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The effect of major cations on the toxicity of cadmium to *Folsomia candida* in a sand-solution medium analyzed by biotic ligand modeling☆

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ABSTRACT

The aim of this study was to assess the effect of major cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, and H^{+}) on cadmium toxicity to the springtail *Folsomia candida*. Survival of the animals was determined after seven days exposure to different cadmium concentrations in an inert sand-solution medium, in different experimental setups with modification of the cation concentrations. Among the cations tested, Ca^{2+} and Mg^{2+} had protective effects on the toxicity of cadmium to the springtails while Na^{+}, K^{+}, and H^{+} showed less competition with free cadmium ions for binding to the uptake sites of the collembolans. Toxicity predicted with a biotic ligand model agreed well with the observed values. Calculated conditional binding constants and the fraction of biotic ligands occupied by cadmium to show 50% effects were similar to values reported in the literature. The results emphasize the important role of solution chemistry in determining metal toxicity to soil invertebrates.

1. Introduction

The widespread release of metals to the environment by human activities makes it important to investigate their adverse effects on living organisms. The effect of metals on organisms is based on their concentrations at the site of action (biological surface). However, more complexity will be added in soil when other parameters are involved in the process of metals reaching the targeted organisms. Therefore, only a small fraction of the total amount of metals present is considered bioavailable (Peijnenburg, 2002). This fraction is responsible for the relationship between metal uptake by soil organisms and effects. Development of the free ion activity model (FIAM) was based on the assumption that toxicity is mainly due to metals being present as free ion (Morel, 1983; Campbell, 1995).

Further steps toward understanding the relationship between metal uptake and effects were made by developing biotic ligand models (BLMs) to assess the bioavailability of metals, first to aquatic organisms (Di Toro et al., 2001; Santore et al., 2001; De Schamphelaere and Janssen, 2002) and later to soil organisms (Steenbergen et al., 2005; Thakali et al., 2006a, b). In these BLMs, the adverse effects were related to the fraction of bioavailable metal bound to the biotic ligand (BL) sites on the targeted organism. In the BLMs, some parameters affecting metal bioavailability are considered, such as competing cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, and H^{+}) and complexing factors (organic and inorganic binding ligands). The accumulation BL sites have been reported for only few test organisms; for example, fish gills (Santore et al., 2001), and plant root apoplasts (Antunes et al., 2006). For small animals, however, the observed effects are linked to the metal accumulation in the whole organism. For instance, Van Gestel and Koolhaas (2004) reported that internal concentration could be a better tool for describing cadmium toxicity to the springtail *Folsomia candida*. No clear BL sites for the springtails were reported in the literature.

Metals may become available for soil organisms through the soil solution or pore water (Van Gestel, 1997). Soil is a complex system

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containing different organic and inorganic ligands to which free metal ions may bind. Solutions embedded in inert sand could be an alternative substrate for assessing the mode of action of metal toxicity to soil organisms, avoiding the complexity of soil (e.g., Steenbergen et al., 2005). These media have controlled conditions to exclude the confounding effects of extra ligands in soil. In a previous study, we showed that collembolans were able to survive in a solution embedded in inert quartz sand for up to 21 days (Ardestani et al., 2014).

Springtails (Collembola, Isotomidae) are soil organisms which have been used in ecotoxicity tests for studying the effects of chemicals (Van Gestel, 2012). The collembolan species *F. candida* has been selected for toxicity tests for more than 40 years (Fountain and Hopkin, 2005). This species is widespread living in different environments. It is easy to collect *F. candida* from natural habitats and to rear it in the laboratory. Therefore, the presence of established cultures in many labs makes them easy to obtain for testing. The selected metal for the present study, cadmium, is important because of its high toxicity and its widespread occurrence in the environment due to human activities (Satarug et al., 2003).

Terrestrial BLMs (t-BLMs) have been developed to investigate metal toxicity to soil organisms (e.g., Thakali et al., 2006a, b), using several soil organisms including plants and animals. However, only few studies have investigated the effect of different parameters on the toxicity of cadmium to springtails applying a t-BLM (Ardestani et al., 2013). In the latter study, only the effect of Ca and pH on cadmium toxicity was investigated. Therefore, the aim of the present study was to assess how other cations compete with the free cadmium ions for uptake in the springtail *F. candida* using a BLM approach with a complete experimental setup including several relevant competing cations.

