VU Research Portal

Dynamic interaction processes of rare earth metal mixtures in terrestrial organisms interpreted by toxicokinetic and toxicodynamic model

Gong, B.; He, E.; Van Gestel, C.A.M.; Tang, Y.; Yang, W.; Yang, J.; Li, Y.; Qiu, H.

published in

Journal of Hazardous Materials
2021

DOI (link to publisher)

[10.1016/j.jhazmat.2021.126281](https://doi.org/10.1016/j.jhazmat.2021.126281)

document version

Publisher's PDF, also known as Version of record

document license

Article 25fa Dutch Copyright Act

[Link to publication in VU Research Portal](#)

citation for published version (APA)

Gong, B., He, E., Van Gestel, C. A. M., Tang, Y., Yang, W., Yang, J., Li, Y., & Qiu, H. (2021). Dynamic interaction processes of rare earth metal mixtures in terrestrial organisms interpreted by toxicokinetic and toxicodynamic model. *Journal of Hazardous Materials*, 418, 1-10. [126281].
<https://doi.org/10.1016/j.jhazmat.2021.126281>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

E-mail address:

vuresearchportal.ub@vu.nl

Research Paper

Dynamic interaction processes of rare earth metal mixtures in terrestrial organisms interpreted by toxicokinetic and toxicodynamic model

Bing Gong^{a,b}, Er kai He^{a,c,*}, Cornelis A.M. Van Gestel^d, Yetao Tang^c, Wenjun Yang^c, Jing Yang^a, Ye Li^a, Hao Qiu^b

^a School of Geographic Sciences, East China Normal University, Shanghai 200241, China

^b School of Environmental Science and Engineering, Shanghai Jiao Tong University, Shanghai 200240, China

^c Guangdong Provincial Key Laboratory of Environmental Pollution Control and Remediation Technology, Sun Yat-sen University, Guangzhou 510006, China

^d Department of Ecological Science, Faculty of Science, Vrije Universiteit, De Boelelaan 1085, 1081HV Amsterdam, The Netherlands

ARTICLE INFO

Editor: Dr. C. LingXin

Keywords:

Rare earth elements

Toxicity

Interaction pattern

Toxicokinetics

Toxicodynamics

ABSTRACT

Despite the progress in explanation of mixture toxicity of rare earth elements (REEs), a large knowledge gap still exists in interpreting their mixed effects from a dynamic perspective. Here, we investigated the effects of La-Ce mixtures in *Enchytraeus crypticus* at different exposure times. The single and mixture toxicity of La and Ce increased with time, as reflected by the reduced LC50/MT50 values. With concentration addition as the reference model, the interactions between La and Ce were quantified by MIXTOX modelling tool, showing a time-dependent pattern with antagonistic effect after 1 and 2 d but additive effects afterwards. The dynamic accumulation and toxicity of La/Ce in organisms exposed to REE mixtures was fitted using a process-based toxicokinetic and toxicodynamic (TK-TD) model to unravel how the elements interacted. Generally, the estimated uptake, elimination, and damage rate constants of La/Ce declined with increasing level of each other, suggesting inhibited uptake and subsequently reduced toxicity of La/Ce due to competition effect. The interplay of La and Ce in TK and TD processes seemed responsible for the observed antagonism. Our study showed that mixture toxicity and interaction of REEs are time-dependent processes and application of TK-TD model may provide more insight into this dynamic effect.

1. Introduction

Rare earth elements (REEs) have been widely used in modern technologies, such as electronics, medicine, high-strength magnets and renewable energy, triggering a huge increasing demand for REEs (Alonso et al., 2012). Elevated emission of REEs to the environment takes place during their use and disposal processes (Migaszewski and Gatuszka, 2014). China tops the list for having the most abundant REEs and serves as the world's largest producer and exporter of REEs, meanwhile it endures high REE pollution levels (Alonso et al., 2012; Gonzalez et al., 2014). For example, Ganzhou of Jiangxi province is the largest heavy REEs production area, with the concentrations of REEs up to 3179 mg kg⁻¹ in tailing soils (Wei et al., 2001) and 928 mg kg⁻¹ in farmland soils (Liu et al., 2019). Bayan Obo mining district in Baotou of Inner Mongolia is the largest light REEs production area, with a mean REE concentration of 4670 mg kg⁻¹ around mine tailings, which is much higher than the average level of REEs in China (181 mg kg⁻¹) (Wang and

Liang, 2015).

The widespread occurrence of REEs in water and soil raises concerns regarding their potential adverse effects on ecosystem and human health. Although a stimulating or protective effect to microorganisms (Pol et al., 2014), plants (Agathokleous et al., 2018) and animals (Pagano et al., 2015) was reported when applying low dosage of REEs, a growing body of evidences have indicated that REEs were toxic to organisms at high dosages (Gonzalez et al., 2014; Gwenzi et al., 2018). Due to the geologic co-occurrence in feedstocks, the contamination of environmental mediums (e.g., sediment, water, soil) with REE mixtures has been reported in recent years and thereafter gained more attention (Amyot et al., 2017; Chao et al., 2016; Romero-Freire et al., 2018). Compared with the extensive ecotoxicity data for individual REEs, studies on the adverse effect of REE mixtures have been mainly focused on fungi (Bergsten-Torralba et al., 2020; d'Aquino et al., 2009), algae (Tai et al., 2010; Tan et al., 2017), microcrustaceans (Bergsten-Torralba et al., 2020), aquatic biota (Romero-Freire et al., 2019), and plants

* Corresponding author at: School of Geographic Sciences, East China Normal University, Shanghai 200241, China.

E-mail address: ekhe@geo.ecnu.edu.cn (E. He).

<https://doi.org/10.1016/j.jhazmat.2021.126281>

Received 30 March 2021; Received in revised form 14 May 2021; Accepted 29 May 2021

Available online 3 June 2021

0304-3894/© 2021 Elsevier B.V. All rights reserved.

(Gong et al., 2020a, 2020b; He et al., 2019; Li et al., 2020). Yet, our current understanding of the combined effects of REEs in the terrestrial environment, especially for soil organisms, is still lacking, which reinforces the demand for further research to obtain a full picture of their potential ecological risk.

For risk assessment of metal mixtures, the basic concept is that mixture toxicity can be predicted based on single component toxicity data. Within this framework, concentration addition (CA) and independent action (IA) are most commonly applied reference models for predicting mixture effects of chemicals, assuming similar or dissimilar modes of action of the elements in the mixture, respectively (Van Gestel et al., 2011). Conceptually, the CA and IA models assumed that mixture components do not interact with each other during the process of intoxication (Backhaus et al., 2000). The coexistence of substances in most cases can lead to an enhanced or diminished toxicity, which is defined as synergistic or antagonistic effects compared to the additive effects predicted by either the CA or the IA model (Vijver et al., 2010). As described above, either CA or IA models are not applicable for predicting the mixture toxicity of chemicals when interactions do occur. A mathematical modelling tool, MIXTOX, was further developed for detecting and quantifying deviations from the CA or IA models by incorporating additional interaction parameters, which could be estimated using a stepwise statistical data analysis procedure (Jonker et al., 2005).

