

Bioavailability and phytotoxicity of rare earth metals to *Triticum aestivum* under various exposure scenarios

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ARTICLE INFO

Keywords:

Bioavailability
Toxicity
Uptake
Rare earth metals
BLM
WHAM- F_{tox}

ABSTRACT

It is a daunting challenge to predict toxicity and accumulation of rare earth metals (REMs) in different exposure scenarios (e.g., varying water chemistry and metal combinations). Herein, we investigated the toxicity and uptake of La and Ce in the presence of various levels of Ca, Mg, Na, K, and at different pH values, as well as the combined effects of La and Ce in wheat *Triticum aestivum*. Major cations (Ca^{2+} and Mg^{2+}) significantly mitigated the toxicity and accumulation of La^{3+}/Ce^{3+} . Toxicity and uptake of La, Ce, and La–Ce mixtures could be well quantified by the multi-metal biotic ligand model (BLM) and by the Langmuir-type uptake model with the consideration of the competitive effects of Ca^{2+} and Mg^{2+} , with more than 85.1% of variations explained. The derived binding constants of Ca, Mg, La, and Ce to wheat root were respectively 3.87, 3.59, 6.97, and 6.48 on the basis of toxicity data, and 3.23, 2.84, 6.07, and 5.27 on the basis of uptake data. The use of the alternative WHAM- F_{tox} approach, requiring fewer model parameters than the BLM but with similar Akaike information criterion (AIC) values, successfully predicted the toxicity and accumulation of La/Ce as well as toxicity of La–Ce mixtures, with at least 76.4% of variations explained. However, caution should be taken when using this approach to explain the uptake of La–Ce mixtures. Our results provided promising tools for delineating REMs toxicity/uptake in the presence of other toxicity-modifying factors or in mixture scenarios.

Author contribution statement

Bing Gong, conceived the idea and designed the experiment, performed the experiment, wrote the manuscript. Erkai He, conceived the idea and designed the experiment, wrote the manuscript, raised the funding. Bing Xia, wrote the manuscript. Rongrong Ying, revised the manuscript. Willie J.G.M. Peijnenburg, revised the manuscript. Yang Liu, revised the manuscript. Hao Qiu, conceived the idea and designed the experiment, wrote the manuscript, raised the funding.

1. Introduction

Rare earth metals (REMs) are a group of 15 lanthanides, yttrium (Y),

and Scandium (Sc). The REMs have a unique place among the elements because of their striking properties (e.g., magnetism, corrosion resistance, luminescence, and electroconductivity) (IUPAC, 2005). Global demand for REMs has rapidly increased since the 1990s (Alonso et al., 2012) because of the widespread use of REMs in many sectors, e.g. high-tech equipment, medicine, electronics, and renewable energy (Gwenzi et al., 2018). This has led to the release of an increasing loads of REMs to the environment. The average concentration of the REMs in the Earth's crust are 150–220 parts per million (Long et al., 2010), whereas the total REMs levels can reach >1000 mg/kg as a result of human activities (Li et al., 2013). The enrichment of REMs poses a great threat to the ecosystem and human health (Gonzalez et al., 2014; Liu et al., 2018). Research efforts towards quantitative modeling of the adverse effects of

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<https://doi.org/10.1016/j.ecoenv.2020.111346>

Received 3 April 2020; Received in revised form 10 September 2020; Accepted 11 September 2020

Available online 22 September 2020

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REMs are therefore desired in the context of conducting appropriate risk assessment.

Compared to conventional metals with well-documented toxicological impacts (Cobbina et al., 2015; Feng et al., 2018; Macoustra et al., 2019; Shahid et al., 2017), little attention has been paid to the ecotoxicity and accumulation of REMs. The available studies dealing with the toxicity and accumulation of REMs mainly focused on individual REMs, ignoring other toxicity-modifying factors (Gonzalez et al., 2014; Herrmann et al., 2016). However, toxicity of REMs may vary with diverse exposure conditions (Gong et al., 2019), and it is thus necessary to take environmental factors such as the coexisting cations into consideration for assessing REMs toxicity at different exposure scenarios. In the real environment, REMs are typically found together in geological deposits (Alonso et al., 2012) and subsequently released into water and soil as mixtures (Weltje et al., 2002). This means that the assessment of mixture effects of REMs is more environmentally relevant than solely assessing the toxicity of individual REMs. Metals can influence each other's toxic effects and the combined toxicity cannot be accurately predicted without proper consideration of mixture interactions (Cremazy et al., 2019). These interactive effects could occur at different levels: i) physicochemical interactions in the exposure media, affecting chemical speciation and hence the bioavailability of metals, ii) physiological interactions during the uptake processes, influencing toxicokinetic processes and thereby the available amount at the sites of action, iii) internal interactions at the target site, affecting toxicodynamic processes and subsequently the combined effect (Qiu and He, 2017). The toxic effects of ternary REMs mixture (Ce, Gd, and Lu) to seven aquatic species have been reported to deviate from strict additivity (Romero-Freire et al., 2019). Another research has shown that a combination of REMs, whose summed dosages were the same as the dose of the individual REMs, induced an equal level of toxicity to the algae *Skeletonema costatum* (Tai et al., 2010). The mixed effects of binary mixtures of REMs (Y, La, and Ce) to wheat (*Triticum aestivum* L.) were dependent on the expressions of exposure (Gong et al., 2018). Moreover, there were strong antagonistic interactions among the ternary mixtures of REMs (Y, La, and Ce) in wheat (*Triticum aestivum* L.) (He et al., 2020). These results prompted us to consider bioavailability and mixture interactions when constructing models for predicting REMs toxicity/uptake in mixture scenarios.

The biotic ligand model (BLM) is a mechanistic-underpinned model for predicting toxicity/uptake of metal as a function of the free metal ion activity (Lavoie et al., 2014). In the BLM, metal toxicity is assumed to be proportional to the fraction of metal bound to the BLs on or within the target organism. Major cations (e.g., Ca^{2+} , Mg^{2+} , Na^+ , K^+) and protons are considered as parameters that may affect metal bioavailability to different extents, thus modifying uptake and toxicity (Chen et al., 2013). The BLM was originally applied to predict the toxicity of individual divalent metal ions by taking both metal speciation and cation competition into account (Di Toro et al., 2001; He et al., 2014; Lin et al., 2018). Assuming that competition is responsible for mixture interactions, the development of a multi-metal BLM for considering mixture interactions and other bioavailability-modifying factors is possible. Attempts have been made to extend the BLM to assess mixture toxicity/uptake of conventional divalent metals (Chen et al., 2010; Liu et al., 2014; Qiu et al., 2015). However, its applicability for trivalent metals remains unclear. Gensemer and Playle (1999) reported that Al^{3+} and Al hydroxy species were needed to evaluate trivalent Al bioavailability and toxicity. For trivalent REMs such as Sc (Cremazy et al., 2014), Eu (Yang et al., 2014), Ce (El-Akl et al., 2015), Tm (Zhao and Wilkinson, 2015), Sm (Tan et al., 2017), Nd (Yang and Wilkinson, 2018), the BLM approach explained the effects of competition but not complexation for biouptake of REMs by the freshwater green alga (*Chlamydomonas reinhardtii*). These intriguing findings motivated us to identify the validity of the BLM theory for quantifying toxicity/uptake of REMs when applied individually (with varying Ca, Mg, Na, K and H levels) and in combination.

