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Stable isotope fractionation during uptake and translocation of cadmium by tolerant *Ricinus communis* and hyperaccumulator *Solanum nigrum* as influenced by EDTA☆

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ABSTRACT

The isotopic fractionation could contribute to understanding the Cd accumulation mechanisms in plant species. However, there are few of systematical investigations with regards to the Cd isotope fractionation in hyperaccumulator plants. The Cd tolerant *Ricinus communis* and hyperaccumulator *Solanum nigrum* were cultivated in nutrient solutions with varying Cd and EDTA concentrations. Cd isotope ratios were determined in the solution, root, stem and leaf. The two investigated plants were systematically enriched in light isotopes relative to their solutions ($\Delta^{114/110}Cd_{plant\text{-}solution} = -0.64‰$ to $-0.29‰$ for *R. communis* and $-0.84‰$ to $-0.31‰$ for *S. nigrum*). Cd isotopes were markedly fractionated among the plant tissues. For both plant species, an enrichment in light Cd isotopes from solution to root was noted, followed by a slight depletion in light Cd isotopes from root to shoot. Noticeably, the chelation process has caused lighter Cd isotope enrichment in the root of *R. communis* and *S. nigrum*. Further, the good fits between $\triangle^{114/110}Cd_{root\text{-}plant}$ and ln F_{root} (or between $\triangle^{114/110}Cd_{shoot\text{-}plant}$ and ln F_{shoot}) indicate that Cd isotopic signatures can be used to study Cd transportation during the metabolic process of plants. This study suggests that knowledge of the Cd isotope ratios could also provide new tool for identifying the Cd-avoiding crop cultivars.

1. Introduction

The health hazards of Cd pollution have attracted global attention since “the itai-itai disease” appeared in Japan in the 1950's (Staessen et al., 1999). Phytoremediation is a cost-effective and environmentally sustainable strategy for Cd extraction from contaminated soils through the accumulation of Cd-hyperaccumulating and Cd-tolerant plant's tissues (Ali et al., 2013). Generally, isotope fractionation occurs during the uptake and transport of metal within plants, and any observed variations in stable isotopic composition can create an isotope “fingerprint” of Cd uptake, transport and redistribution in the plants (von Blanckenburg et al., 2009; Wiggenhauser et al., 2016). Thus, detailed study of isotopic fractionation can contribute to understand the mechanisms of Cd accumulation and tolerance in plant species.

Instrumental developments and methodological refinements over the last two decades have now expanded the application of high-precision metal isotope analyses, thereby triggering the development of stable metal isotopes as novel geochemical tracers (Pallavicini et al., 2014; Wiederhold, 2015). Stable metal isotopes (such as Hg (Yin et al., 2015), Fe (Kiczka et al., 2010; Guelke and Von Blanckenburg, 2007), Mg (Black et al., 2008; Bolou-Bi et al., 2010), Cu (Weinstein et al., 2011; Jouvin et al., 2012), Zn (Weiss et al., 2005; Moynier et al., 2009; Arnold et al., 2010; Aucour et al., 2011; Tang et al., 2012, 2016; Deng et al., 2014), Ni (Deng et al., 2014), Ca (Page et al., 2008; Cobert et al., 2011; Hindshaw et al., 2012; Schmitt

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et al., 2013)) have been applied in many fields of ecosystem research, such as the potential application for tracing the processes of metal transport in the environment. The stable metal isotope fractionation in higher plants has also been studied for Zn (Moynier et al., 2009; Aucour et al., 2011; Jouvin et al., 2012; Tang et al., 2012, 2016; Deng et al., 2014), Mg (Black et al., 2008; Bolou-Bi et al., 2010), Fe (Kiczka et al., 2010; Guelke and Von Blanckenburg, 2007), Cu (Weinstein et al., 2011; Jouvin et al., 2012), Ca (Cobert et al., 2011; Hindshaw et al., 2012; Schmitt et al., 2013) and Ni (Deng et al., 2014). Moreover, the processes (absorption, complexation, diffusion, reduction) which could lead to metal isotope fractionation within plants have been demonstrated (Bolou-Bi et al., 2010; Kiczka et al., 2010; Cobert et al., 2011; Jouvin et al., 2012; Tang et al., 2012, 2016; Deng et al., 2014; Wiederhold, 2015).