To date, the available ecotoxicity data for REEs was mainly obtained from a static perspective. The accumulation and toxicity of chemicals, however, is a time-dependent process. Once the uptake rate of a given substance exceeds its excretion or detoxification rate, it will be accumulated in an organism, and eventually cause toxic effects when the critical body threshold is reached (McGeer et al., 2003). It has been shown that the combined effects and interaction patterns of metal mixtures differ between acute and chronic exposures, proving the importance of exposure time in metal mixture toxicity assessment (Nys et al., 2017). Conventional mathematical models based on the dose-response relationships of chemicals at fixed exposure times cannot explicitly take the temporal dimension of toxicity into consideration, obscuring the fact that mixture toxicity depends on exposure time (Baas et al., 2007; Cedergreen and Streibig, 2005). Hence, approaches that incorporate the factor of time are needed to improve the understanding of the dynamic process of metal mixture intoxication in organisms and the interpretation of the interactions taking place between the mixture components.

As an alternative to the mathematical-based methods, a process-based mechanistic toxicokinetic and toxicodynamic (TK-TD) model, which can simulate the time course of accumulation and toxicity of chemicals in organisms, is a promising tool to quantify dynamic metal toxicity (Ashauer and Escher, 2010). Toxicokinetics links the exposure concentration of a given substance to a body concentration, describing the time-course processes of uptake, distribution, biotransformation and elimination. Toxicodynamics builds bridges between the internal concentration and adverse effects on the organism, describing the time-course processes of damage. In this way, the process-based model provides an alternative tool for the real-time prediction of metal toxicity with more mechanistic and biological relevance by taking into account the bioaccumulation and corresponding toxicity of metals over time (Jager et al., 2011). TK-TD models have been increasingly used in simulating and predicting single metal toxicity over time (Cedergreen et al., 2017; Jager et al., 2011). However, their application for describing the dynamic accumulation and toxicity of metal mixtures is still limited. Antagonistic/synergistic effects of metals in mixtures are mainly caused by their interaction at uptake/target sites, reducing/increasing their uptake and toxic effect (Vijver et al., 2011; Wang et al., 2013). Hence, we suppose that the observed interactions could be explained by comparing the uptake, elimination and damage rate constants of metals in mixtures, which could be obtained by fitting a TK-TD model to the accumulation and toxicity data.

Here, lanthanum (La) and cerium (Ce) were chosen as representative

elements of REEs as their relatively high crustal abundance (31 mg kg⁻¹ for La and 63 mg kg⁻¹ for Ce) (Brown et al., 1990). The combined effects of La and Ce on the terrestrial organism *Enchytraeus crypticus* were investigated in a simulated soil solution-sand system at a range of exposure times. Using the MIXTOX modelling tool, the extended CA model was applied to quantitatively determine the time dependence of toxic effects and the interaction patterns of La and Ce in the mixture based on the derived deviation parameters. The process-based TK-TD model was used to describe and interpret the dynamic interaction processes at uptake and target level by comparing the estimated uptake, elimination and damage rate constants of La/Ce at different dose ratios in the mixture. The objective of this study was to refine the risk assessment of REE mixtures by improving the interpretation of the dynamic interactions between the individual components using a modelling approach.

2. Materials and methods

2.1. Test organism

Enchytraeus crypticus (Enchytraeidae; Oligochaeta; Annelida) were used as the test species. As ecologically relevant soil-living worm, it has been widely applied as the model species for ecotoxicological studies, since it is sensitive to numerous stressors, has excellent control performance and a short generation time (Castro-Ferreira et al., 2012). *E. crypticus* were maintained in agar medium in a climate chamber at 20 °C, 75% relative humidity, and complete darkness. The worms were fed regularly with oatmeal and yolk powder as the food source. Adults (body length: 0.8–1.2 cm, wet weight: 1.0–1.5 mg) with white spots in the clitellum region were selected for the experiments.

2.2. Preparation of exposure medium

A simulated soil solution-quartz sand system was used as the exposure medium to avoid the impact of complex soil processes on REE speciation. Quartz sand was selected as test matrix and pretreated according to He and van Gestel (2013). The basic solution mainly contained Ca, Mg, Na and K. Stock solutions of LaCl₃ and CeCl₃ were prepared by adding different amounts of LaCl₃·6H₂O and CeCl₃·7H₂O to deionized water. A series of treatments were prepared by adding different volumes of stock solutions to the basic solution. All used chemicals (purity >99%) were obtained from Sigma-Aldrich. All test solutions were adjusted to the target pH of 6.0 using diluted NaOH/HNO₃ (0.10 mM) and 0.75 mg L⁻¹ 2-(N-morpholino)-ethanesulfonic acid (MES) buffer. 5.5 mL of test solution containing different concentrations of La and/or Ce was added to 20.0 g of pretreated quartz sand in a 100 mL glass jar to prepare each treatment. All test treatments were equilibrated for 24 h before use.

2.3. Toxicity tests

The toxicity tests included three simultaneous sets: La alone, Ce alone, and La-Ce mixtures. For the individual treatments, nominal concentrations of La or Ce ranged from 0 to 4.0 mM. For the La-Ce mixtures, nominal concentrations ranged from 0.2 to 1.6 mM for La and from 0.2 to 2.4 mM for Ce (Table S1). To evaluate the impact of time on the toxicity of the REEs, five exposure times (1, 2, 4, 7 and 14 d) were selected. Ten adults were introduced into each glass jar and three replicates were used for each treatment and exposure time. The jars were wrapped in perforated aluminum foils to reduce water evaporation and to prevent escape of worms. The toxicity tests were carried out in a climate chamber at 20 °C and 75% relative humidity with a 12:12 h light: dark cycle. In order to minimize variability, all treatments of the same set with same exposure time were performed simultaneously. The glass jars were weighed every 2 d and deionized water was added to compensate water loss. After supplement to deionized water, the jars

were shaken gently to maintain the exposure media homogenized. During the experiment, the worms were not fed to avoid the potential impacts of food on the concentration and speciation of REEs. After different exposure times, the surviving animals from three replicates of each treatment were recorded, collected, rinsed with demineralized water, and frozen at -20°C for further analysis of the body concentration of REEs to describe the toxicokinetic process of La and Ce in organisms (Fig. S1).

2.4. Chemical measurements and speciation calculations

For each treatment, solution pH was measured with a pH meter (Mettler Delta 320, Switzerland). The actual concentrations of dissolved REEs (La and Ce) in the initial test solutions were measured by inductively coupled plasma optical emission spectrometry (ICP-OES, 5300DV, PerkinElmer, detection limit of 0.05 mg L^{-1}). At the end of the test, the frozen worms were freeze-dried for more than 48 h, weighed with a microbalance (Mettler-Toledo XPR2U) and digested with HNO_3 (65%–68%, GR). Inductively coupled plasma mass spectrometry (ICP-MS, NexION 350D, PerkinElmer) was used to determine the internal concentrations of La and Ce in the animals. For quality control, a certified reference material (Multi-Element Calibration Std. #1, J&K Scientific Ltd.) and a reagent blank were run every 20 samples.