2. Materials and methods

2.1. Test medium

Test solutions embedded in an inert quartz matrix were used as described earlier (Ardestani et al., 2014). The chemical characteristics of this medium are shown in Table S1 (Supporting Information). Stock control solutions (background solutions) were prepared by adding 0.2 mM Ca as calcium nitrate ($Ca(NO_3)_2 . 4H_2O$, 99% pure, Riedel-de Haën, GmbH), 0.1 mM Mg as magnesium sulfate ($MgSO_4 . 7H_2O$, $\geq$ 99% pure, Sigma-Aldrich, GmbH), 1.0 mM Na as sodium nitrate ($NaNO_3$, 99.5% pure, zur Analyse, Merck), and 0.1 mM K as potassium nitrate (KNO_3, 99% pure, zur Analyse, Merck) at pH 6.00. The selected concentration ranges for each cation were based on the naturally occurring concentrations in soil pore water (e.g., Van Gestel and Koolhaas, 2004). Five cation sets were prepared: Ca-set, Mg-set, Na-set, K-set, and pH-set (Table 1). For each cation-concentration set, the concentrations of the other cations and pH level (as background solution) were kept constant. For each set, one control and five nominal cadmium concentrations of 1.6, 3.1, 6.2, 12.5, and 25 mM were prepared using cadmium nitrate salt ($Cd(NO_3)_2 . 4H_2O$, 98% pure, Sigma-Aldrich, GmbH). The selected cadmium concentrations were based on our previous study (Ardestani et al., 2014) in which a complete dose-response relationship for the effects of cadmium on the survival of *F. candida* could be observed. The selected exposure levels were also in the range of the calculated LC_{50} values of up to 30.1 mM and up to 0.83 mM based on porewater concentrations for *F. candida* exposed for 28 and 50 days, respectively in different soils (Van Gestel and Koolhaas, 2004; Bur et al., 2010).

Except for the pH-set, which had different pH levels (see Table 1), the pH of the stock solution in other test solutions was adjusted to 6.00 ± 0.1 using MOPS/MES buffers or diluted NaOH when necessary. The pH of the test solutions was analytically determined using a pH meter (InoLab pH 7110).

2.2. Test organism

The springtail *F. candida* (Berlin strain, Vrije University Amsterdam, The Netherlands) was cultured in plastic containers on a base of plaster of Paris and charcoal (9:1) in climate chambers at 16 °C, 75% relative humidity (RH), and 12:12h light/dark regime. Animals were fed with dried baker's yeast (Dr. Oetker). Age-synchronized animals were obtained by allowing adults to lay eggs for 2 days at 20 °C, after which they were removed and eggs were allowed to hatch. Twenty-day old synchronized springtails were used for the different experiments.

2.3. Experimental set up

Cadmium toxicity to *F. candida* was investigated after seven days exposure. For each cation-concentration set, four replicates were prepared. Five springtails were added into each replicate 100 ml glass jars filled with 5.4 ml test solution embedded in 20 g prepared inert quartz sand, and equilibrated for one day before introducing the test organisms. Test jars were placed in climate chambers at 20 °C, 75% RH and a 12:12h light/dark regime. All glass jars were aerated twice a week. Survival of the animals was recorded at the end of the experiment and one animal from each replicate (in total two replicates for each cadmium concentration) was taken for analysis (cadmium uptake).

2.4. Metal analysis

At the end of the experiment, test animals walking on the solution surface were sampled randomly from each replicate (using a small spoon) and cadmium concentrations in the animals were measured (See method in Ardestani et al., 2014). Quality of the analysis was controlled using the reference material Dolt-4, NRC-CNRC, Canada and measured cadmium concentrations were always within 15% of the certified reference value. The concentrations of dissolved cadmium, Ca, Mg, Na, and K in the test solutions were measured by flame AAS (Perkin Elmer, AAnalyst 100). The detection limit of the flame AAS was 0.01 mg/L and measured concentrations were always higher than this level.