Compared with other metal isotopes (such as Zn, Cu, Fe, Mg), the study of stable Cd isotope fractionation is still in its infancy. The Cd isotopes have been applied on samples of different nature, such as meteorites (Wombacher et al., 2003, 2008), sea waters (Lacan et al., 2006; Ripperger et al., 2007; Gault-Ringold and Stirling, 2012; Xue et al., 2012; Yang et al., 2012; Lambelet et al., 2013; Abouchami et al., 2014; Conway and John, 2015; Hohl et al., 2017), geological and environmental matrices (soil (Cloquet et al., 2005, 2006; Shiel et al., 2010; Chrastný et al., 2015; Wen et al., 2015; Martinkova et al., 2016; Zhang et al., 2016; Zhu et al., 2016), sediments (Gao et al., 2013) and plants (Pallavicini et al., 2014; Wiggenhauser et al., 2016)). However, the mechanisms reported to influence Cd isotopic fractionation included partial evaporation/condensation (Wombacher et al., 2003, 2008), biological activity (such as adsorption processes, uptake) (Ripperger et al., 2007; Pallavicini et al., 2014; Wei et al., 2015, 2016; Wiggenhauser et al., 2016) and weathering (Zhang et al., 2016). For example, Wombacher et al. (2003, 2008) suggested that the substantial natural Cd isotope fractionations were generated by evaporation and (or) condensation processes. Also, Ripperger et al. (2007) reported the Cd isotopic fractionation from +0.3‰ to +3.8‰ for $\delta^{114/110}$Cd in near-surface waters, which was attributed to the preferential uptake of dissolved isotopically light Cd by phytoplankton. Moreover, Zhang et al. (2016) found significant Cd isotope fractionation during weathering processes. Wiggenhauser et al. (2016) suggested that the enrichment of heavy isotopes in the wheat grains were attributed to processes avoiding the accumulation of Cd in grains. Our previous studies have reported that Cd isotope fractionation during Cd transport from stem to leaf differs between the Cd-tolerant and -hyperaccumulator species (Wei et al., 2015, 2016). Furthermore, we suggested that the Cd isotope fractionation could provide the information for identifying the hyperaccumulator plant cultivars. In this case, three important issues must be addressed before Cd isotopes can be used to study Cd biochemical processes, viz: (1) the Cd translocation processes within the plants should be ascertained, (2) the factors which affect Cd isotope fractionation in the plants should be identified, and (3) the relationship between Cd isotope fractionation and Cd uptake, transport and redistribution within plants should be stated (von Blanckenburg et al., 2009).

Ricinus communis have high tolerance to Cd while *Solanum nigrum* have higher Cd enrichment capability (Wei et al., 2006; Huang et al., 2011). The major trait for the hyperaccumulator lies in its high efficiency in Cd translocation into shoots while the tolerant species would retain more Cd in roots. EDTA has been suggested to be the most effective and efficient organic ligand in solubilizing soil-bound metals, and could improve the phytoremediation efficiency by increasing the uptake and translocation of heavy metals towards shoot tissues (Evangelou et al., 2007; Leleyter et al., 2012; Shahid et al., 2013).

In a previous study, we have measured the Cd isotope ratios of the tissues in *R. communis* and *S. nigrum* (Wei et al., 2016). In this study, we conducted hydroponic culture experiments of the Cd tolerant *R. communis* and the hyperaccumulator *S. nigrum* using nutrient solutions with differing Cd and EDTA concentrations. The objectives of this study were to: (1) characterize Cd isotopic fractionation between solutions and plants, and among different plant tissues, and compare the fractionation of the two plant species; (2) discuss the processes which are responsible for the observed fractionations associated with Cd and EDTA supply and plant species; and (3) assess the relationship between Cd isotopic fractionation and the Cd translocation within plants.