Alternatively, the Windermere Humic Aqueous Model coupled with the toxicity function (WHAM- F_{tox}) may serve as a new option for integrating bioavailability into toxicity modeling (Tipping and Lofts, 2013). It assumes that the interactions of metals with biological surfaces can be mimicked by their interactions with particulate humic acid (HA) (Stockdale et al., 2014). There are various functional groups in HA, which can represent the heterogeneous distribution of BLs. The concentrations of metals binding to particulate HA can be easily calculated by WHAM VII (Tipping et al., 2011). The acquisition of the required parameters of the WHAM- F_{tox} approach is convenient based on the available particulate HA-phase database of WHAM VII. Particularly, this model is favoured under the circumstances that lack of information on the binding constants for binding of a metal to an organism might hamper the application of BLM for quantifying the toxicity of mixtures. A few studies have reported the feasibility of WHAM- F_{tox} to predict the individual/mixture toxicity and accumulation of traditional divalent metals (He and Van Gestel, 2015; Le et al., 2015; Tipping and Lofts, 2013). On one hand, the WHAM- F_{tox} approach requires fewer model parameters than that for the BLM, and thus this lower parameterized model is superior in case of lower data availability. On the other hand, considering its different mechanistic perspective as compared to the BLM, it is worth to explore whether the WHAM- F_{tox} approach can act as a surrogate in modelling toxicity and uptake of REMs.

Two light REMs with relatively high average crustal abundance (30 mg/kg for La and 60 mg/kg for Ce) were selected in the present study (Brown et al., 1990). The toxicity and accumulation of La, Ce and their binary mixtures in wheat were investigated. The main objectives were to determine the impacts of coexisting cations (Ca^{2+} , Mg^{2+} , Na^+ , K^+ , and H^+) and mixture interactions on the bioavailability and toxicity of REMs, and to develop bioavailable-based models (extended BLM and WHAM- F_{tox} approach) for normalizing toxicity and uptake of REMs under different exposure scenarios. The Akaike information criterion (AIC) was further used to evaluate the model performance.

2. Materials and methods

2.1. Test species

Triticum aestivum L. NAU 9918 (Nanjing Agricultural University) seeds were used as the test species in the current study. The detailed information regarding the sterilization and pre-germination of wheat seeds was documented by Wang et al. (2013). After germination, seedlings with root lengths between 1.00 and 1.50 cm were carefully chosen for the experiments.

2.2. Test solution and treatments

The nutrient solution used in the current study was the same as earlier reported by Gong et al. (2018). Its principal composition (nominal concentration) was: $[\text{Ca}] = 0.3 \text{ mM}$, $[\text{Mg}] = 0.2 \text{ mM}$, $[\text{K}] = 1.2 \text{ mM}$, and $[\text{BO}_3] = 15 \text{ }\mu\text{M}$. The detailed composition of the nutrient solution is shown in Table S1. The concentration ranges of selected cations were based on the concentrations that occur in natural soil pore waters (Van Gestel and Koolhaas, 2004). For identifying the impact of different cations, a univariate experimental design was chosen in single REMs toxicity tests. Toxicity tests were performed with La or Ce and five sets of individual cations (Ca-set, Mg-set, Na-set, K-set and pH-set) (Table S2). For each set, a range of REMs concentrations were selected to guarantee a complete dose response relationship for the effects of REMs on *Triticum aestivum* L. For the La-Ce binary mixture, three treatment series (i.e., single La, single Ce, and La-Ce mixture) were designed based on a full factorial design (Table S3). A series of specific treatments were prepared by adding several volumes of stock solutions of $\text{LaCl}_3 \cdot 6\text{H}_2\text{O}$, $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, NaCl, and KNO_3 to the nutrient solution. A preliminary experiment showed that the impact of anion (Cl^-) on the root elongation of *Triticum aestivum* was negligible

(Fig. S4). All treatments were buffered at the target pH values 6.00 using 0.75 mg/L 2-(*N*-morpholino)-ethanesulfonic acid (MES: for pH < 7.00 treatments), 0.75 g/L 3-(*N*-morpholino)-propanesulfonic acid (MOPS: for pH > 7.00 treatments) and diluted NaOH (0.20 mM) if necessary. All test solutions were equilibrated for 1 d before use.

2.3. Toxicity tests

The wheat root elongation test was conducted in the phytotron using a regime of 16:8 h light:dark (20 °C, 75% humidity). After germination, four seedlings were transferred to parafilm fixed on the surface of a glass beaker wrapped in aluminum foil. Each beaker was filled with 250 mL test solution, which was changed daily to maintain the exposure concentrations. Three replicates were applied for each treatment. At the end of the exposure (4 days), the wheat roots were taken out of the test solutions (De Schampelaere et al., 2005), and then washed with deionized water. Before being dried at 80 °C for further analysis, the longest root length of individual seedlings was measured and the relative root elongation (RRE, % of the control) was calculated as the toxicological endpoint:

$$RRE(\%) = \frac{L_a}{L_c} \times 100 \quad (1)$$

where L_a (cm) is the average longest root of the four seedlings exposed to REMs, and L_c (cm) is the mean longest root of the control treatments.

2.4. Chemical measurements and speciation calculations

For REMs analysis, *aqua regia* was used to digest the dried plant roots. Inductively coupled plasma optical emission spectrometry (ICP-OES, iCAP7600, Thermo Fisher) was used to determine the concentration of La, Ce, and other cations (Na, Mg, K, and Ca, etc) in the test solutions and in the plant root digests. These measured metal concentrations were used for all speciation modelling calculations. The detection limits of La and Ce by ICP-OES were 10 and 50 µg/L, respectively. A calibration standard (Multi-Element Calibration Std. #1, J&K Scientific Ltd.) was used as the ICP-OES standard reference in analyzing the sample. Two certified reference samples, diluted Multi-Element Calibration Std. #1 (AG-MECAL1-ASL-5) with certified La or Ce concentrations of 0.01 and 5 mg/L, respectively, were analyzed at the beginning and at the end of each series of La or Ce measurements. Measured La or Ce concentrations were within 85–115% of the certified reference value. A pH meter (Mettler Delta 320, Switzerland) was used to determine the pH values of the test solutions at the beginning and the end of toxicity tests. The WHAM VII (CEH, UK) was used to calculate the free ion activities of REMs (La and Ce) and major cations in the test solutions (Tipping et al., 2011). Input parameters for WHAM VII included the mean pH values, temperature (20 °C), $p\text{CO}_2$ ($10^{-3.5}$ atm), and dissolved concentrations of elements and anions in the test solutions (for an example of speciation calculation see Table S6).

2.5. Data analysis and modeling

A log-logistic function was selected to describe the RRE of wheat exposed to a single REMs in test solutions in Origin 9.0 (Haanstra et al., 1985):

$$RRE(\%) = \frac{A}{1 + \left(\frac{c_i}{EC50}\right)^{\beta_i}} \quad (2)$$

where A represents the average response of the control, c_i represents different dose expressions, β_i represents the slope of the dose-response curve, and $EC50$ represents the dose inducing 50% toxic effect.