Table 1
Chemical characteristics of the test media used in the different toxicity test sets with *Folsomia candida*.

Cation set	Varied cation concentrations and pH levels	Cation concentrations of background solutions at each varied cation level
Ca	0.2, 1.0, 2.0, 4.0 mM	0.1 mM Mg, 1.0 mM Na, 0.1 mM K, pH 6.00
Mg	0.1, 1.0, 2.0, 4.0 mM	0.2 mM Ca, 1.0 mM Na, 0.1 mM K, pH 6.00
Na	1.0, 3.0, 5.0, 7.0 mM	0.2 mM Ca, 0.1 mM Mg, 0.1 mM K, pH 6.00
K	0.1, 1.0, 1.5, 2.0 mM	0.2 mM Ca, 0.1 mM Mg, 1.0 mM Na, pH 6.00
pH	5.0, 6.0, 7.0, 8.0	0.2 mM Ca, 0.1 mM Mg, 1.0 mM Na, 0.1 mM K

2.5. Data analysis

The LC_{50} values for the effect of cadmium on *F. candida* survival were calculated using the trimmed Spearman-Karber method (Hamilton et al., 1977). The values were estimated based on the average measured cadmium concentrations at the beginning and at the end of the tests. The activity of cadmium and other cations in the test solutions were calculated using the Visual Minteq program (Gustafsson, 2007). The activities were based on the average measured cation and anion concentrations and pH level. LC_{50} values were also calculated based on the activity of free cadmium ions. All analyses were run in Excel and Statistica 13.2 program package.

2.6. Biotic ligand modeling approach

For the biotic ligand modeling part, cadmium toxicity data were modeled as described by e.g., Ardestani et al. (2015) and De Schamphelaere and Janssen (2002). A full description of the model is given in the Supporting Information. A logistic dose-response model (Haanstra et al., 1985) was used to describe the survival of *F. candida* as a function of cadmium uptake at the BL sites.

3. Results

3.1. Cadmium toxicity and the effect of cations

Control springtail survival was 100% in all different cation-concentration sets. A proper dose-response relationship could be obtained in all experimental sets (Fig. S1, Supporting Information). When plotting survival against free cadmium ion activity, the fit of the logistic dose-response model was slightly improved ($R^2 = 0.79$; Fig. S1B) compared to that using measured cadmium concentrations in the solutions ($R^2 = 0.74$; Fig. S1A).

The LC_{50} values based on average measured cadmium concentrations and free ion activities (Table S2, Supporting Information) ranged from 1.27 to 3.30 mM and from 0.72 to 1.53 mM, respectively. Increasing calcium concentrations from 0.2 mM to 4.0 mM and Mg concentrations from 0.1 to 4.0 mM (expressed as their activities in Fig. 1) resulted in a linear increase in $LC_{50}\{Cd^{2+}\}$ with R^2 values of 0.73 and 0.77 ($p < 0.05$), respectively (Fig. 1A and B). An