2. Experimental and analytical methods

2.1. Plant growth experiment

The seeds of *R. communis* and *S. nigrum* were germinated on the substrate in a greenhouse. Two weeks after germination, seedlings were then transferred into polycarbonate pots containing half strength Hoagland's solution (Zhang et al., 2014). The macronutrient solution consisted of 2 mM $Ca(NO_3)_2$, 2.5 mM KNO_3, 0.5 mM KH_2PO_4, 1 mM $MgSO_4$ and 0.5 mM NH_4NO_3, as well as the micronutrient solution consisted of 0.25 μM H_3BO_3, 0.25 μM $MnSO_4$, 0.25 nM $CoCl_2$, 12.5 nM KI, 75 nM $ZnSO_4$, 0.25 nM $CuSO_4$ and 2.5 nM Na_2MoO_4. After 7 d, $CdCl_2 \cdot 2.5H_2O$ was added to the solution with Cd concentration of 0.5 mg L^{-1} and 5 mg L^{-1}. In addition, 0.5 mg L^{-1} or 5 mg L^{-1} Na-EDTA were added, respectively. Thus, six experiments were conducted with ① Cd-0.5, EDTA-0 (no EDTA added), ② Cd-5, EDTA-0 (no EDTA added), ③ Cd-0.5, EDTA-0.5, ④ Cd-0.5, EDTA-5, ⑤ Cd-5, EDTA-0.5, ⑥ Cd-5, EDTA-5. Each plant was treated as a single replicate, and there were four replicates of each condition.

Plants were cultivated under controlled conditions for 30 d (16 h photoperiod with a white light intensity of 350 μmol photons m^{-2} s^{-1}; day: night temperature 25 °C: 18 °C; relative humidity 60%–70%). Root, stem and leaf from four individual plants per experiments were separated, washed several times with ultrapure (18.2 MΩ) water in order to release Cd adsorbed on roots (Wei et al., 2015, 2016). Plant materials were freeze-dried and weighed prior to analysis.

2.2. Sample preparation and analyses

Plant samples were digested on a hot plate with HNO_3+HF + $HClO_4$ and purified by anionic exchange chromatography following the procedures of Wei et al. (2015). Each plant sample was taken to determine the concentrations of Cd and macro- and micronutrients. The concentrations of Cd and macro- and micronutrients for each of the four replicate plant samples were determined by inductively coupled plasma quadrupole mass spectrometry (ICP-QMS) (Elan DRC-e, Perkin Elmer, USA) before and after Cd purification. The Cd recovery was quantitatively monitored and yielded a range from 95% to 104% in the samples.

2.3. Cd isotope analyses and reference materials

The Cd isotope compositions were measured by multiple collector inductively coupled plasma mass spectrometry (MC-ICPMS) and the instrumental mass bias was corrected with standard-sample-standard bracketing technology. The total analytical blank was negligible in all cases at 375–789 pg, equivalent to <0.1% of the Cd present on any plant samples (1.9–269 μg).

In order to compare the Cd isotope values with other laboratories, the NIST SRM 3108 was used as an internal reference standard and three other standard solutions (Münster Cd, Nancy-Spex Cd, Spex-1 Cd standard solution) were used as the second reference

materials for quality control. The $\delta^{114/110}Cd_{NIST\ 3108}$ of Münster Cd, Nancy-Spex Cd and Spex Cd standard solutions were 4.45 ± 0.08‰ (2SD, n = 12), −0.09 ± 0.01‰ (2SD, n = 2) and −1.25 ± 0.06‰ (2SD, n = 3), respectively. The $\delta^{114/110}Cd_{NIST\ 3108}$ values of Münster Cd matched with the results of previously published methods ($\delta^{114/110}Cd_{NIST\ 3108}$ = 4.46–4.55‰) (Wombacher and Rehkämper, 2004; Ripperger and Rehkamper, 2007; Gault-Ringold and Stirling, 2012; Xue et al., 2012). This suggested that the Cd isotope values of standard solutions in this study were accurate.

Long-term stability throughout all analytical sessions as measured on NIST SRM 3108 was presented in Fig. 1A. The external reproducibility of the 136 independent measurements throughout all analytical sessions was better than 0.07‰ for $\delta^{114/110}Cd_{NIST\ 3108}$ (2SD, Fig. 1A). The obtained linearity between $\delta^{112/110}Cd_{NIST\ 3108}$ and $\delta^{114/110}Cd_{NIST\ 3108}$ values of the samples and Cd standard solutions was presented in Fig. 1B. The slope (2.0023 with 99.98% confidence interval) of mass-dependent fractionation is agreed well with that of the theoretical equilibrium fractionation. The excellent agreement of $\delta^{112/110}Cd_{NIST\ 3108}$ and $\delta^{114/110}Cd_{NIST\ 3108}$ values suggested that there is no mass-independent fractionation during the measurements.

