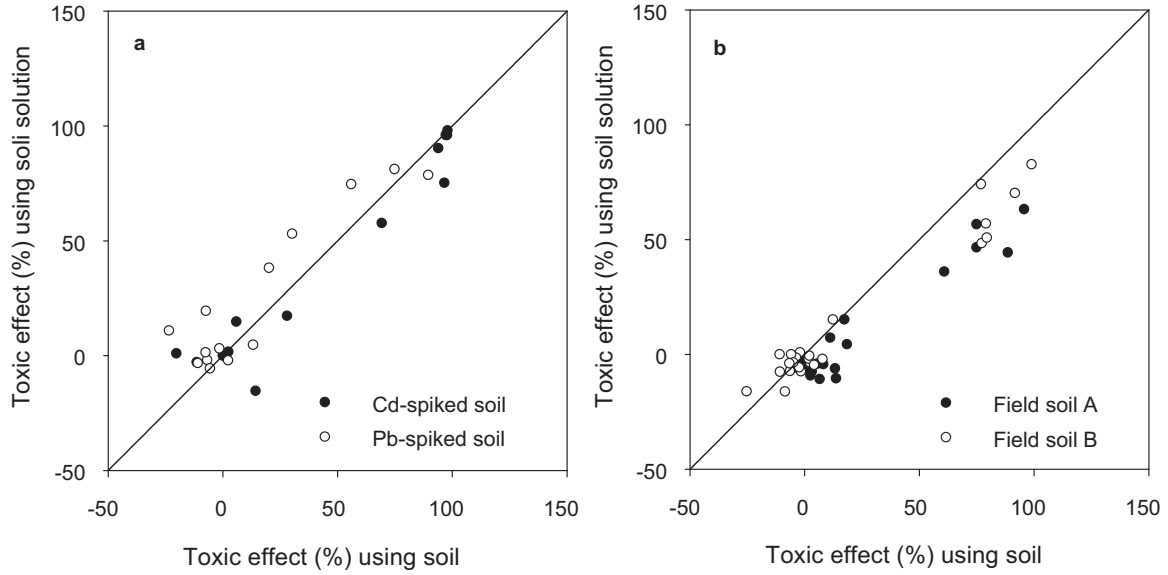

Fig. 4. Comparison of experimentally determined toxic effects (i.e., % bioluminescence inhibition) between soil and soil solution after 5-min exposure: (a) pristine soils freshly spiked with Cd or Pb and (b) field-aged soils contaminated with various heavy metals (i.e., Cd, Pb, Cu, As). Open and closed circles on the solid line means that the toxicity in soil and soil solution is the same.

volume of recovered porewater is often not enough to determine for chemical properties. Although centrifugation is most widely used for porewater recovery [28], it tends to extract higher metal concentrations in comparison to other means such as Rhizon samplers [29] and zero-tension lysimeters [30]. Moreover, it is plausible to cause re-equilibration of the soil during extraction process of porewater due to the altered soil properties [31]. Especially, porewater recovered by high speed centrifugation (i.e., $9700 \times g$) tends to exhibit significantly higher pH and fluoride content [32].

Such technical problems involved in porewater recovery and analysis can be overcome when the 'soil solution' as previously defined in Section 2 is used. To test whether or not the use of soil solution was valid, Cd and Pb toxicity in soil solution was compared to their toxicity in soil. The inhibition of bioluminescence was experimentally determined in soil and soil solution after 5-min exposure to *V. fischeri*. For this experiment, pristine soil freshly spiked with Cd or Pb and field-aged soil contaminated with various metals (i.e., Cd, Pb, Cu, As) were used. The former was considered to have easily desorbable metals and the latter as a medium containing strongly sorbed metals. Toxicity data from both soil samples resulted in strong positive linear relationships of toxic effects (i.e., bioluminescence inhibition) between the soil and the soil solution with the correlation coefficients of above 0.9 ($p < 0.005$) (Fig. 4). Student's *t*-test results (i.e., $p > 0.05$ in all cases) confirmed no significant difference in toxic effects between the soil and the soil solution. The results clearly demonstrate that metal toxicity to *V. fischeri* only originates from the dissolved metal ions and, if any, the metal ions associated with particles passed through the 0.45-μm GHP syringe filter. It indicates that soil solution toxicity can be reliably used to represent the toxicity in both freshly