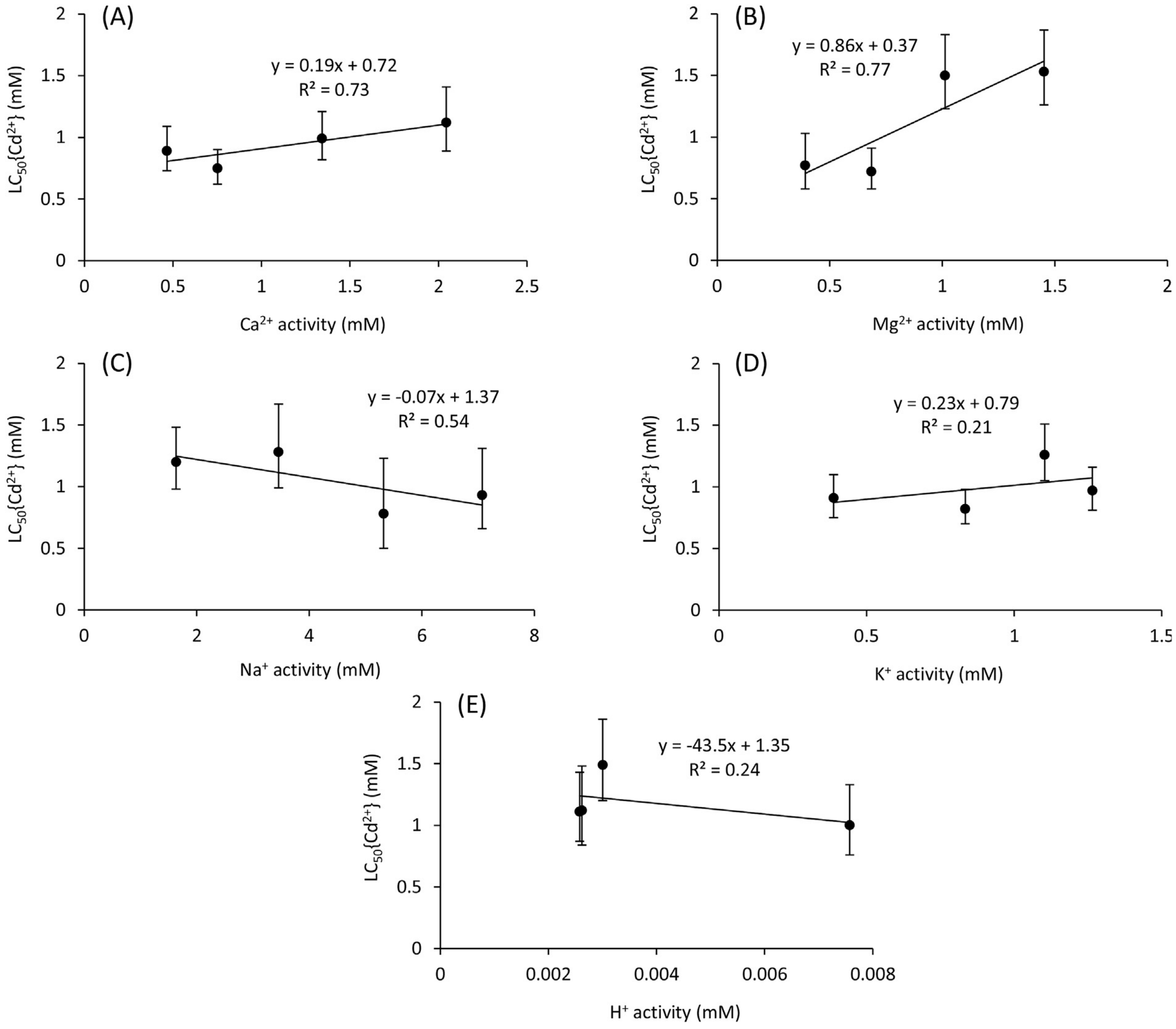

Fig. 1. The 7-d $LC_{50}\{Cd^{2+}\}$ for the effect of cadmium on the survival of *Folsomia candida* as a function of the activities of Ca^{2+} (A), Mg^{2+} (B), Na^{+} (C), K^{+} (D), and H^{+} (E) in test solutions (Table 1) embedded in an inert quartz sand medium. Data points show the calculated LC_{50}, error bars are 95% confidence intervals. The solid lines show the linear regression.

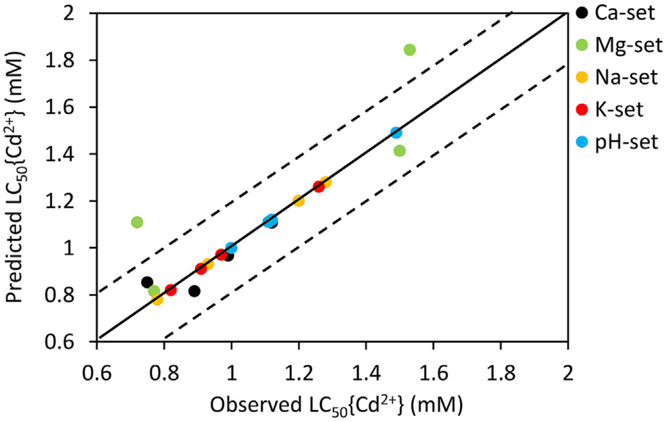

Fig. 2. The relationship between the predicted and observed 7-d $LC_{50}\{Cd^{2+}\}$ for the effect of cadmium on the survival of *Folsomia candida* exposed in different solutions embedded in an inert quartz sand matrix. The solid line is the 1:1 line and the dashed lines indicate a factor of two differences between the predicted and observed values.