2.4. Data calculations

The isotope compositions are expressed in δ (‰) relative to the NIST SRM 3108:

$$\delta^{114/110}Cd = [2\ (^{114}Cd/^{110}Cd)_{sample}/((^{114}Cd/^{110}Cd)_{standard1}+(^{114}Cd/^{110}Cd)_{standard2})\text{-}1] \times 1000 \quad (1)$$

Standard 1 and Standard 2 represented the standard solution measured before and after the sample.

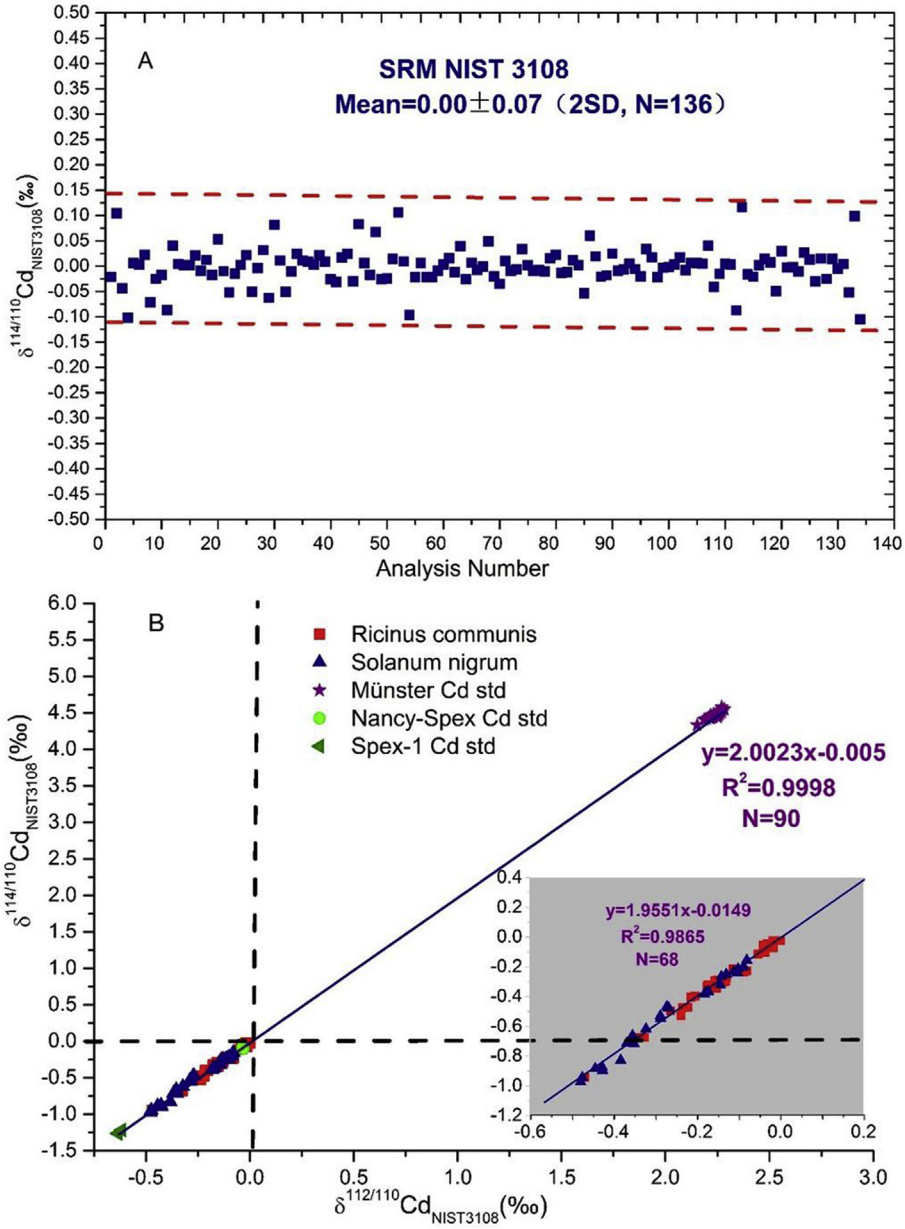

Fig. 1. A-long-term reproducibility of $\delta^{114/110}Cd_{NIST\ 3108}$ values for the NIST 3108 Cd standard (mean±2SD, n = 136, internal reproducibility). B- linearity achieved between $\delta^{112/110}Cd_{NIST\ 3108}$ and $\delta^{114/110}Cd_{NIST\ 3108}$ values.

The $\delta^{114/110}Cd$ values for whole plant or shoot (stem + leaf) are calculated as (Wei et al., 2016):

$$\delta^{114/110}Cd_{\text{whole plant or shoot}} = \sum_i m_i c_i \delta^{114/110}Cd_i / \sum_i m_i c_i \quad (2)$$

where m, c and i represent the mass of biomass (g), Cd concentration ($ng \cdot g^{-1}$), and the different plant parts, respectively.

The apparent isotopic fractionation between the two components A and B is calculated as:

$$\triangle^{114/110}Cd_{A\text{-}B} = \delta^{114/110}Cd_A - \delta^{114/110}Cd_B \quad (3)$$

2.5. Data analysis

All data were analyzed using one-way analysis of variance (ANOVA) with a SPSS statistical software package (version 11.5). Duncan's test was used for multiple comparisons between treatment means at the $p < 0.05$ level. Correlation coefficients (r) between quantitative variables for the field experiment were determined by Pearson's bivariate correlation analysis at the $p < 0.01$ or $p < 0.05$ levels.

3. Results

3.1. Plant growth and Cd distribution among the tissues

Cd concentrations of *R. communis* grown in different nutrient solutions decreased in the order of root > stem > leaf, regardless of the initial EDTA or Cd concentrations. In contrast, Cd concentration in the leaf of *S. nigrum* was higher than that in the stem when *S. nigrum* grew in the 0.5 mg L^{-1} Cd solution (Fig. 