2.5. Data analysis and modeling

2.5.1. Dose-response relationship

A logistic equation was used to describe the fraction of worms surviving exposure to different concentrations of La and Ce, single and in binary mixtures.

$$S = \frac{S_{\max}}{1 + \left(\frac{C_w}{\text{LC}_{50}}\right)^b} \quad (1)$$

where S represents the survival fraction at each exposure time, $S_{\max}$ the survival fraction in the control (%), C_w the measured concentrations of single La or single Ce or the concentration of La-Ce in mixtures expressed as toxic units (MT), b the slope parameter, and LC_{50} the concentration causing 50% of mortality.

2.5.2. Concentration addition (CA) model

Given the similarity in physicochemical properties of La and Ce (Blinova et al., 2018), the CA model is preferred to predict their mixture toxicity. The toxic potency of the mixture of REEs is expressed as the sum of the toxic units (MT), which was calculated as:

$$\text{MT} = \sum_{i=1}^n \text{TU}_i = \sum_{i=1}^n \frac{C_i}{\text{LC}_{50_i}} = \frac{C_{\text{La}}}{\text{LC}_{50_{\text{La}}}} + \frac{C_{\text{Ce}}}{\text{LC}_{50_{\text{Ce}}}} \quad (2)$$

where C_{La} and C_{Ce} are the measured exposure concentrations of La and Ce in a mixture, $\text{LC}_{50_{\text{La}}}$ or $\text{LC}_{50_{\text{Ce}}}$ the effective concentrations of La (or Ce) causing 50% mortality when applied singly. If a mixture produces 50% mortality at $\text{MT} = 1$, then CA holds (Backhaus and Faust, 2012).

2.5.3. MIXTOX modelling

The MIXTOX modelling tool was developed to quantify the deviations from CA (Eq. (2)) and can be rewritten as follows (Haas et al., 2010):

$$\sum_{i=1}^n \frac{C_i}{\text{LC}_{50_i}} = \exp(G) \quad (3)$$

Here, the quantity G describes the degree of deviation from CA. If $G = 0$, the right side of Eq. (3) equals 1, i.e., Eq. (2) in the main text.

Individual contributions of chemicals to the toxicity of the mixture rather than their real concentrations should be incorporated in the deviation function G . The relative contribution to toxicity z_i of each chemical i in a mixture can be calculated as follows:

Fig. 1. Dose-response relationships for the effect on the survival of *Enchytraeus crypticus* of single La (A), single Ce (B), and La-Ce mixtures (C) at different exposure times. All exposures were performed in a sand-solution medium. For the La-Ce mixtures, exposure concentrations are expressed as toxic units.

$$G(z_1, \dots, z_n) = a \prod_{i=1}^n z_i \quad (4)$$

$$z_i = \frac{\text{TU}_{x_i}}{\sum_{j=1}^n \text{TU}_{x_j}}, \quad \text{where} \quad \text{TU}_{x_i} = \frac{C_i}{\text{LC}_{50_i}} \quad (5)$$

The deviation function G is described by the parameter a (i.e., $a > 0$ indicate antagonism, $a < 0$ indicate synergism, and $a = 0$ indicate additivity). In this study, a stepwise approach was used for the model fitting. First, the single toxicity of La and Ce was assessed by CA model, and the obtained parameters were used to predict their combined effect. Then, all data was fitted with two nested models: CA model and its extension model with a deviation function by introducing an additional parameter a . A Chi-Square test was applied to compare the fitting goodness of two models. Significant interactions can be detected if the predictive ability with the extension model is significantly improved than that with CA model. More detailed information regarding the seeking of the optimal model fit to the mixture toxicity data used in the MIXTOX modelling tool analysis have been clearly and comprehensively described by Jonker et al. (2005).

2.5.4. Toxicokinetic and toxicodynamic model

The accumulation of La and Ce in the test organisms with time can be described with a first-order one-compartment equation, as follows:

$$C_{\text{Body}} = C_w \times \frac{K_u}{K_e} \times (1 - e^{-K_e \times t}) \quad (6)$$

where C_{Body} represents the La or Ce concentration in the test organism

Fig. 2. The relationship between the predicted and observed survival fraction of *Enchytraeus crypticus* exposed to La-Ce mixtures in a sand-solution medium and for different exposure times. The predicted values were obtained by fitting MIXTOX modelling tool to the data. The solid lines are the 1:1 relationship. The dashed lines are a 20% difference between the predicted and observed values. R^2 indicates the coefficient of determination.

after different exposure times (mg kg^{-1}), C_w the measured exposure concentration of La and Ce in test solutions (mg L^{-1}), t the exposure time (d), K_u the uptake rate constant ($\text{L kg}^{-1} \text{d}^{-1}$), and K_e the bioaccumulation-based elimination rate constant (d^{-1}).

The relationship between toxicity and time can be expressed as:

$$\text{LC50}(t) = \frac{\text{LC50}_\infty}{1 - e^{-K_d \times t}} \quad (7)$$

where $\text{LC50}(t)$ represents the LC50 at different exposure times (mg L^{-1}), LC50_∞ the ultimate LC50 value (mg L^{-1}), and K_d the damage rate constant (d^{-1}).

All parameters were estimated with the SOLVER option in JMP 16.0 (SAS institute) by minimizing the sum of squared residuals of observed and predicted values.

3. Results and discussion

3.1. Time-dependent toxicity of La, Ce, and their mixtures

The co-existence of REEs in terrestrial environment and long-term exposure durations of REEs in organisms are the real situations (Romero-Freire et al., 2018; Liang et al., 2021). This strengthens the need to accurately assess the adverse effects of REEs on terrestrial organisms in a time course. Therefore, we focused on investigating the dynamic toxicity of REEs and their mixtures to soil organisms. In the present study, all data analyses were expressed based on the measured concentrations (Table S1). The observed effects on enchytraeid survival of single La, single Ce, and La-Ce mixtures after different exposure times are plotted against measured concentrations of the individual REEs and toxic units for the mixtures (Fig. 1). For single exposures, LC50 values decreased gradually from 536 mg L^{-1} at 1 d to 277 mg L^{-1} at 14 d for La,

Table 1

The parameters of the MIXTOX modelling tool estimated when fitting the concentration addition model to data on the toxicity of La-Ce mixtures to *Enchytraeus crypticus*, at different exposure times. The parameter a indicates antagonisms ($a > 0$), synergism ($a < 0$) or additivity ($a \sim 0$).

Time (day)		R^2	a	Significance	Interaction pattern
1	CA	0.54	1.29	$p < 0.01$	Antagonism
	CA(S/A)	0.86			
2	CA	0.59	0.41	$p < 0.01$	Antagonism
	CA(S/A)	0.73			
4	CA	0.86	-0.07	$p > 0.05$	Additivity
	CA(S/A)	0.89			
7	CA	0.92	-0.21	$p > 0.05$	Additivity
	CA(S/A)	0.92			
14	CA	0.97	0.12	$p > 0.05$	Additivity
	CA(S/A)	0.97			

and from 581 mg L⁻¹ at 1 d to 326 mg L⁻¹ at 14 d for Ce (Table S2). The LC50 values of La were smaller than for Ce irrespective of the exposure times, indicating La was more toxic to *E. crypticus* than Ce. For the La-Ce mixtures, the estimated MT50s were 1.32, 0.78, 0.67, 0.62, and 0.54 after 1, 2, 4, 7, and 14 d, respectively (Table S2).