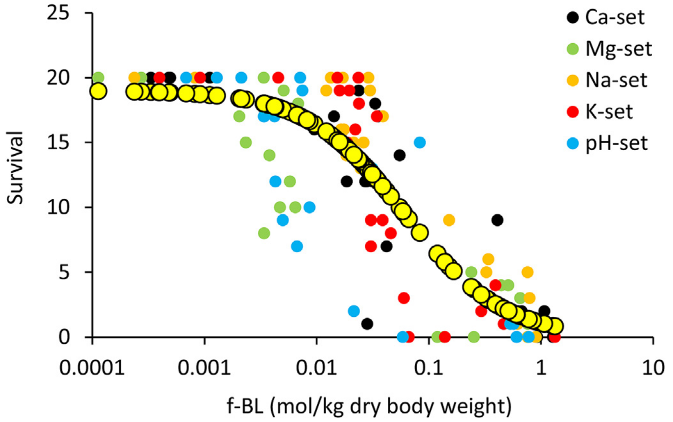

Fig. 3. Dose-response relationships for the effect of cadmium on the survival of *Folsomia candida* after seven days exposure in test solutions embedded in an inert quartz sand matrix at different Ca, Mg, Na, K, and pH levels. Survival is plotted against the fraction of biotic ligand sites occupied by cadmium (mol/kg dry body weight). The dotted line represents the fit of a logistic model (Eq. (S11)) to all data.

increase in Na concentrations from 1.0 mM to 7.0 mM, K concentrations from 0.1 mM to 2.0 mM, and pH from 5.0 to 8.0, did not lead to an increase in $LC_{50}\{Cd^{2+}\}$ (Table S2 and Fig. 1; C, D, and E). In case of the Na-set and pH-set, increasing cation concentrations in fact slightly increased cadmium toxicity (Fig. 1).

3.2. Modeling cadmium toxicity and BLM parameters

Since no protective effects of Na^+, K^+, and H^+ were observed (Fig. 1), only data for Ca and Mg was used for further analyses and model development. Solving matrix calculations by considering the slope (Eq. (S9), see Supporting Information) and intercept (Eq. (S10)) from the linear relationships between $LC_{50}\{Cd^{2+}\}$ and free cation activities (Fig. 1, Eq. (S8)) resulted in conditional binding constants log $K_{Ca\text{-}BL}$ and log $K_{Mg\text{-}BL}$ of 0.58 and 3.00 L/mol, respectively. The conditional binding constant for cadmium (log $K_{Cd\text{-}BL}$) and f_{BL50} were calculated using Eq. (S7) by changing the value of $K_{Cd\text{-}BL}$ to get the best fit for the relationship between the logit (percent of mortality) and f_{BL50} (Fig. S2, Supporting Information). The calculated values were 1.98 L/mol and 0.06 for log $K_{Cd\text{-}BL}$ and f_{BL50}, respectively with $R^2 = 0.77$ (Fig. S2).

All model parameters were used in Eq. (S8) to give the following equation for cadmium toxicity:

$$LC_{50}\{Cd^{2+}\} = 0.033 + 0.019\,\{Ca^{2+}\} + 0.098\,\{Mg^{2+}\} \quad (1)$$

To validate the BLM for cadmium toxicity, $LC_{50}\{Cd^{2+}\}$ values predicted using Eq. (1) and all free cation activities were plotted against measured $LC_{50}\{Cd^{2+}\}$ values (both in mM): Fig. (2). A good match was obtained between predicted and observed toxicity data with differences being less than a factor of two (except for two data points from the Mg-set).