2.5.1. Multi-metal biotic ligand model for predicting toxicity

Based on the BLM theory, considering the competing cations X^{Z+} (Ca^{2+} , Mg^{2+} , Na^+ , K^+ and H^+), the fraction (f) of the BLs bound by an REMs ion (M^{3+}) is described by

$$f_{MBL} = \frac{K_{MBL} \times \{M^{3+}\}}{1 + K_{MBL} \times \{M^{3+}\} + \sum K_{XBL} \times \{X^{Z+}\}} \quad (3)$$

where K_{MBL} and K_{XBL} are the stability binding constant of M^{3+} and X^{Z+} with the BLs, respectively, and curly brackets $\{\}$ represent free ion activities (mol/L).

When f_{MBL} is used as the dose descriptor in Eq. (2), REMs toxicity can be described as follows:

$$RRE(\%) = \frac{A}{1 + \left(\frac{f_{MBL}}{f_{MBL}^{50\%}}\right)^{\beta_i}} \quad (4)$$

The estimations of the BLM parameters ($\log K_{XBL}$, $\log K_{MBL}$, β_i , and $f_{MBL}^{50\%}$) are based on the global fitting of the entire set of single La and Ce toxicity data together to Eq. (4). The optimal model parameters can be obtained based on the best correlation between the predicted and the observed RRE by solving the values of $\log K_{XBL}$, $\log K_{MBL}$, β_i , and $f_{MBL}^{50\%}$ to minimize the root-mean-square error (RMSE) in JMP 16.0 (SAS institute) (Wang et al., 2010). The obtained stability binding constants ($\log K_{XBL}$ and $\log K_{MBL}$) from single-REMs BLMs are directly used for the mixture scenarios.

It is assumed that competition dominates interactions between La^{3+} and Ce^{3+} , and an extended BLM was developed to predict mixture toxicity:

$$f_{mix} = \frac{K_{LaBL} \times \{\text{La}^{3+}\} + K_{CeBL} \times \{\text{Ce}^{3+}\}}{1 + K_{LaBL} \times \{\text{La}^{3+}\} + K_{CeBL} \times \{\text{Ce}^{3+}\} + \sum K_{XBL} \times \{X^{Z+}\}} \quad (5)$$

where f_{mix} indicates the fraction of BLs occupied by La^{3+} and Ce^{3+} ; K_{LaBL} , K_{CeBL} , and K_{XBL} are the stability binding constants of La^{3+} , Ce^{3+} and X^{Z+} to the BLs (L/mol), respectively. When f_{mix} was used as the dose descriptor in Eq. (2), REMs mixture toxicity can be linked to f_{mix} :

$$RRE(\%) = \frac{A}{1 + \left(\frac{f_{mix}}{f_{mix50}}\right)^{\beta_i}} \quad (6)$$

The estimations of the extended BLM parameters (β_i , and f_{mix50}) are based on fitting the La and Ce mixture toxicity data to Eq. (6).

2.5.2. Langmuir-type model for predicting uptake

A basic Langmuir equation was used to predict the uptake of REMs based on the free ion activity in the test solutions:

$$c_{root} = \frac{K_{MS} \times \{M^{3+}\} \times C_{max}}{1 + K_{MS} \times \{M^{3+}\}} \quad (7)$$

where C_{root} is the concentration of REMs accumulated in the root (mol/g dry root weight), C_{max} is the concentration of REMs at saturation (mol/g dry root weight), curly brackets $\{\}$ represent free REM ion activities (M), K_{MS} is the binding constant of REMs for uptake sites (L/mol). The estimations of C_{max} and K_{MS} are based on fitting single La or Ce toxicity data to Eq. (7).

The Langmuir-type equation can be extended to Eq. (8) to describe the role of competing cations on REMs uptake:

$$c_{root} = \frac{K_{MS} \times \{M^{3+}\} \times C_{max}}{1 + K_{MS} \times \{M^{3+}\} + \sum K_{XS} \times \{X^{Z+}\}} \quad (8)$$

where $\{X^{Z+}\}$ is the free ion activity of competing cations (M), K_{XS} is the binding constant of competing cations for uptake sites (L/mol). The estimations of C_{max} and K_{XS} are based on globally fitting the entire single La and Ce toxicity data together to Eq. (8).

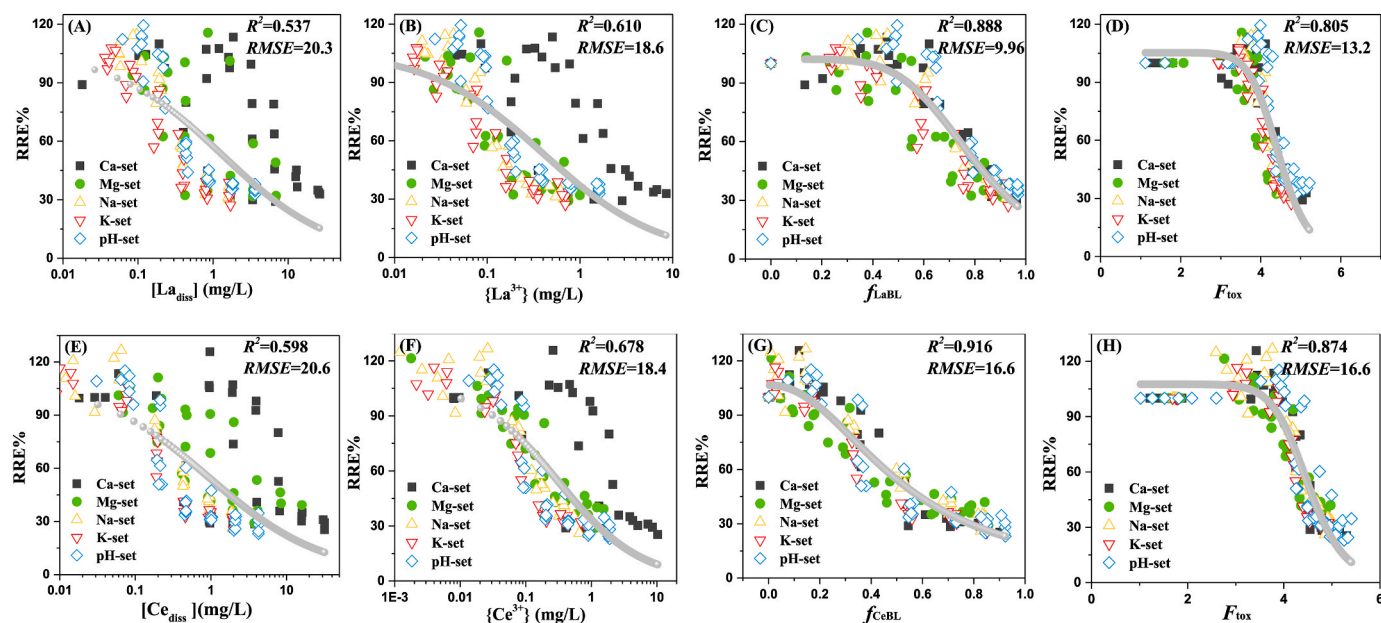


Fig. 1. Dose response relationships for the effect of single REM (La and Ce) on the relative root elongation (% of control) of *Triticum aestivum* L. after 4-d exposure in different solutions. Dose-response relationships based on dissolved concentration (A, E), free REM ion activity (B, F), the fraction of biotic ligand sites occupied by REM ions (La^{3+} and Ce^{3+}) (C, G) and the toxicity function (D, H). Data points represent the observed values. The grey dotted lines represent the fit of the logistic regression model (Eq. (2)) based on all data.