Generally, with increasing exposure concentration and exposure time, single and mixture toxicity of La and Ce increased as evidenced by the decreasing LC50 and MT50 values. Consistently, Paola et al. (2007) found that REEs were toxic to *Lemna minor* L. upon prolonged exposure but not in an acute toxicity test. Bioavailability of metals has been considered as a dynamic process, in which exposure to the metal results in bioaccumulation and subsequent toxic effects when the body metal concentration exceeds the critical body residue (Broerse et al., 2012). The accumulation of metal is regarded as the rate-limiting step for metal toxicity (Martins and Boaventura, 2002). Our previous work also proved that the accumulation of REEs in *E. crypticus* depends on the exposure time, resulting in time-varying toxicity (Huang et al., 2020). Similar conclusions on the relevance of exposure time for metal toxicity have also been reported for conventional metals (e.g., Ni, Zn, Fe, Pb and Cd) (Abdel Gawad, 2018; He and van Gestel, 2013; Li et al., 2016). Li et al. (2016) found that the sea cucumber exhibited different physiological adaption mechanisms (e.g., regulating oxygen consumption rates and metabolic enzyme activities) in response to acute or chronic heavy metal (Cu, Cd, and Zn) stress. These findings highlight the importance of considering time as a factor for accurately assessing the environmental risk of REEs.

3.2. Quantifying interactions of La-Ce mixtures in a time course

The CA model was fitted to the data on the toxicity of La-Ce mixtures at different exposure times, based on the estimated single toxicity of La and Ce, to detect any interactions. As shown in Fig. S2, data points above and below the 1:1 relationship of predicted and observed values represent an under- and over-prediction of toxic effects by the CA model, indicating the existence of synergistic and antagonistic interactions, respectively. It can be seen the interaction pattern of La and Ce was mainly antagonistic after 1 and 2 d exposure, but nearly additive after 4, 7, and 14 d exposure. The MIXTOX modelling tool was applied to describe the mixture toxicity of La and Ce by incorporating the interactions for different exposure times separately (Fig. 2). The estimated interaction parameter (a), the goodness of fitting (R^2), and the statistic outcomes (p) are listed in Table 1. The estimated parameter a were 1.29 and 0.41 for 1 d and 2 d, respectively, quantitatively confirming the antagonistic interaction for La and Ce and showing that the magnitude of antagonism decreased with time. Compared to the CA model, incorporating the parameter a for antagonism significantly improved the prediction of the toxicity of the mixture of La-Ce at 1 and 2 d. The model fits (R^2) improved from 0.54 to 0.86 at 1 d, and from 0.59 to 0.73 at 2 d (Table 1). Adding the deviation parameter a did not significantly

improve the model fits for other exposure times (4, 7 and 14 d). To conclude, the type of interaction of La-Ce mixtures varied from antagonistic to non-interactive effects with exposure time.

Our previous work showed that most of the observed interaction patterns of REE mixtures in wheat plants were antagonistic or additive, depending on the mixture combinations and the way exposure was expressed (Gong et al., 2018; He et al., 2019). Competitive interaction of REE ions for binding sites on plant roots during toxicokinetic processes might be the reason for the observed antagonistic effect (Wang et al., 2013). While, synergistic effects of binary and ternary mixtures of REEs in algae have also been reported, which could be explained by the impairment of physiological processes during the toxicodynamic phase of the process of intoxication (Bergsten-Torralba et al., 2020; Romero-Freire et al., 2019). The differences in interaction patterns may be attributed to the varying combinations and doses of metals, test species, and toxicological endpoint examined (Norwood et al., 2003; Vijver et al., 2010). Until now, available researches of interactions among chemicals were mainly based on the combined effects at a fixed time point without considering the influence of time.

The present study further showed that the interaction pattern of REE mixtures was time-dependent. Consistently, the magnitude of antagonistic interaction of Ni and Co showed a decreasing trend with increased exposure time, suggesting additive effects after long-term exposure (He et al., 2015). The interaction types among Cu-Zn, Cu-Cd, Cd-Pb and Ni-Co mixtures were time dependent for their toxicity to zebrafish larvae (Feng et al., 2018). Varying interactive effects between Cu and Cd to *Folsomia candida* were observed over the whole time period (Baas et al., 2007). Nys et al. (2017) stated that the observed interactions of metal mixtures were different between acute and chronic exposures because of the occurrence of metal detoxification upon long-term exposure. The interaction types of Cd and Pb varied from synergistic to antagonistic with time, which possibly due to the occurrence of competition between metals during the toxicodynamic process after certain time (Rosal et al., 2010).

For La and Ce, because of their similar physicochemical properties, it is assumed that they could compete for common transport sites of *E. crypticus*, resulting in less accumulation of either one metal or both two metals in organisms and therefore antagonistic effect (Adeel et al., 2019; Gong et al., 2018). In the present study, the time-varying interaction of La and Ce from antagonism to additivity might be explained by the equilibrium of metal body concentration reached after a certain exposure time (Tan et al., 2017; Wang et al., 2013). According to the saturation binding principles, the uptake ability of metals by organism will eventually reach its peak due to the limited number of binding sites (Borgmann et al., 2004; Croteau and Luoma, 2007). Once the steady state was achieved, the antagonistic interaction resulting from the inhibited uptake will disappear (Gopalapillai and Hale, 2017). In studies on the dynamic accumulation of chemicals, it has been widely reported that the accumulation of metals (e.g., Cu, Ni and REEs) could reach equilibrium inside of organisms (He and van Gestel, 2013; Huang et al., 2020; Santos et al., 2021). Similarly, interaction types of Cd and Pb mixtures varied from synergistic to antagonistic with time, which were explained by the occurrence of competition of the metals during the toxicodynamic process (Feng et al., 2018). Thus, the understanding of toxicokinetic and toxicodynamic processes could offer greater insights into the time-course development of toxicity and interactions of metals in mixtures.

3.3. Interpreting dynamic interaction processes by toxicokinetic and toxicodynamic models

To explain the varying interactions of La and Ce over time, the toxicokinetic (time-course body concentration) and toxicodynamic (time-course toxicity) process of La and Ce in *E. crypticus* exposed to their mixtures were analyzed by a TK-TD model to determine the dynamic effect of La on Ce and vice versa.

Table 2

The estimated uptake rate constant (K_u) and bioaccumulation-based elimination rate constant (K_e) for the uptake of La and Ce in *Enchytraeus crypticus*, exposed in a sand-solution medium. The parameters were obtained by fitting a first-order one-compartment TK model to the body concentrations over time of worms exposed to single La, single Ce or La-Ce mixtures with different La/Ce concentrations.