To check for the accuracy of the f_{BL50} calculated from Eq. (S7) describing the relationship between the logit (percent of mortality) and f_{BL}, survival of animals from all data sets were plotted against the f_{BL} values (Eq. (S6)), as shown separately for each cation in Fig. 3. A logistic dose-response curve (Eq. S11) fitted to all experimental data sets, as shown in Fig. 3. The f_{BL50} calculated from the best fit of the logistic regression was similar to the previous value (from Eq. (S7)). In the final step, predicted survival data was plotted against observed values using the conditional binding constants calculated from the matrix calculations as described above incorporated into Eq. (S12). This analysis was performed for the Ca– and Mg-sets, first separately and then using all Ca and Mg data together (Fig. S3, Supporting Information, A-C). This resulted in f_{BL50} values of 0.07, 0.05, and 0.06 with R^2 values of 0.88, 0.89, and 0.76 for Ca-set, Mg-set, and Ca + Mg data together, respectively (Fig. S3, A-C). In the present study, the applicability of using the cadmium BLM was double checked; first, by comparing predicted and measured $LC_{50}\{Cd^{2+}\}$ values (Fig. 2) and second, by comparing predicted and observed survival data (Fig. S3).

4. Discussion

4.1. Cadmium toxicity and the effect of cations

The high survival of the springtails in all control solutions of the different cation-concentration sets suggests that the solution embedded in inert sand was a suitable medium for investigating cadmium toxicity to *F. candida*. This is in agreement with a previous study in which collembolan survival was 100% up to 21 days of incubation in the basic control sand-solution medium (Ardestani et al., 2014).

The LC_{50}s based on measured cadmium concentrations and free cadmium ion activities (1.27–3.30 mM and 0.72–1.53 mM, respectively) for the different experimental sets are consistent with those of 2.51 mM and 1.51 mM, respectively found after seven days exposure in a sand-solution medium (Ardestani et al., 2014). The obtained LC_{50}s are also in the range of the values reported for *F. candida* exposed for seven days to cadmium in simulated soil solutions at different calcium and pH levels (Ardestani et al., 2013). Bur et al. (2010) reported LC_{50}s of 0.31 mM–0.83 mM based on porewater concentrations when the springtails were exposed for 50 days in natural soils. The agreement of the values from our study and literature data supports the validity of the solution embedded in inert sand medium system for assessing the toxicity of cadmium to *F. candida* and suggests sand-solution as a replacement for soil pore water testing.

Among the cations tested, Ca^{2+} and Mg^{2+} were the most competitive with the free cadmium ions for binding to the uptake sites of *F. candida*. This supports the main assumption of the BLM approach in which the bivalent cations mostly compete with free cadmium ions for binding to the biotic ligand sites on the organisms (Playle et al., 1993; Paquin et al., 2002). The latter authors attributed these effects to the similar ion atomic radius of Ca^{2+},

Mg^{2+} and cadmium ions to enter ion channels of the fish gill epithelial cells, which are known as transport sites for ion exchange. This would result in a lower toxicity of cadmium when higher cation concentrations are present in the medium. Cadmium and calcium ions have similar ionic charge and an ionic radius of approximately 0.95 Å and 1.00 Å, respectively, which suggests free cadmium ions most likely enter cells via Ca^{2+} channels, explaining for the strong competition between these two ions (Markich and Jeffree, 1994). This is similar for Mg^{2+}, which with an ionic radius of approximately 0.72 Å might be an even stronger competitor for binding to the biotic ligands (See below). This is consistent with the results of the present study, which also showed a stronger competitive effect of Mg^{2+} compared to Ca^{2+} (Fig. 1). Another possible pathway which may reduce the adverse effects of cadmium ions on the surface of biotic ligands is the relationship between the influx and efflux rate of other metallic ions (such as Ca^{2+}). This is not simply due to the competing effects of ions; for example, higher concentrations of Ca^{2+} in the surrounding solution may result in a reduced efflux of other cations from the epithelial cells (BL site). This keeps the homeostasis of ions in the body constant (Paquin et al., 2002). This regulatory effect at the BL surface is performed by active influx/efflux mechanisms when cation concentration is in excess. It has been reported that cadmium ions especially inhibited Ca^{2+} influx in rainbow trout, but did not affect Ca^{2+} efflux and had only minor effects on Na^+ fluxes (Reid and McDonald, 1988). This, however, may be different in other organisms.