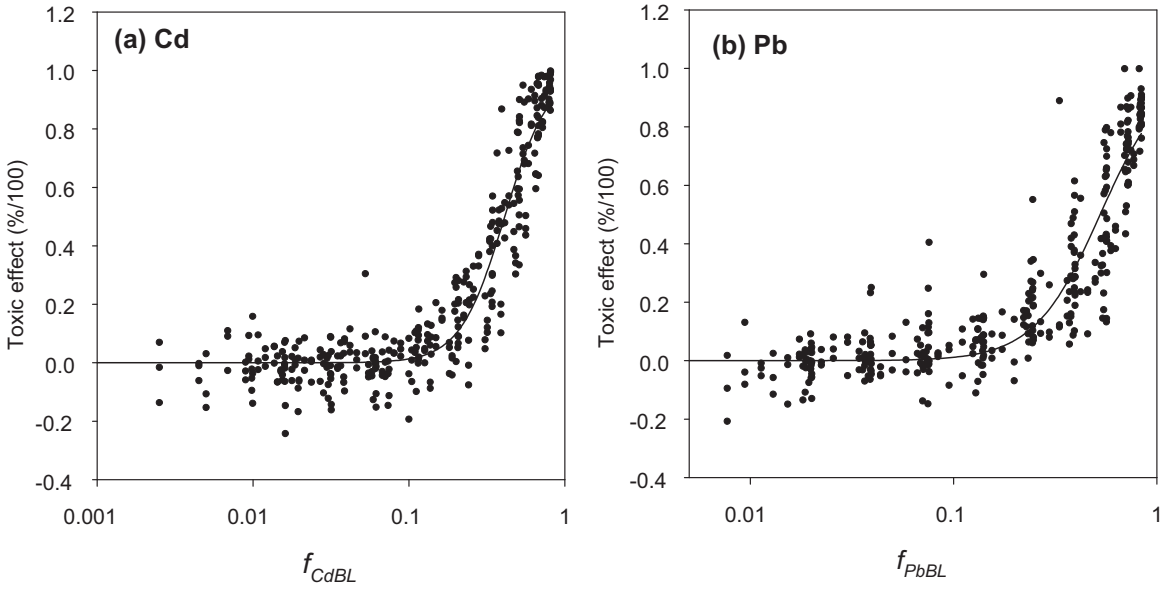

Fig. 5. Dose–response relationships of Cd (a) and Pb (b) between toxic effect and fraction of BL sites occupied by metals (f). Toxic effect was experimentally determined using *V. fischeri* toxicity test. The solid lines show the fitting results using Eq. (8).

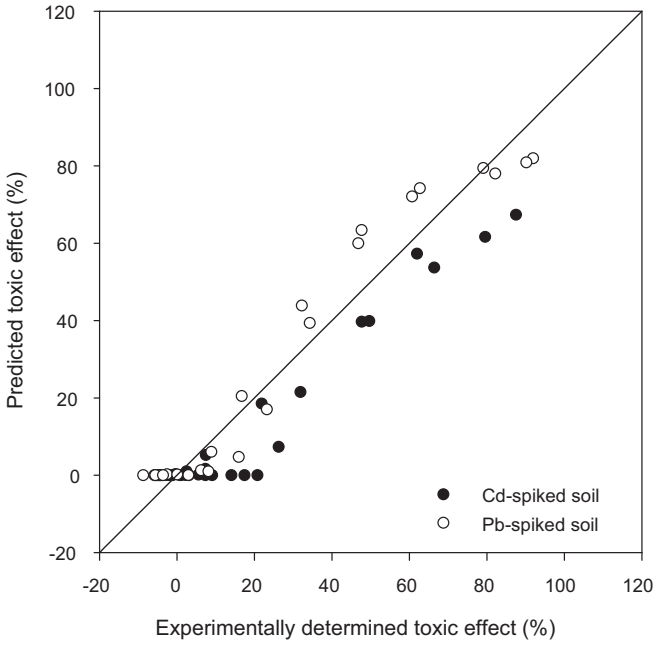

Fig. 6. Validation of the established biotic ligand-based models by comparing experimentally determined toxic effects (i.e., % bioluminescence inhibition) to the toxic effects predicted from the metal and cations activities in soil solution of Cd- and Pb-spiked soil. The solid line represents 1:1 ratio between the experimentally determined and predicted toxic effect values.

spiked and field-aged soils. Therefore, parameters of the biotic ligand-based model determined from aqueous phase tests were valid for predicting metal toxicity in soil.

3.4. Applicability of prediction models to soil

Scrutinizing Eq. (7) reveals that EC_{50} value is calculated from the activities of competing cations only. Toxic effect value (R) calculated from Eq. (9) is more meaningful in that it represents the free ion activity of a metal of interest as well as the activities of competing cations.

Model-predicted toxic effect data (expressed as the % bioluminescence inhibition) were fitted to a logistic dose–response curve to determine fitting parameters (i.e., β and f_{50}) of Eq. (9) (Fig. 5). The binding constant parameters were used from Eq. (7). The derived fitting parameter β was −3.0522 for Cd and −2.6783 for Pb, respectively. The f_{50} values were 0.4113 for Cd and 0.5267 for Pb, and these values were in good agreement with the previously calculated values (i.e., $f_{CdBL}^{50\%} = 0.435$ and $f_{PbBL}^{50\%} = 0.547$) from Eq. (6).

With the soils freshly spiked with Cd or Pb, toxic effects (i.e., % bioluminescence inhibition at given cations and metal activities) were experimentally determined using the Microtox® solid phase toxicity test method. In order to predict the toxic effect of soil solutions by using Eq. (9), soil solution samples were recovered from the soils, and their chemical properties including the concentrations of Cd or Pb, competing cations, DOC, and pH were determined. The activities of free Cd or Pb ions and competing cations were calculated by the WinhumicV speciation model using chemical properties as input data, and the values were used to predict toxic effect of Cd or Pb in soil solutions. Finally, model-predicted toxic effects and experimentally determined toxic effects were compared and strong linear relationships are found both in Cd- and Pb-spiked soils (Fig. 6). The results indicate that toxicity of the Cd- or Pb-contaminated soil can be predicted by using the chemical data of soil solutions and the developed biotic ligand-based model.

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