La			Ce		
Treatment	K_u (L kg ⁻¹ d ⁻¹)	K_e (d ⁻¹)	Treatment	K_u (L kg ⁻¹ d ⁻¹)	K_e (d ⁻¹)
La	1.81	0.95	Ce	1.14	0.68
La+Ce	1.58	0.92	Ce+La	1.19	0.62
45.2			35.3		
La+Ce	1.55	0.86	Ce+La	1.36	0.77
86.3			68.8		
La+Ce 180	1.43	0.47	Ce+La 146	1.17	0.60
La+Ce 271	1.10	0.42	Ce+La 222	1.06	0.51
La+Ce 345	1.17	0.27	Ce+La 287	1.02	0.34

For the toxicokinetic process, the one-compartment model (Eq. 6) was fitted to the dynamic data of REE accumulation in the enchytraeids. The estimated uptake (K_u) and elimination (K_e) rate constants are shown in Table 2. Overall, the estimated K_u and K_e values were larger for La than for Ce upon single exposure, suggesting that La is more easily taken up and eliminated by *E. crypticus* than Ce. This agrees with Huang et al. (2020) who concluded that the uptake and eliminate ability of REEs in *E. crypticus* reduces with increasing atomic number. When exposed to La-Ce mixtures, the K_u and K_e value for La declined from 1.81 to 1.17 L kg⁻¹ d⁻¹ and from 0.95 to 0.27 d⁻¹, respectively, with ascending level of Ce. For Ce, the K_u and K_e values increased at lower level of La, and then reduced from 1.17 to 1.02 L kg⁻¹ d⁻¹ and from 0.77 to 0.34 d⁻¹ with further increasing exposure concentrations of La.

The decrease of K_u values may indicate a reduced uptake rate of La/Ce caused by a reduced availability of binding or transporting sites when more competing cations are present (Li et al., 2009). The development with time of body concentrations of La and Ce in the enchytraeids exposed to mixtures with different La/Ce ratios are shown in Figs. 3 and 4, respectively. Generally, a decreasing trend in REE bioaccumulation levels was observed with increasing concentration of the other element, confirming that the reduced uptake of REEs was due to competitive interaction. Similar results have been reported previously, for instance, Tan et al. (2017) reported that the uptake of Sm in *Chlamydomonas reinhardtii* decreased by nearly 50% with the addition of equimolar concentrations of REEs (La, Ce, Eu). Komjarova and Blust (2009) demonstrated that the occurrence of metal interactions in the zebrafish *Danio rerio* during uptake processes was largely correlated with the competition for binding sites and the induction/suppression of metal transferring proteins. Gopalapillai and Hale (2017) indicated that Ni, Cu and Cd competed for uptake sites in *Lemna minor*, and Cu and Cd shared binding sites inside this plant. The decreased K_e value indicated weaker excretion ability of La/Ce of *E. crypticus* with decreasing ratio of La-Ce concentrations in the mixtures. The elimination rate constant of individual chemicals depends mostly on the kind of pollution and the organism's physiology (Crommentuyn et al., 1994; He and van Gestel, 2013). For metal mixtures, intracellular competition may cause a reduction in chemical efflux as the regulation ability of organisms to excrete toxicants is limited (Cedergreen et al., 2017). Hence, the K_e value of La/Ce showed a decreasing trend with the elevated concentration of the other in the present study.

For the toxicodynamic process, the influence of the coexistent element on the toxicity of La or Ce, expressed as the LC50, in a time course was described by a toxicity-time relationship model (Eq. 7) and shown in Fig. 5. With increasing concentration of the other element, the toxicity of La/Ce increased, and the time required to achieve steady state of the LC50 of La (or Ce) gradually increased with increasing exposure concentration of Ce (or La). As shown in Table 3, the estimated

LC50 ∞ and damage rate constant (K_d) decreased from 361 to 29.6 mg L⁻¹ and from 0.85 to 0.09 d⁻¹ for La with increasing concentrations of Ce. Similarly, for Ce LC50 ∞ and K_d decreased from 379 to 39.6 mg L⁻¹ and from 1.05 to 0.10 d⁻¹, respectively. Here, the LC50 ∞ represents the combined toxic effect of La-Ce related to the exposure concentration of La or Ce separately. The obvious decrease of the LC50 ∞ with increasing concentration of the coexisting REE indicates an increased contribution to toxicity of the coexisting element. The reduced damage rate of La/Ce reflects the existence of antagonistic interactions at the target level between La and Ce during the toxicodynamic process. Previous studies found that whether the presence of one metal could interact with other metals at the target level depends on the differences in the properties and modes of action of metals (Son et al., 2016). Charles et al. (2014) demonstrated that synergetic and antagonistic interactions occurred between Cu and Ni during the toxicodynamic process, depending on their respective concentrations in the mixture. Feng et al. (2018) also reported that joint actions occurred among metal mixtures (Cu-Zn, Cu-Cd, Cd-Pb, and Ni-Co) in the toxicodynamic process with the type of interaction changing with time. The results of the present study highlight the significance for understanding the dynamic interaction of REE in mixtures of interactive effects occurring during toxicokinetic and toxicodynamic processes.

As discussed above, the deviation from the CA or IA reference models can result from interactions among components in a mixture during the toxicokinetic and toxicodynamic process (Lee and Landrum, 2006). Several attempts have been made to develop models using a TK-TD approach to predict the dynamic toxicity of chemical mixtures and to provide better understanding of the observed interactions. A simple model for the energy budget (DEB_{kiss}) was applied for modelling the sublethal mixture effect of Cd and fluoranthene in nematodes (*Caenorhabditis elegans*), providing more insight into the relevant dynamic processes underlying the observed toxicity (Jager et al., 2014). Cedergreen et al. (2017) indicated that TK-TD modelling could serve as a promising tool to understand and predict the synergistic interactions between an azole fungicide and an insecticide on the survival of the crustacean *Daphnia magna*, although the predictive ability is still uncertain. The difference in sensitivity to the pyrethroid of aquatic invertebrates was tested and modeled by TK-TD, showing that sensitivity is more related to the TD process (Dalhoff et al., 2020). In the present study, the dynamic accumulation and toxicity of La-Ce mixtures were well described by a process-based TK-TD model, with R^2 value ranging from 0.79 to 0.93 and from 0.92 to 0.94 for TK (Figs. 3 and 4) and TD (Fig. 5) processes, respectively. We for the first time proved the potential applicability of a TK-TD model for predicting the toxicity of REE mixtures to a terrestrial invertebrate in a time course by considering the impact of interactions on relevant parameters. This work contributes to elucidating the time-varying toxicity of REE mixtures that was not yet comprehensively recognized.