Table 1

Summary of optimum parameters using the BLM and the WHAM- F_{tox} approach for the toxicity and bioaccumulation of single REM (La and Ce) to *Triticum aestivum* L. based on free ion activities.

Model	H	Ca	Mg	La	Ce
BLM		$\log K_{\text{Ca}}$	$\log K_{\text{Mg}}$	$\log K_{\text{La}}$	$\log K_{\text{Ce}}$
Toxicity		3.87 (0.03) ^a	3.59 (0.06)	6.97 (0.02)	6.48 (0.02)
Uptake		3.23 (0.04)	2.84 (0.02)	6.07 (0.04)	5.27 (0.01)
WHAM- F_{tox}	α_{H}			α_{La}	α_{Ce}
	1.00			3.38	3.43

^a Standard errors are indicated in brackets.

2.5.3. WHAM- F_{tox} model

The basis of this model is the assumption that the toxicity of REMs can be described by a linear toxicity function F_{tox} (mol/g) (Tipping and Lofts, 2013):

$$F_{\text{tox}} = \sum \alpha_i \nu_i \quad (9)$$

where ν_i (mol/g) is the concentration of REMs ions or proton bound to particulate HA, which is calculated by WHAM VII, and α_i (dimensionless) represents the toxicity coefficient. The concentration of particulate HA was assigned at a sufficiently low level (5.0×10^{-6} g/L) to calculate ν_i while avoiding to affect the REMs speciation (Stockdale et al., 2010). It should be noted that only the relative values of ν_i rather than the absolute values are of interest in this approach.

Specifically, for the binary mixture of La and Ce in the present study:

$$F_{\text{tox}} = \alpha_{\text{H}} \nu_{\text{H}} + \alpha_{\text{La}} \nu_{\text{La}} + \alpha_{\text{Ce}} \nu_{\text{Ce}} \quad (10)$$

When F_{tox} was used as the dose descriptor in Eq. (2), the mixture toxicity of REMs can be described as follows:

$$\text{RRE}(\%) = \frac{100}{1 + \left(\frac{F_{\text{tox}}}{F_{\text{tox}50\text{-mix}}} \right)^{\beta_i}} = \frac{100}{1 + \left(\frac{\alpha_{\text{H}} \nu_{\text{H}} + \alpha_{\text{La}} \nu_{\text{La}} + \alpha_{\text{Ce}} \nu_{\text{Ce}}}{F_{\text{tox}50\text{-mix}}} \right)^{\beta_i}} \quad (11)$$

where $F_{\text{tox}50\text{-mix}}$ is the $F_{\text{tox,mix}}$ giving 50% inhibition of root elongation. The estimations of ν_i and α_i are based on globally fitting the entire single and binary mixture toxicity data together to Eq. (11).

To compare the outcomes of different models, the AIC was introduced. The AIC considers both the model fitness and the number of parameters to evaluate the model performance (Burnham and Anderson, 2004). It can be described as follows:

$$\text{AIC} = N \log \left(\frac{\text{SS}}{N} \right) + 2n \quad (12)$$

where N represents the sample size, SS represents the residual sum of square, and n represents the total number of model parameters.

The linear regression analysis was performed to determine the relationship between the model predicted and observed response. In the present study, RMSE was used as the indicator of model deviation for all the models.

3. Results and discussion

3.1. Prediction of individual toxicity of La/Ce

The different dose descriptors ($[\text{M}_{\text{diss}}]$, $\{\text{M}^{3+}\}$, f_{MBL} and F_{tox}) are used to predict the toxicity of single La and Ce under different exposure conditions (Fig. 1). Of the four models tested, an increasing correlation to RRE with the deeper understanding of bioavailability was observed: f_{MBL} or $F_{\text{tox}} > \{\text{M}^{3+}\} > [\text{M}_{\text{diss}}]$. It is no surprise that the use of dissolved concentration as dose was insufficient to normalize toxicity variations without taking REMs bioavailability into account (Qiu and He, 2017). The better model fits were obtained with free ion activity as a basis, supporting that free REMs ions were the dominant toxic species. This also complies with the assumption of the BLM (Ardestani et al., 2013). Satisfying fits were obtained by applying BLM or WHAM- F_{tox} theory. This indicates that these theories are applicable for quantifying trivalent REMs toxicity considering other toxicity-modifying factors. The values of $\text{EC}_{50}\{\text{La}^{3+}\}$ increased linearly from 3.00 to 24.2 μM with an increase of $\{\text{Ca}^{2+}\}$ ($R^2 = 0.931$, $p < 0.01$, Fig. S1A and Table S2) and from 1.29 to 3.21 μM with an increase of $\{\text{Mg}^{2+}\}$ ($R^2 = 0.692$, $p < 0.05$, Fig. S1B and

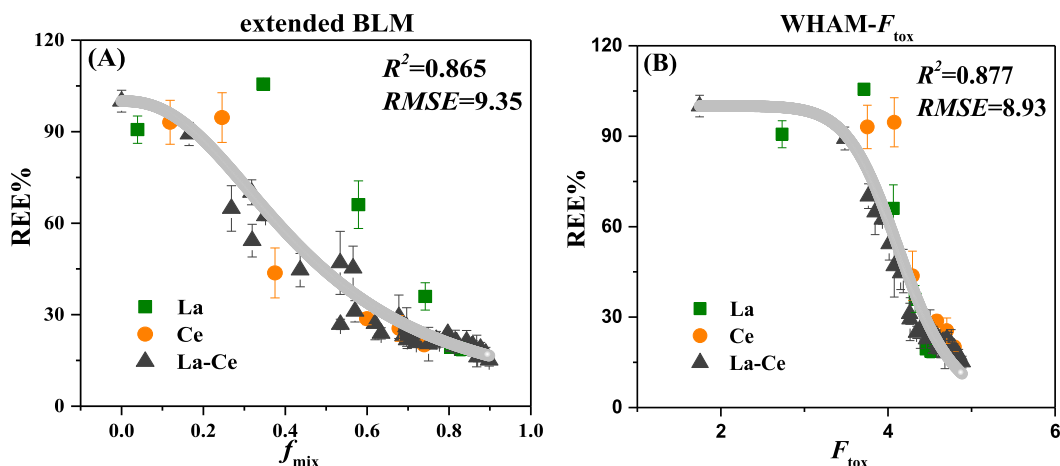


Fig. 2. Relationships between the relative root elongation (% of control) of *Triticum aestivum* L. exposed to La and Ce mixtures for 4-d, and f_{mix} (A), as well as F_{tox} (B). The data points represent the observed values. The grey dotted line represents the fit for all toxicity data of a logistic dose-response model.

Table S2). However, there were no consistent linear correlations between the $\{Na^+\}$, $\{K^+\}$, and $\{H^+\}$ and the $EC_{50}\{La^{3+}\}$ (Fig. S1C and D). Therefore, competition between Na^+ , K^+ , H^+ and La^{3+} for binding sites on wheat roots could be ignored when the BLM was developed. Our previous study showed a consistent competition effect of Ca^{2+} and Mg^{2+} on Ce toxicity, while Na^+ , K^+ , and H^+ did not affect Ce toxicity (Gong et al., 2019). The developed BLM, which incorporates the competition between Ca^{2+} , Mg^{2+} and La^{3+}/Ce^{3+} yielded the best fits with $R^2 = 0.888$, $RMSE=9.96$ for La and $R^2 = 0.916$, $RMSE=16.6$ for Ce. The obtained values of $\log K_{CaBL}$, $\log K_{MgBL}$, $\log K_{LaBL}$ and $\log K_{CeBL}$ were 3.87, 3.59, 6.97 and 6.48, respectively (Table 1). The optimal values of the BLM were obtained as follows: $f_{LaBL}^{50\%} = 0.803$, $f_{CeBL}^{50\%} = 0.511$, $\beta_{La} = 5.18$, and $\beta_{Ce} = 2.18$ (Table S4).