The protective effect of cations has been extensively studied in the literature (e.g., De Schamphelaere and Janssen, 2002; Lock et al., 2007; Thakali et al., 2006a, b; Li et al., 2008). Generally, toxicity of metals is decreased with increasing concentrations (activities) of cations. In some studies, the protective effects of Ca^{2+} and Mg^{2+} have been confirmed, but in others these effects were absent or were much less evident for either one or both divalent cations. For example, Clifford and McGeer (2010) showed protective effects of Ca^{2+} and Mg^{2+} and to some extent H^+, but not of Na^+ on cadmium toxicity to *Daphnia pulex* when exposed for two days in test solutions, which is in agreement with the present study. Steenbergen et al. (2005), however, found competitive effects of protons and Na ions on copper toxicity to the earthworm *Aporrectodea caliginosa* after seven days of exposure in an aqueous Steiner nutrient solution. In another study, ameliorating effects of all cations (Ca^{2+}, Mg^{2+}, Na^+, K^+, and H^+) on cadmium toxicity to the earthworm *Eisenia fetida* were reported when the animals were exposed in solution media for two days (Li et al., 2008). Therefore, the competing effects can be different in different test organisms (collembolans vs. earthworms or daphnids) and for different metals (copper vs. cadmium). This emphasizes the strong role of physiological mechanisms inside organisms when encountering ionic imbalances in their living environment.

4.2. Modeling cadmium toxicity and BLM parameters

The conditional binding constants for cadmium and two main competing metallic ions (Ca^{2+} and Mg^{2+}) to *F. candida* are summarized in Table 2. Using simulated soil solutions, a log $K_{Cd\text{-}BL}$ value of 1.62 L/mol was determined (Ardestani et al., 2013), which is consistent with the value obtained in the present study (1.98 L/mol). However, the estimated log $K_{Cd\text{-}BL}$ was much lower than the values reported in the literature for soil or aquatic organisms (Table 2). One explanation for this can be related to the difference between test species used. Each test organism may have a specific binding capacity for metals at biotic ligand sites. So, metals may reach the uptake sites easier with higher binding affinity for aquatic species than for soil organisms. In case of barley, values may be similar since plants are grown in solutions instead of soil (Table 2), but the exact mechanisms remain unclear. Soil animals, such as earthworms and collembolans, may show lower binding affinities for metals, unless they are exposed in soil which is a more complex system. Another explanation may be attributed to the solution composition. In this case, each organism may be more sensitive to one or two metallic cation(s) than to other cations, leading to expected higher or lower binding affinities. This also concerns the competing cations (Ca^{2+}, Mg^{2+}, etc.) and their affinities for binding to the biotic ligand sites of the test organisms. They therefore may reduce the negativity of the electrical charge at the biotic ligand surface. As a result, the activity of free cadmium ions and its binding affinity at the membrane surface will be reduced.

For calcium, the log $K_{Ca\text{-}BL}$ value (0.58 L/mol) found in the present study was much lower than the 2.87 mol/L estimated previously for *F. candida* (Ardestani et al., 2013) and that of other studies on different soil organisms (Table 2). This may be partly due to the higher cation concentration (higher ionic strength) of the sand solution compared to simulated soil solutions, thereby reducing the singular effect of calcium for binding to the uptake sites of the organism. Consistent with our results, Yermiyahu and Kinraide (2005) reported that ionic strength may directly affect the electrostatic binding of cations by plant root surfaces. However, the log $K_{Mg\text{-}BL}$ of 3.00 calculated in the present study is consistent with the previous reported values for other soil organisms (Table 2). Here, we re-emphasize that the values reported for plants are just for showing results from different tests and not for direct comparison with other soil organisms such as earthworms, collembolans, etc., as their uptake mechanisms are completely different.

The higher log $K_{Mg\text{-}BL}$ compared to the log $K_{Ca\text{-}BL}$ value in the present study is in agreement with other studies in which the toxicity of cadmium to two plant species, *Hordeum vulgare* and *Glycine max,* was investigated after 5 and 7 days of exposure in aqueous media, respectively (Wang et al., 2016; Chen et al., 2017). Similar higher Mg affinities compared to Ca have been observed in studies on zinc, copper, and nickel toxicity to barley (Thakali et al.,

Table 2
Summary of biotic ligand model-based parameters obtained when exposing different test organisms to cadmium in different substrates, at different test durations, and assessing different endpoints.