4. Conclusions

The present study determined the dynamic mixture toxicity of La and Ce to *Enchytraeus crypticus*. Based on the CA model, the MIXTOX modelling tool was applied to quantify the interactions in the La-Ce mixtures at different time scales. The individual and mixture toxicity of La and Ce increased with increasing exposure time, with the interactive effects varying from antagonistic (1 and 2 d) to additive (4, 7 and 14 d). These observed dynamic accumulation and toxicity processes were analyzed with a process-based TK-TD approach to interpret the occurrence of interactions. By comparing the estimated uptake, elimination, and damage rate constants of La or Ce with increasing concentrations of the other element, we found that interactions of La and Ce occurred at both the toxicokinetic and the toxicodynamic level to affect the uptake and toxicity of La-Ce mixtures. Such findings would not have been obvious based on traditional analysis of dose-response relationships. The obtained ecotoxicity data of REEs for terrestrial organisms is

Fig. 3. Dynamic bioaccumulation of La in *Enchytraeus crypticus* exposed in a sand-solution medium to La with increasing levels of Ce (A to E). Lines show the fit of a toxicokinetic (TK) model (Eq. (6)) to the data; data points show the mean value of the measurements.

valuable and crucial for establishing the environmental quality standards for REE contaminated soils. Moreover, the present work demonstrates the importance of considering the factor of time for risk assessment of REEs, subsequently, will be helpful for establishing more reliable and conservative environmental standards of REEs, especially for long term exposure scenarios. Also, a new alternative approach was provided to the traditional methods for revealing the underlying interaction processes of REEs in mixtures in relation to exposure time. Further studies are also underway to elucidate the time-course toxicity of REEs at the molecular level, promoting a more comprehensive understanding of the molecular mechanisms involved in the time-dependent mixture toxicity.

Supporting information

Figures showing the brief description of the experiment performed to describe the toxicokinetic (TK) process of REEs and the relationships

between the predicted (by CA model) and observed survival of *E. crypticus* exposed for different periods of time to La-Ce mixtures. Tables showing the measured concentrations of REEs in the test solutions and the estimated LC50 values for the toxicity to *E. crypticus* of single La, single Ce and La-Ce mixtures at different exposure times.

CRediT authorship contribution statement

EH, BG, and HQ conceived the idea and designed the experiment; EH performed the experiment; GB, EH, and HQ wrote the manuscript; CVG, YT, WY, JY, and YL revised the manuscript; EH and HQ raised the funding.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

Fig. 4. Dynamic bioaccumulation of Ce in *Enchytraeus crypticus* exposed in a sand-solution medium to Ce with increasing levels of La (A to E). Lines show the fit of a toxicokinetic (TK) model (Eq. (6)) to the data; data points show the mean value of the measurements.

Fig. 5. The influence of the coexistent element on the toxicity of single La (left) or single Ce (right) to *Enchytraeus crypticus* exposed in a sand-solution medium, in a time course. The data points are estimated LC50 values from observed toxicity data; the solid lines show the fit of TD model (Eq. 7).

Table 3

The estimated ultimate LC50 value (LC50 ∞) and damage rate constant (K_d) for the toxicity of La and Ce, single and in mixtures, to *Enchytraeus crypticus*, exposed in a sand-solution medium. The LC50 ∞ and K_d values were obtained by fitting a toxicodynamic (TD) model to the LC50 values for different exposure times.

La			Ce		
Treatment	K_d (d ⁻¹)	LC50 ∞ (mg L ⁻¹)	Treatment	K_d (d ⁻¹)	LC50 ∞ (mg L ⁻¹)
La	0.85	361	Ce	1.05	379
La+Ce	0.71	291	Ce+La	0.91	331
45.2			35.3		
La+Ce	0.54	222	Ce+La	0.62	274
86.3			68.8		
La+Ce	0.38	145	Ce+La	0.44	183
180			146		
La+Ce	0.20	74.6	Ce+La	0.10	50.5
271			222		
La+Ce	0.09	29.6	Ce+La	0.10	39.6
345			287		

the work reported in this paper.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 41977115, No. 41877500, and No. 42022057), Shanghai Rising-Star Program (No. 20QA1404500), the Science and Technology Program of Guangzhou, China (No. 201904010116), and the Research Fund Program of Guangdong Provincial Key Laboratory of Environmental Pollution Control and Remediation Technology (2020B1212060022).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2021.126281.