Statistically significant influences of Ca^{2+} and Mg^{2+} on La/Ce toxicity were observed in the present study, indicating that competition indeed occurred between REM ions (La^{3+} or Ce^{3+}) and cations (Ca^{2+} and Mg^{2+}) for binding to the transport sites or sites of toxic action on the root surfaces. Similarly, the alleviations of samarium (Sm) uptake by alga (*Chlamydomonas reinhardtii*) by Ca^{2+} and Mg^{2+} were found by Tan et al. (2017), which can be attributed to the competition between Ca^{2+} , Mg^{2+} and Sm^{3+} for transport sites. The stronger interaction of Ca^{2+} with Sm indicates that Ca transporters are crucial in the internalization of REMs. In the present study, the higher affinity of Ca^{2+} ($\log K_{CaBL} = 3.87$) for the BLs is consistent with the observed stronger impacts of Ca^{2+} on toxicity of La^{3+} and Ce^{3+} compared with Mg^{2+} . Our observation can be attributed to the more similar ionic radius between Ca^{2+} and REMs ions (Das et al., 1988). Generally, REMs are considered non-essential elements in plants, whereas they may disturb the Ca-mediated physiological processes through competing with Ca in Ca channels. Based on their trivalent charges and resulting higher charge density, the divalent Ca with a relatively lower charge density can likely be displaced by REMs at biological binding sites (Thomas et al., 2014). Brown et al. (1990) reported that REMs induced inhibition of various enzymes and other functional proteins by replacing Ca from extra-cellular binding sites, which in turn induced adverse effects on the plant. Sneller et al. (2000) also reported that the toxicity mechanisms of REMs include competition and/or replacement with Ca/Mg.

Although the BLM can provide satisfactory predictions for La and Ce toxicity, it should be noted that such a model requires a large amount of data to obtain all model parameters. The WHAM- F_{tox} approach was therefore proposed here as a surrogate to the BLM with three main advantages (Le and Peijnenburg, 2017). First, it owns superiority of handling less data due to the already existing binding constants for a large number of metal cations in WHAM. Second, it has the potential of application to various plant species. Third, it accounts for both

electrostatic interactions and specific binding at heterogeneous binding sites. This approach was initially developed to describe mixture toxicity of metals and protons to aquatic organisms. Later, the WHAM- F_{tox} approach has been used to predict metal toxicity to terrestrial organisms. For example, its applicability to quantify adverse effects of Cu on the root elongation of *Lemna minor* has been demonstrated (Antunes et al., 2012). The toxicity of Ni and Co to *Enchytraeus crypticus* at different exposure times was successfully predicted by the WHAM- F_{tox} approach (He and Van Gestel, 2015). In the present study, the WHAM- F_{tox} approach is applied for the first time to describe REMs toxicity. This model predicted REMs toxicity reasonably well with $R^2 = 0.805$, $RMSE=13.2$ for La and $R^2 = 0.874$, $RMSE=16.6$ for Ce (Fig. 1 and Table S4). The parameters α_H , α_{La} and α_{Ce} of the WHAM- F_{tox} model were obtained as follows: $\alpha_H = 1.00$, $\alpha_{La} = 3.38$ and $\alpha_{Ce} = 3.43$ (Table 1). The optimal values of the WHAM- F_{tox} were obtained as follows: F_{tox50} (La) = 4.45 mol/g, F_{tox50} (Ce) = 4.50 mol/g, $\beta_{La} = 11.8$, and $\beta_{Ce} = 11.8$ (Table S4). Our results demonstrated the capacity of the WHAM- F_{tox} approach to effectively normalize the effect of other coexisting major cations for La or Ce with at least 80.5% of the variance in toxicity explained. The estimated toxicity coefficient α_{Ce} was slightly larger than α_{La} , suggesting that Ce was more toxic to *T. aestivum* than La.

It is commonly accepted that only bioavailable metals can cause a toxic effect (Shahid et al., 2012). Available evidence demonstrates that the internal metal concentration of organisms can represent metal bioavailability (Van Straalen et al., 2005). Besides, metals bioavailability also depends upon the exposure duration, the environment conditions, physicochemical variables and metal speciation. The concentration of metal taken up is expected to be a better indicator of metal toxicity than the free ion activity (He et al., 2014). Our results showed that when relating La/Ce uptake concentrations to RRE under different exposure scenarios with the log-logistic model, the resulting R^2 and $RMSE$ values were 0.828 and 12.3 respectively for La, and 0.855 and 12.4 respectively for Ce (Fig. S3). This finding reflected that REMs toxicity to *T. aestivum* can be well described with REMs accumulation regardless of exposure scenarios.

3.2. Prediction of the uptake of single La/Ce (with varying Ca, Mg, Na, K and pH levels)

The measured data on La or Ce accumulation in *Triticum aestivum* was fitted to the basic Langmuir model (Eq.(8)) for single REMs (Fig. S2). The obtained of R^2 values were 0.713 and 0.752 for La and Ce, respectively. Linear regression relationships between the predicted and measured accumulation of REMs in plant roots based on the BLM-based extended Langmuir model (Eq. (9)) are shown in Fig. 3. Considerable

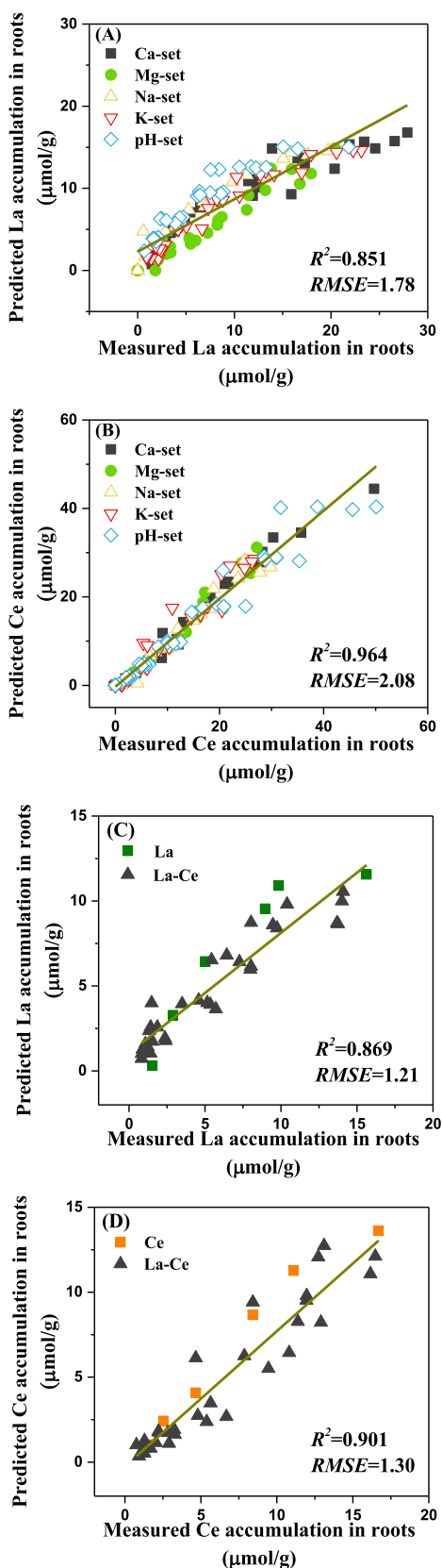


Fig. 3. Linear regression relationships between the predicted and measured REM accumulation in roots of *Triticum aestivum* L. after 4-d exposure to single REMs (A, B) and in REMs mixture (C, D) based on a BLM-based extended Langmuir model which corrected for the competing effects of Ca^{2+} , Mg^{2+} (A, B), of Ca^{2+} , Mg^{2+} , Ce^{3+} (C) and of Ca^{2+} , Mg^{2+} , La^{3+} (D) (Eq. (9)).