2A and B). Compared with EDTA-0, after adding EDTA (0.5 or 5 mg L^{-1}), the Cd concentration decreased in plant tissues of *S. nigrum*. In contrast, the variations of Cd concentration in the different tissues of *R. communis* were significantly correlated with the EDTA concentrations. Thus, compared with the EDTA-0, the Cd concentration in the stem and leaf of *R. communis* increased after adding 0.5 mg L^{-1} EDTA, while it was equal or lower after adding 5 mg L^{-1} EDTA.

For the treatments without the addition of EDTA, Cd concentration in all tissues of *S. nigrum* was higher than that in *R. communis* (Fig. 2A and B). After adding EDTA (0.5 or 5 mg L^{-1}), the Cd concentration in leaf of *S. nigrum* was higher than that in leaf of *R. communis*, while it was reverse in the stem. However, in 0.5 mg L^{-1} Cd treatments, after adding EDTA (0.5 or 5 mg L^{-1}), Cd concentration in the root of *S. nigrum* was lower than that in the root of *R. communis*. Overall, the effect of EDTA on Cd concentration was significantly lower in *R. communis* than that in *S. nigrum* under low Cd treatments.

Shown as Fig. 2 (C and D), the Cd mass in *R. communis* grown in different nutrient solutions decreased in the order of root > stem > leaf regardless of the presence of EDTA or Cd in the nutrient solutions. In contrast, when *S. nigrum* was subjected to the same treatments, the Cd mass was higher in leaf than that in stem. In addition, the Cd mass in all the plant tissues of *R. communis* increased after adding 0.5 mg L^{-1} EDTA compared with EDTA-0. However, compared to EDTA-0, after adding 5 mg L^{-1} EDTA, the Cd mass in the stem of *R. communis* decreased, but it increased in the leaf. In contrast, the Cd mass decreased in all plant tissues of *S. nigrum* after adding 5 mg L^{-1} EDTA compared with EDTA-0. However, compared with EDTA-0, after adding 0.5 mg L^{-1} EDTA, the Cd mass for the root and leaf of *S. nigrum* reduced in the 0.5 mg L^{-1} Cd treatments while it increased in 5 mg L^{-1} Cd treatments.