References

- Abdel Gawad, S.S., 2018. Acute toxicity of some heavy metals to the fresh water snail, *Theodoxus niloticus* (Reeve, 1856). Egypt. J. Aquat. Res. 44 (2), 83–87.
- Adeel, M., Lee, J.Y., Zain, M., Rizwan, M., Nawab, A., Ahmad, M.A., Shafiq, M., Yi, H., Jilani, G., Javed, R., Horton, R., Rui, Y., Tsang, D.C.W., Xing, B., 2019. Cryptic footprints of rare earth elements on natural resources and living organisms. Environ. Int. 127, 785–800.
- Agathokleous, E., Kitao, M., Calabrese, E.J., 2018. The rare earth element (REE) lanthanum (La) induces hormesis in plants. Environ. Pollut. 238, 1044–1047.
- Alonso, E., Sherman, A.M., Wallington, T.J., Everson, M.P., Field, F.R., Roth, R., Kirchain, R.E., 2012. Evaluating rare earth element availability: a case with revolutionary demand from clean technologies. Environ. Sci. Technol. 46 (6), 3406–3414.
- Amyot, M., Clayden, M.G., MacMillan, G.A., Perron, T., Arscott-Gauvin, A., 2017. Fate and trophic transfer of rare earth elements in temperate lake food webs. Environ. Sci. Technol. 51 (11), 6009–6017.
- Ashauer, R., Escher, B.I., 2010. Advantages of toxicokinetic and toxicodynamic modelling in aquatic ecotoxicology and risk assessment. J. Environ. Monit. 12 (11), 2056–2061.
- Baas, J., van Houte, B., Gestel, C., Kooijman, S., 2007. Modeling the effects of binary mixtures on survival in time. Environ. Toxicol. Chem. 26 (6), 1320–1327.
- Backhaus, T., Faust, M., 2012. Predictive Environmental risk assessment of chemical mixtures: a conceptual framework. Environ. Sci. Technol. 46 (5), 2564–2573.
- Backhaus, T., Altenburger, R., Boedeker, W., Faust, M., Grimme, L.H., 2000. Predictability of the toxicity of a multiple mixture of dissimilarly acting chemicals to *Vibrio fischeri*. Environ. Toxicol. Chem. 19 (9), 2348–2356.
- Bergsten-Torralba, L.R., Magalhaes, D.P., Giese, E.C., Nascimento, C.R.S., Pinho, J.V.A., Buss, D.F., 2020. Toxicity of three rare earth elements, and their combinations to algae, microcrustaceans, and fungi. Ecotoxicol. Environ. Saf. 201, 110795.
- Blinova, I., Vija, H., Lukjanova, A., Muna, M., Syvertsen-Wiig, G., Kahru, A., 2018. Assessment of the hazard of nine (doped) lanthanides-based ceramic oxides to four aquatic species. Sci. Total Environ. 612, 1171–1176.
- Borgmann, U., Norwood, W.P., Dixon, D.G., 2004. Re-evaluation of metal bioaccumulation and chronic toxicity in *Hyalella azteca* using saturation curves and the biotic ligand model. Environ. Pollut. 131 (3), 469–484.
- Broerse, M., Oorsprong, H., van Gestel, C.M., 2012. Cadmium affects toxicokinetics of pyrene in the collembolan *Folsomia candida*. Ecotoxicology 21 (3), 795–802.
- Brown, P.H., Rathjen, A.H., Graham, R.D., Tribe, D.E., 1990. Rare earth elements in biological systems. Handbook on the Physics and Chemistry of Rare Earths. Elsevier, pp. 423–452.
- Castro-Ferreira, M.P., Roelofs, D., van Gestel, C.A., Verweij, R.A., Soares, A.M., Amorim, M.J., 2012. *Enchytraeus crypticus* as model species in soil ecotoxicology. Chemosphere 87 (11), 1222–1227.
- Cedergreen, N., Streibig, J.C., 2005. Can the choice of endpoint lead to contradictory results of mixture-toxicity experiments? Environ. Toxicol. Chem. 24 (7), 1676–1683.
- Cedergreen, N., Dalhoff, K., Li, D., Gottardi, M., Kretschmann, A.C., 2017. Can toxicokinetic and toxicodynamic modeling be used to understand and predict synergistic interactions between chemicals? Environ. Sci. Technol. 51 (24), 14379–14389.
- Chao, Y., Liu, W., Chen, Y., Chen, W., Zhao, L., Ding, Q., Wang, S., Tang, Y.T., Zhang, T., Qiu, R.L., 2016. Structure, variation, and co-occurrence of soil microbial communities in abandoned sites of a rare earth elements mine. Environ. Sci. Technol. 50 (21), 11481–11490.
- Charles, J., Crini, G., Degiorgi, F., Sancey, B., Morin-Crini, N., Badot, P.-M., 2014. Unexpected toxic interactions in the freshwater amphipod *Gammarus pulex* (L.) exposed to binary copper and nickel mixtures. Environ. Sci. Pollut. Res. 21 (2), 1099–1111.
- Crommentuijn, T., Doodeman, C.J.A.M., Doornekamp, A., Van Der Pol, J.J.C., Van Gestel, C.A.M., Bedaux, J.J.M., 1994. Lethal body concentrations and accumulation patterns determine time-dependent toxicity of cadmium in soil arthropods. Environ. Toxicol. Chem. 13 (11), 1781–1789.
- Croteau, M.N., Luoma, S.N., 2007. Characterizing dissolved Cu and Cd uptake in terms of the biotic ligand and biodynamics using enriched stable isotopes. Environ. Sci. Technol. 41 (9), 3140–3145.
- Dalhoff, K., Hansen, A.M.B., Rasmussen, J.J., Focks, A., Strobel, B.W., Cedergreen, N., 2020. Linking morphology, toxicokinetic, and toxicodynamic traits of aquatic invertebrates to pyrethroid sensitivity. Environ. Sci. Technol. 54 (9), 5687–5699.
- d'Aquino, L., Morgana, M., Carboni, M.A., Staiano, M., Antisari, M.V., Re, M., Lorito, M., Vinale, F., Abadi, K.M., Woo, S.L., 2009. Effect of some rare earth elements on the growth and lanthanide accumulation in different *Trichoderma* strains. Soil Biol. Biochem. 41 (12), 2406–2413.
- Feng, J., Gao, Y., Ji, Y., Zhu, L., 2018. Quantifying the interactions among metal mixtures in toxicodynamic process with generalized linear model. J. Hazard. Mater. 345, 97–106.
- Gong, B., He, E., Qiu, H., Li, J., Ji, J., Zhao, L., Cao, X., 2018. Phytotoxicity of individual and binary mixtures of rare earth elements (Y, La, and Ce) in relation to bioavailability. Environ. Pollut. 246, 114–121.
- Gong, B., He, E., Peijnenburg, W., Iwasaki, Y., Van Gestel, C.A.M., Cao, X., Zhao, L., Xu, X., Qiu, H., 2020a. Coupling mixture reference models with DGT-perceived metal flux for deciphering the nonadditive effects of rare earth mixtures to wheat in soils. Environ. Res. 188, 109736.
- Gong, B., He, E., Xia, B., Ying, R., Peijnenburg, W.J.G.M., Liu, Y., Qiu, H., 2020b. Bioavailability and phytotoxicity of rare earth metals to *Triticum aestivum* under various exposure scenarios. Ecotoxicol. Environ. Saf. 205, 111346.
- Gonzalez, V., Vignati, D.A.L., Leyval, C., Giamberini, L., 2014. Environmental fate and ecotoxicity of lanthanides: are they a uniform group beyond chemistry? Environ. Int. 71, 148–157.
- Gopalapillai, Y., Hale, B.A., 2017. Internal versus external dose for describing ternary metal mixture (Ni, Cu, Cd) chronic toxicity to *Lemna minor*. Environ. Sci. Technol. 51 (9), 5233–5241.
- Gwenzi, W., Mangori, L., Danha, C., Chaukura, N., Dunjana, N., Sanganyado, E., 2018. Sources, behaviour, and environmental and human health risks of high-technology rare earth elements as emerging contaminants. Sci. Total Environ. 636, 299–313.
- Haas, C.N., Cidambi, K., Kersten, S., Wright, K., 2010. Quantitative description of mixture toxicity: effect of level of response on interactions. Environ. Toxicol. Chem. 15 (8), 1429–1437.
- He, E., van Gestel, C.A., 2013. Toxicokinetics and toxicodynamics of nickel in *Enchytraeus crypticus*. Environ. Toxicol. Chem. 32 (8), 1835–1841.
- He, E., Baas, J., Van Gestel, C.A., 2015. Interaction between nickel and cobalt toxicity in *Enchytraeus crypticus* is due to competitive uptake. Environ. Toxicol. Chem. 34 (2), 328–337.
- He, E., Gong, B., Qiu, H., Van Gestel, C.A.M., Ruan, J., Tang, Y., Huang, X., Xiao, X., Li, M., Qiu, R., 2019. Model-based rationalization of mixture toxicity and accumulation in *Triticum aestivum* upon concurrent exposure to yttrium, lanthanum, and cerium. J. Hazard. Mater. 389, 121940.
- Huang, X., He, E., Qiu, H., Zhang, L., Tang, Y., Zhao, C., Li, M., Xiao, X., Qiu, R., 2020. Do toxicokinetic and toxicodynamic processes hold the same for light and heavy rare earth elements in terrestrial organism *Enchytraeus crypticus*? Environ. Pollut. 262, 114234.
- Jager, T., Albert, C., Preuss, T.G., Ashauer, R., 2011. General unified threshold model of survival—a toxicokinetic-toxicodynamic framework for ecotoxicology. Environ. Sci. Technol. 45 (7), 2529–2540.
- Jager, T., Gudmundsdottir, E.M., Cedergreen, N., 2014. Dynamic modeling of sublethal mixture toxicity in the nematode *Caenorhabditis elegans*. Environ. Sci. Technol. 48 (12), 7026–7033.
- Jonker, M.J., Svendsen, C., Bedaux, J.J.M., Bongers, M., Kammenga, J.E., 2005. Significance testing of synergistic/antagonistic, dose level-dependent, or dose ratio-dependent effects in mixture dose-response analysis. Environ. Toxicol. Chem. 24 (10), 2701–2713.
- Komjarova, I., Blust, R., 2009. Multimetal Interactions between Cd, Cu, Ni, Pb, and Zn Uptake from Water in the Zebrafish *Danio rerio*. Environ. Sci. Technol. 43 (19), 7225–7229.