improvement in model fits were observed with $R^2 = 0.851$, $RMSE=1.78$ for La and $R^2 = 0.964$, $RMSE=2.08$ for Ce in single REM exposures. The resulting binding constants $\log K_{\text{CaS}}$, $\log K_{\text{MgS}}$, $\log K_{\text{LaS}}$ and $\log K_{\text{CeS}}$ were 3.23, 2.84, 6.07 and 5.27, respectively (Table 1). In the present study, REM ions alone were not sufficient to explain the overall variation of REMs accumulation in *T. aestivum* exposed to test solution with various chemistry. When the extended Langmuir model was developed to predict REMs uptake, the R^2 values were improved when considering the competitive cations (Ca^{2+} and Mg^{2+}). This indicates that the competition effect of Ca^{2+} and Mg^{2+} also plays an important role in REMs uptake. In previous publications, He et al. (2014) reported a competition effect of cations (Ca^{2+} , Mg^{2+} and Na^{+}) on Ni uptake to *E. crypticus*. The pH and coexisting cations were demonstrated to influence Cu uptake by plants (Chen et al., 2013). Goecke et al. (2015) reported that Ca inhibited lanthanides uptake by algae. Generally, metals enter organisms via the uptake pathways of essential channels or carriers for cations (Bridges and Zalups, 2005). The binding constants of $\log K_{\text{CaS}}$ (3.23) and $\log K_{\text{MgS}}$ (2.84) are dissimilar to previous observations ($\log K_{\text{CaS}} = 4.5$ (Kola and Wilkinson, 2005), $\log K_{\text{CaS}} = 4.0$ (Lavoie et al., 2012), $\log K_{\text{CaS}} = 4.8$ (Francois et al., 2007), $\log K_{\text{CaS}} = 3.0$ and $\log K_{\text{MgS}} = 5.1$ (Worms and Wilkinson, 2007), $\log K_{\text{CaS}} = 2.6$ and $\log K_{\text{MgS}} = 3.4$ (Yang and Wilkinson, 2018)). A possible explanation for these discrepancies is that the estimated binding constants vary across test organisms.

According to our results, the toxicity-modifying cations are the same as the uptake-modifying cations. The competition between metal ions and cations for BLs can inhibit metal uptake. Borgmann et al. (2008) proposed that the location of the site of toxic action plays a significant role in describing toxicity based on the uptake concentration. In their study, metals are found to enter the organism through the same uptake pathways if the sites of toxic action are close to the surface of the organisms, while they may pass through completely different uptake pathways if the sites of toxic action are inside the organisms. Our results showed that there were no significant differences between $\log K_{\text{CaS}}$ and $\log K_{\text{CaBL}}$ and between $\log K_{\text{MgS}}$ and $\log K_{\text{MgBL}}$ (Table 1), which suggests that the protective effects of Ca and Mg on REMs toxicity were mainly due to competition at the transport sites on the surface of wheat root.

The computed amounts of metal ions binding to HA based on WHAM have been recognized as promising indicators of their accumulation in Enchytraeidae (He and Van Gestel, 2015) and in plant roots (Le et al., 2015). The linear regression relationships between La/Ce bound to HA and the measured La/Ce accumulation in roots of *Triticum aestivum* L. exposed to La, Ce and their binary mixtures are shown in Fig. 4. Generally, the estimated HA-bound REMs concentrations were lower than the measured REMs concentrations in both single and mixture exposures. By applying the WHAM- F_{tox} approach to predict single REMs uptake, the obtained of R^2 values were 0.764 and 0.810 for La and Ce, respectively. The AIC scores of these two models indicated that the performance of WHAM- F_{tox} was similar to that of BLM in assessing the uptake of single La/Ce (Table S5). Our results showed that HA-bound Ce well predicted the Ce accumulation in plant roots, with 81.0% of the variance in the data explained. A slightly weak performance was observed when predicting La uptake ($R^2 = 0.764$) in single metal exposures. The explained variance increased from 76.4% to 85.3% for La accumulation and from 81.0% to 88.4% for Ce accumulation when excluding the pH-set data (data not shown). This suggested that the pH effect cannot be predicted appropriately by nonspecific competition between REM ions and H^{+} at the reversible binding sites. Changes in pH might influence REMs uptake process by altering the protonation of the binding sites (Bervoets and Blust, 2000). In the bulk solution, variations in pH would result in changes in the root surface potential, which in turn might influence the partitioning of the REM ions between the root surface and bulk solution (McLaughlin, 1977), and hence affect the trans-membrane potential (Smith and Raven, 1979). Moreover, under changing pH conditions, REMs might undergo different speciation which might have different behavior regarding uptake. Not only free REM ions but also the formation of other REM species (e.g.,