Test organism	Endpoint	Duration (days)	Medium	Conditional binding constants (log units, L/mol)			f_{BL50}	References
				$K_{Cd\text{-}BL}$	$K_{Ca\text{-}BL}$	$K_{Mg\text{-}BL}$		
Hordeum vulgare	Root elongation	5	Solution	5.19	2.87	2.98	0.29	Wang et al. (2016)
Glycine max	Root elongation	7	Solution	5.95	2.67	3.31	0.40	Chen et al. (2017)
Daphnia pulex	Survival (immobilization)	2	Solution	6.97	4.08	3.71	–	Clifford and McGeer (2010)
Vibrio fischeri	Bioluminescence inhibition	5 min.	Solution	5.02	2.84	2.19	0.44	An et al. (2012)
Eisenia fetida	Survival	2	Solution	4.0	3.35	2.82	0.72	Li et al. (2008)
Folsomia candida	Survival	7	Solution	1.62	2.87	–	0.04	Ardestani et al. (2013)
Folsomia candida	Survival	7	Sand-solution	1.98	0.58	3.00	0.06	Present study

2006a; Li et al., 2009a; Antunes and Kreager, 2009; Wang et al., 2010; Wang et al., 2012), for copper toxicity to wheat (Luo et al., 2008), for cadmium toxicity to *E. fetida* (Li et al., 2009b), for copper toxicity to *D. magna* (De Schamphelaere and Janssen, 2002), for zinc toxicity to *Pseudokirchneriella subcapitata* (Heijerick et al., 2002), and for nickel toxicity to *Chlamydomonas reinhardtii* (Worms and Wilkinson, 2007). As mentioned above and discussed in few of the above-cited papers on different metals and test organisms, the higher log $K_{\text{Mg-BL}}$ compared to the log $K_{\text{Ca-BL}}$ could be explained by its smaller ionic radius, thus increasing the affinity of Mg for binding to the biotic ligand surface and entering ionic channels.

The estimated f_{BL50} value of 0.06 is in agreement with previous studies on *F. candida* in which values of 0.04 and 0.05 were obtained when the animals were exposed to metals in simulated soil solution and in soil, respectively (Ardestani et al., 2013; Thakali et al., 2006a). Other studies however, showed higher f_{BL50} values (Table 2), suggesting the intrinsic species-dependent characteristic of this value. The smaller f_{BL50} value may indicate that *F. candida* is more sensitive to cadmium than other soil organisms with 50% effects being reached when 6% of the biotic ligand sites of the animals are occupied. Therefore, this value plus the conditional binding constants for cadmium and other cations as input for the development of a BLM can be used as a good predictor of the relationship between metal uptake and effects for each test organism. This was confirmed by the good relationship between predicted and observed effects with R^2 values ranging from 0.76 to 0.89 in the present study (Fig. S3). Our developed BLM to predict cadmium toxicity to *F. candida*, using solutions embedded in an inert sand medium, was validated by less than a factor of two difference between predicted and estimated $LC_{50}\{Cd^{2+}\}$ values (Fig. 2). This is in agreement with other studies in the literature, supporting the suitability of using a sand-solution medium for obtaining a proper and relevant estimate of cadmium toxicity to *F. candida*.

5. Conclusions

The present study showed a protective effect of Ca^{2+} and Mg^{2+}, but not of Na^{+}, K^{+}, and H^{+} on the toxicity of cadmium to the springtails when exposed to cadmium in test solutions embedded in an inert quartz sand medium. Using a biotic ligand approach, conditional binding constants for cadmium, calcium, and magnesium were estimated which were comparable with the values reported in the literature. Predicted effects of cadmium on springtail survival were in good agreement with the observed values. These results confirm the applicability of the BLM developed for toxicity of cadmium to collembolans using sand-solution medium.

Conflict of interest

There is no conflict of interest.

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Appendix A. Supplementary data

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