- Lee, J.-H., Landrum, P.F., 2006. Development of a multi-component damage assessment model (mdam) for time-dependent mixture toxicity with toxicokinetic interactions. *Environ. Sci. Technol.* 40 (4), 1341–1349.
- Li, J., He, E., Romero-Freire, A., Cao, X., Zhao, L., Qiu, H., 2020. Coherent toxicity prediction framework for deciphering the joint effects of rare earth metals (La and Ce) under varied levels of calcium and NTA. *Chemosphere* 254, 126905.
- Li, L., Zhou, D., Wang, P., Peijnenburg, W.J., 2009. Kinetics of cadmium uptake and subcellular partitioning in the earthworm *Eisenia fetida* exposed to cadmium-contaminated soil. *Arch. Environ. Contam. Toxicol.* 57 (4), 718–724.
- Li, L., Tian, X., Yu, X., Dong, S., 2016. Effects of acute and chronic heavy metal (Cu, Cd, and Zn) exposure on sea cucumbers (*Apostichopus japonicus*). *Biomed. Res. Int.* 2016, 4532697.
- Liang, W.-Q., Xie, M., Tan, Q.-G., 2021. Making the biotic ligand model kinetic, easier to develop, and more flexible for deriving water quality criteria. *Water Res.* 188, 116548.
- Liu, W.S., Guo, M.N., Liu, C., Yuan, M., Chen, X.T., Huot, H., Zhao, C.M., Tang, Y.T., Morel, J.L., Qiu, R.L., 2019. Water, sediment and agricultural soil contamination from an ion-adsorption rare earth mining area. *Chemosphere* 216, 75–83.
- Martins, R.J.E., Boaventura, R.A.R., 2002. Uptake and release of zinc by aquatic bryophytes (*Fontinalis antipyretica* L. ex. Hedw.). *Water Res.* 36 (20), 5005–5012.
- McGeer, J.C., Brix, K.V., Skeaff, J.M., DeForest, D.K., Brigham, S.I., Adams, W.J., Green, A., 2003. Inverse relationship between bioconcentration factor and exposure concentration for metals: implications for hazard assessment of metals in the aquatic environment. *Environ. Toxicol. Chem.* 22 (5), 1017–1037.
- Migaszwski, Z.M., Galsuska, A., 2014. The characteristics, occurrence, and geochemical behavior of rare earth elements in the environment: a review. *Crit. Rev. Environ. Sci. Technol.* 45 (5), 429–471.
- Norwood, W.P., Borgmann, U., Dixon, D.G., Wallace, A., 2003. Effects of metal mixtures on aquatic biota: a review of observations and methods. *Hum. Ecol. Risk Assess.* Int. J. 9 (4), 795–811.
- Nys, C., Versieren, L., Cordery, K.I., Blust, R., Smolders, E., De Schampelaere, K.A.C., 2017. Systematic evaluation of chronic metal-mixture toxicity to three species and implications for risk assessment. *Environ. Sci. Technol.* 51 (8), 4615–4623.
- Pagano, G., Guida, M., Tommasi, F., Oral, R., 2015. Health effects and toxicity mechanisms of rare earth elements—knowledge gaps and research prospects. *Ecotoxicol. Environ. Saf.* 115, 40–48.
- Paola, I.M., Paciolla, C., d'Aquino, L., Morgana, M., Tommasi, F., 2007. Effect of rare earth elements on growth and antioxidant metabolism in *Lemna minor* L. *Caryologia* 60 (1–2), 125–128.
- Pol, A., Barends, T.R.M., Dietl, A., Khadem, A.F., Eygensteyn, J., Jetten, M.S.M., Op den Camp, H.J.M., 2014. Rare earth metals essential for methanotrophic life. *Environ. Microbiol.* 16, 255–264.
- Romero-Freire, A., Minguez, L., Pelletier, M., Cayer, A., Caillet, C., Devin, S., Gross, E.M., Guerold, F., Pain-Devin, S., Vignati, D.A.L., Giamberini, L., 2018. Assessment of baseline ecotoxicity of sediments from a prospective mining area enriched in light rare earth elements. *Sci. Total Environ.* 612, 831–839.
- Romero-Freire, A., Joonas, E., Muna, M., Cossu-Leguille, C., Vignati, D.A.L., Giamberini, L., 2019. Assessment of the toxic effects of mixtures of three lanthanides (Ce, Gd, Lu) to aquatic biota. *Sci. Total Environ.* 661, 276–284.
- Rosal, R., Rodea-Palomaes, I., Boltes, K., Fernández-Piñas, F., Leganés, F., Petre, A., 2010. Ecotoxicological assessment of surfactants in the aquatic environment: combined toxicity of docusate sodium with chlorinated pollutants. *Chemosphere* 81 (2), 288–293.
- Santos, F.C.F., van Gestel, C.A.M., Amorim, M.J.B., 2021. Toxicokinetics of copper and cadmium in the soil model *Enchytraeus crypticus* (Oligochaeta). *Chemosphere* 270, 129433.
- Son, J., Lee, Y.-s., Kim, Y., Shin, K.-i., Hyun, S., Cho, K., 2016. Joint toxic action of binary metal mixtures of copper, manganese and nickel to *Paronychiurus kimi* (Collembola). *Ecotoxicol. Environ. Saf.* 132, 164–169.
- Tai, P., Zhao, Q., Su, D., Li, P., Stagnitti, F., 2010. Biological toxicity of lanthanide elements on algae. *Chemosphere* 80 (9), 1031–1035.
- Tan, Q.G., Yang, G., Wilkinson, K.J., 2017. Biotic ligand model explains the effects of competition but not complexation for Sm biouptake by *Chlamydomonas reinhardtii*. *Chemosphere* 168, 426–434.
- Van Gestel, C.A.M., Jonker, M.J., Kammenga, J.E., Laskowski, R., Svendsen, C., 2011. Mixture toxicity: linking approaches from ecological and human toxicology. *Integr. Environ. Assess. Manag.* 7 (2), 308.
- Vijver, M.G., Peijnenburg, W.J.G.M., De Snoo, G.R., 2010. Toxicological mixture models are based on inadequate assumptions. *Environ. Sci. Technol.* 44 (13), 4841–4842.
- Vijver, M.G., Elliott, E.G., Peijnenburg, W.J.G.M., de Snoo, G.R., 2011. Response predictions for organisms water-exposed to metal mixtures: a meta-analysis. *Environ. Toxicol. Chem.* 30 (6), 1482–1487.
- Wang, L., Liang, T., 2015. Geochemical fractions of rare earth elements in soil around a mine tailing in Baotou. *Sci. Rep.* 5, 12483.
- Wang, Y.-M., Kinraide, T.B., Wang, P., Zhou, D.-M., Hao, X.-Z., 2013. Modeling rhizotoxicity and uptake of Zn and Cu singly and in binary mixture in wheat in terms of the cell membrane surface electrical potential. *Environ. Sci. Technol.* 47 (6), 2831–2838.
- Wei, Z., Yin, M., Zhang, X., Hong, F., Li, B., Tao, Y., Zhao, G., Yan, C., 2001. Rare earth elements in naturally grown fern *Dicranopteris linearis* in relation to their variation in soils in south-Jiangxi region (southern China). *Environ. Pollut.* 114, 345–355.