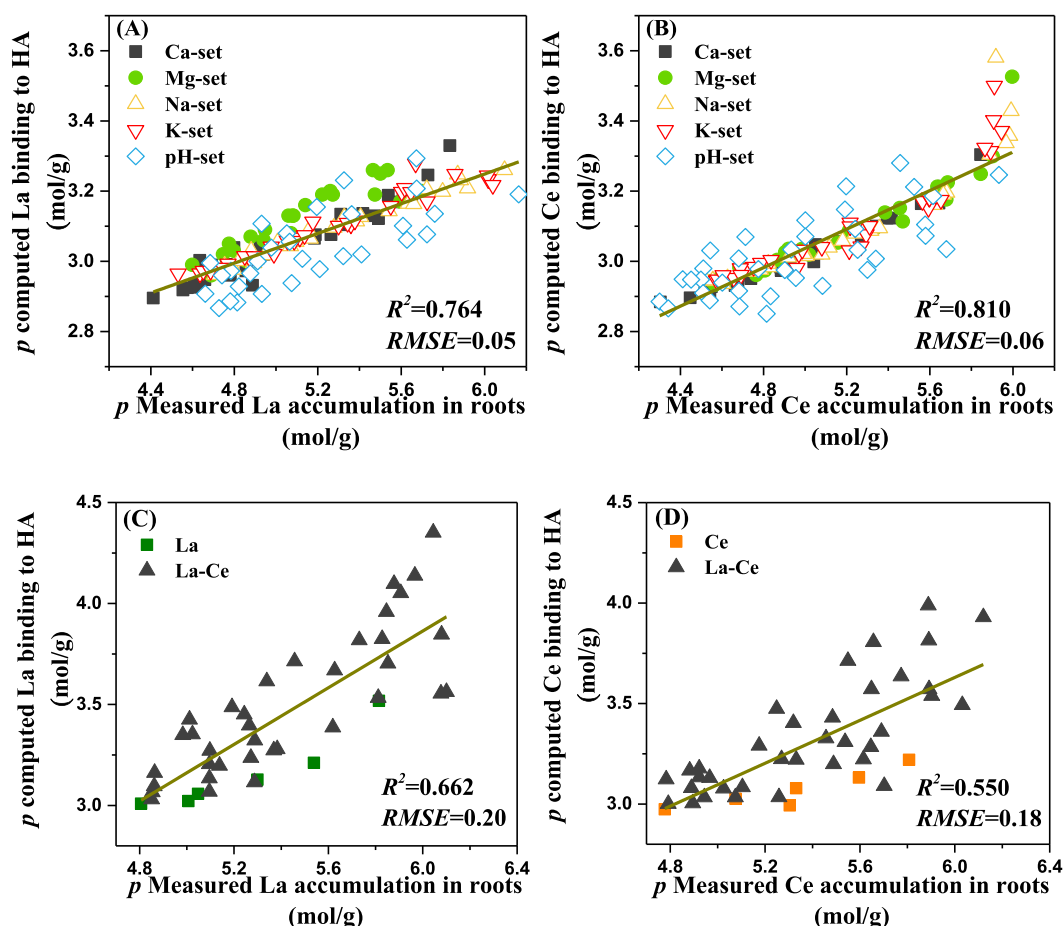


Fig. 4. Linear regression relationships between the concentrations of REMs (La and Ce) bound to humic acid calculated using WHAM- F_{tox} and measured REMs accumulations in roots of *Triticum aestivum* L. after 4-d exposure in single REMs (A, B) and in REMs mixture (C, D).

Table 2

Summary of dose-response parameters (EC50 and β) and model fits (R^2 and RMSE) for La and Ce mixture using the fraction of biotic ligands sites occupied by REM ions and the toxicity function as dose^c resp^dctively.

	La-Ce			
	f_{mix50}^a	β^b	R^2^c	RMSE ^d
fmixrowhead	0.452 (0.0) ^e	2.36 (0.22)	0.865	9.35
rowhead	$F_{tox50,mix}$ (mol/g) ^f	β	R2	RMS ^e
Ftox_mixrowhead	4.16 (0.03)	12.8 (1.06)	0.877	8.93

^a The f_{mix50} is the^efraction of biotic ligands occupi^{bd} by La³⁺ and Ce³⁺ that cause 50% growth reduction.

^b β is the model slope.

^c R^2 indicates the goodness of fit.

^d RMSE represents the root-mean-squared error associated with the predicted response.

^e Standard errors are indicated in brackets.

^f $F_{tox50,mix}$ (mol/g) is the toxicity function $F_{tox,mix}$ producing a 50% growth reduction.

hydroxo-complexes) might be responsible for the accumulation of REMs in the range of pH-values studied (Cremazy et al., 2013). Our results showed that WHAM- F_{tox} approach provided reasonable performance (with $R^2 \geq 0.764$) for estimating La/Ce concentrations in wheat roots with fewer parameters when compared with the BLM-based model.

3.3. Modeling toxicity of La-Ce mixtures

The mixture toxicity data of REMs were analyzed using the extended

BLM and the WHAM- F_{tox} approach, respectively (Fig. 2). The estimated values of the parameters (EC50 and β) and the model fits (R^2 and RMSE) for La-Ce mixture are listed in Table 2. Based on the EC50 values, the combined toxicity was larger than that for individual element, which was attributed to their interactive effects. Assuming that competition acts as a mechanism for metal mixture interactions (Soumya and Wood, 2004), the use of BLM-based approaches for interpreting mixture effects is suitable. For example, the extended BLM approach has been applied successfully for quantifying mixture toxicity of Cd and Pb at different concentrations of Ca, which can be attributed to the fact that this model takes into account not only the competition between Cd and Pb but also the competition between Ca²⁺ and Cd or Pb for BLs (Jho et al., 2011). Attempts have been made to develop extended BLMs for soil invertebrates (earthworms and enchytraeids) (Li et al., 2008; Lock et al., 2006; Steenbergen et al., 2005) and plants (Li et al., 2009; Lock et al., 2007; Wang et al., 2017; Wu et al., 2017; Versieren et al., 2014) in solution or in solution-sand systems. These studies showed that the application of the extend BLM in predicting metal mixture toxicity to terrestrial organisms is theoretically and empirically feasible. It is expected that the derived stability binding constants from single exposure are applicable to mixture scenarios, which can avoid a huge amount of experiments to determine the parameters for metal mixtures. In our study, the extended BLM in which the La-Ce interactions and the competitive effects of Ca²⁺ and Mg²⁺ were included, explained 86.5% of the variance in the data on La-Ce mixture toxicity with the parameters estimated from single REMs toxicity data. Similar to our results, some publications have verified the extended BLM for predicting mixture toxicity with the derived parameters from toxicity data of single metals (Hatano and Shoji, 2008; Jho et al., 2011). Our findings demonstrated

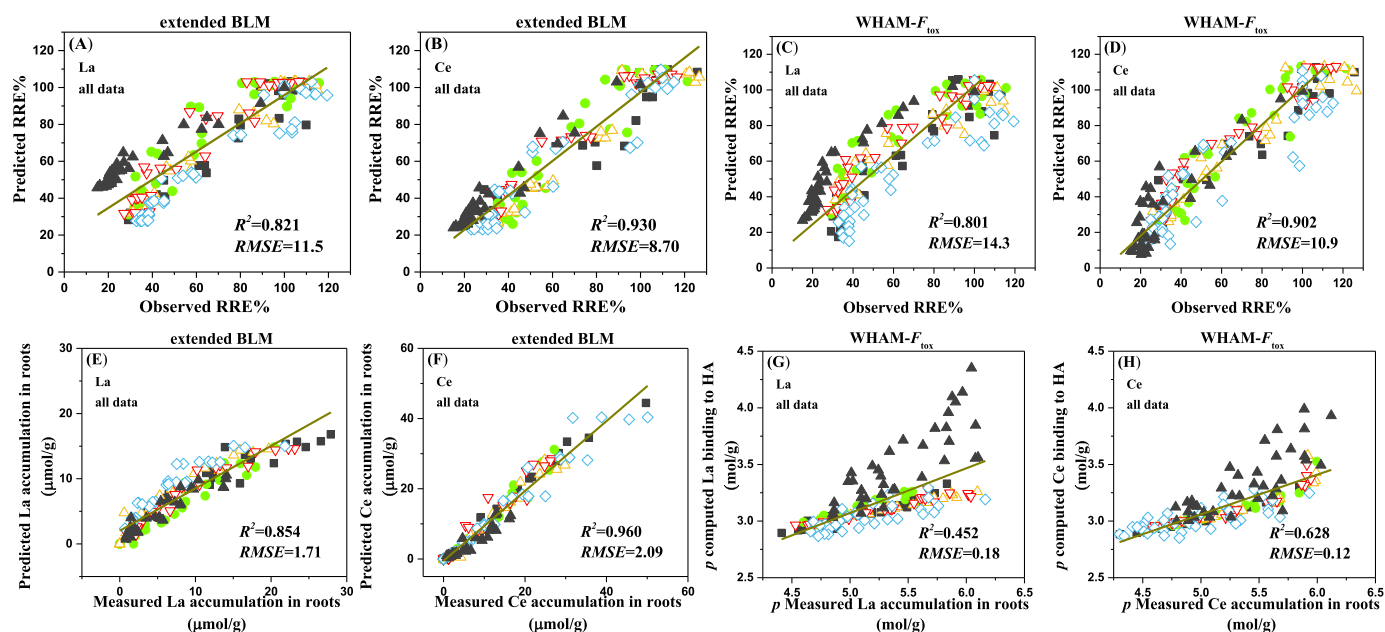


Fig. 5. Linear regression relationship between the observed and predicted relative root elongation (% of control) of *Triticum aestivum* L. after 4-d exposure for all sets together based on the BLM-based extended Langmuir model (A, B) and the WHAM- F_{tox} approach (C, D). Linear regression relationship between the measured and predicted REM accumulations in roots of *Triticum aestivum* L. after 4-d exposure for all sets together based on the BLM-based extended Langmuir model (E, F) and the WHAM- F_{tox} approach (G, H).

the capacity of the cation competition theory to effectively predict REMs mixture toxicity (Fig. 2 and Table 2). When relating the observed and predicted RRE of *Triticum aestivum* L. for all data sets together with the extended BLM, the resulting R^2 values were 0.821 for La and 0.930 for Ce (Fig. 5A and B).

Compared with BLM, the WHAM- F_{tox} approach provides a simpler option for predicting mixture toxicity without performing a mass of univariate experiments to determine model parameters. The toxicity function F_{tox} was determined by the values of ν_1 and α_1 . The contribution of metal ions and protons to toxicity was considered strictly additive. Therefore, the linear toxicity function F_{tox} can be readily extended to describe the toxicity of mixtures. Qiu et al. (2015) reported that the WHAM- F_{tox} approach can successfully predict mixture toxicity to plants across different soils. Our results showed that on the basis of $F_{tox,mix}$ as the dose descriptor, the WHAM- F_{tox} approach explained 87.7% of the observed variations in La-Ce mixture toxicity. When the WHAM- F_{tox} approach was applied to all toxicity data together, the resulting R^2 values were 0.801 for La and 0.902 for Ce, which were comparable to the performance of the extended BLM (Fig. 5C and D). Hence, the WHAM- F_{tox} approach is recommended as a potential surrogate to BLM to effectively quantify mixture toxicity of REMs.

3.4. Modeling uptake of La-Ce mixtures

The BLM has been extensively used to analyze uptake of mixtures of divalent metal ions by different species (Brix et al., 2017; Chen et al., 2010; Cremazy et al., 2019; Niyogi et al., 2015). For example, Pb and Cu uptake in the green alga *Chlamydomonas reinhardtii* after exposure to a mixture of these metals could properly be interpreted by the BLM (Chen et al., 2010). Another research showed that the BLM has the potential to explain the metal uptake of most combinations of binary mixtures (Ag, Cd, Cu, Ni, Pb and Zn) in the great pond snail *Lymnaea stagnalis* (Cremazy et al., 2019). In the present study, the BLM-based extended Langmuir model based on estimated parameters from single REMs exposures was used to predict REMs uptake after exposure to a mixture of La and Ce. This model successfully predicted uptake of the La-Ce mixtures with $R^2 = 0.869$, $RMSE=1.21$ for La and $R^2 = 0.901$, $RMSE=1.30$

for Ce (Fig. 3C and D), confirming that the competition of REMs and cations for uptake sites plays an important role in REMs uptake. When relating the measured and predicted REMs accumulation in plant roots for all data sets together with the extended Langmuir model, the resulting R^2 (or $RMSE$) values were 0.854 (or 1.71) for La and 0.960 (or 2.09) for Ce (Fig. 5E and F). Therefore, the BLM-based model can offer a suitable conceptual framework for quantifying REMs mixtures uptake.

The WHAM- F_{tox} approach provides a different perspective of quantifying and rationalizing toxicity and uptake of metal mixtures. A previous publication has verified that the WHAM- F_{tox} approach has the potential to describe the uptake of metal mixtures, offering a satisfying prediction exposed to metal mixtures (Zn, Cd and Cu) on macro-invertebrates under varying water chemistries (Iwasaki et al., 2013). In contrast, Qiu et al. (2015) reported that the WHAM- F_{tox} approach failed to delineate Cu-Ag toxicity to *Lactuca sativa*, indicating that the competition assumption between Cu^{2+} and Ag^+ is invalid. The predictive ability of the WHAM- F_{tox} approach may differ for different binary metal mixtures. In general, the HA-proxy depends on the chemical properties of the exposure conditions (hydroponic/soil solution) by affecting the amount of metals available for binding to HA and the characteristics of the root surface (Huang et al., 2013). Several of the discrepancies in the model predictive capacity can be interpreted by the model's approximation of metal mixture accumulation using HA as a proxy, or by oversimplification of the relationship between accumulated cations binding to HA and mixture accumulation (Tipping and Lofts, 2015). Except for the competition, other interaction mechanisms (e.g. electrostatic theory) might be responsible for the mixed effects that were observed (Wang et al., 2013). Based on our findings, the WHAM- F_{tox} approach provided relatively weak model fits ($R^2 = 0.662$ for La and $R^2 = 0.550$ for Ce) (Fig. 4C and D). When the WHAM- F_{tox} approach was applied to all uptake data together, the resulting R^2 values were 0.452 for La and 0.628 for Ce (Fig. 5G and H). This indicates that the WHAM- F_{tox} approach is unsuitable for delineating the uptake of La-Ce mixtures with the relatively low model performance. Our results stressed that the WHAM- F_{tox} approach can successfully describe the toxicity but not the uptake of La-Ce mixtures. Caution is thus needed when using this method to interpret the uptake of REMs in mixture

scenarios.

4. Conclusions

The results showed that Ca^{2+} and Mg^{2+} significantly alleviate the toxicity and uptake of La/Ce. Our results proved the effectiveness of the multi-metal BLM framework and demonstrated its feasibility for interpreting toxicity and uptake of single trivalent La/Ce ions (in the presence of various levels of other major cations) and La–Ce mixture exposure. Except for quantifying the uptake of La–Ce mixture, the bioavailability-based WHAM- F_{tox} approach can offer a similar level of precision with the BLM while requiring fewer model parameters. We thus recommend the WHAM- F_{tox} approach as an alternative method in describing individual/mixture toxicity and individual uptake of REMs. Our results provided basic data for establishing relevant environmental quality criteria for REMs. Given that metal bioavailability affected by interactive effects is a dynamic process, further research on exploring the toxicity and accumulation of REMs in the dynamic environment is required. This contributes to better understanding of the mechanisms about REMs toxicity during toxicokinetic and toxicodynamic processes, and thus serves to build up a solid foundation for considering the bioavailability-based approaches into accurate risk assessment of impacts of REMs on organisms and human health.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (No. 41701573, No. 41701571, No. 41877500, and No. 41977115), Shanghai Rising-Star Program (No. 20QA1404500), the National Key R&D Program of China (No. 2018YFC1800600, No. 2018YFD0800700), the Research Fund Program of Guangdong Provincial Key Laboratory of Environmental Pollution Control and Remediation Technology (No. 2018K01), Science and Technology Program of Guangzhou, China (No. 201904010116), and Fundamental Research Funds for the Central Universities (No.19lgpy150).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecoenv.2020.111346>.

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