Overall, after adding Cd (0.5 or 5 mg L^{-1}), the Cd mass and concentration in the two investigated plants have increased while the dry weight have decreased. In contrast, compared with EDTA-0, after adding EDTA (0.5 or 5 mg L^{-1}), the dry weight increased in plant tissues of *S. nigrum*.

3.2. Cd isotope fractionation in nutrient solutions, R. communis and S. nigrum

The Cd isotopic composition ($\delta^{114/110}Cd_{NIST}$) of the initial nutrient solutions was +0.04 ± 0.06‰. In all treatments, the whole plants tended to be enriched in light isotopes relative to the final solution ($\Delta^{114/110}Cd_{\text{plant-solution}} = -0.64$ to −0.29‰ for *R. communis* and −0.84 to −0.31‰ for *S. nigrum*) (Table 1). Cd isotopes were markedly fractionated among all plant tissues. Both the tested plant species also exhibited similar enrichment in light Cd isotopes from the solution to root ($\Delta^{114/110}Cd_{\text{root-solution}} = -0.70$ to −0.32‰ for *R. communis* and −0.97 to −0.37‰ for *S. nigrum*), followed by a further slight depletion in the light Cd isotopes from the root to shoot ($\Delta^{114/110}Cd_{\text{shoot-root}} = +0.15$ to +0.22‰ for *R. communis* and +0.13 to +0.16‰ for *S. nigrum*). Noticeably, the Cd isotopic composition in leaf of *S. nigrum* was heavier than that in stem, whereas isotopic composition in stem was heavier than that in leaf of *R. communis*.

The Cd isotopic fractionation between solution and plant and among various plant tissues differed with EDTA supply, Cd supply and plant species in three major ways. Firstly, all the tissues (root, stem, leaf) of *S. nigrum* showed more enrichment of light Cd isotopes with increased concentration of EDTA in all Cd treatments (Fig. 3C and D). Similar trends were noted for the *R. communis* in the 0.5 mg L^{-1} Cd treatments (Fig. 3A), but not in the higher 5 mg L^{-1} Cd treatments (Fig. 3B). Secondly, Cd supply had a substantial impact on Cd isotope fractionation. The extent of light isotope fractionation in both species was more significant for the 0.5 mg L^{-1} Cd treatments than that for the 5 mg L^{-1} Cd treatments. Thirdly, for all Cd treatments, the Cd isotope composition of *S. nigrum* relative to solutions was significantly lighter than that of *R. communis*.

4. Discussion

4.1. Cadmium isotope fractionation in R. communis and S. nigrum

Cd isotopic mass balance between whole plant and solution yielded an overall depletion in heavy isotopes with $\Delta^{114/110}Cd_{\text{plant-solution}}$ values ranging from −0.64‰ to −0.29‰ for *R. communis* and from −0.84‰ to −0.31‰ for *S. nigrum* (Table 1). Similar to Cd, the plants were enriched in light isotopes for Fe (Guelke and Von Blanckenburg, 2007), Cu (Jouvin et al., 2012), Zn (Weiss et al., 2005; Aucour et al., 2011), Ca (Cobert et al., 2011; Schmitt et al., 2013). In contrast, a previous study suggested that plants were enriched with heavy Mg isotopes (Black et al., 2008). Noticeably, the magnitude of this depletion was more pronounced in the low Cd treatments ($\Delta^{114/110}Cd_{\text{plant-solution}} = -0.64$ to −0.29‰ for *R. communis* and −0.84 to −0.53‰ for *S. nigrum*) than that in high Cd treatments ($\Delta^{114/110}Cd_{\text{plant-solution}} = -0.32$ to −0.30‰ for *R. communis* and −0.43 to −0.31‰ for *S. nigrum*). Consequently, the Cd supply level has affected Cd isotope fractionation in the whole plant grown under hydroponic conditions.

The root and shoot represent a flow-through system (Wiggenhauser et al., 2016). Correspondingly, the Cd isotope fractionation during the Cd translocation between the root and shoot of *R. communis* and *S. nigrum* could be estimated by a Rayleigh-type mass balance equation:

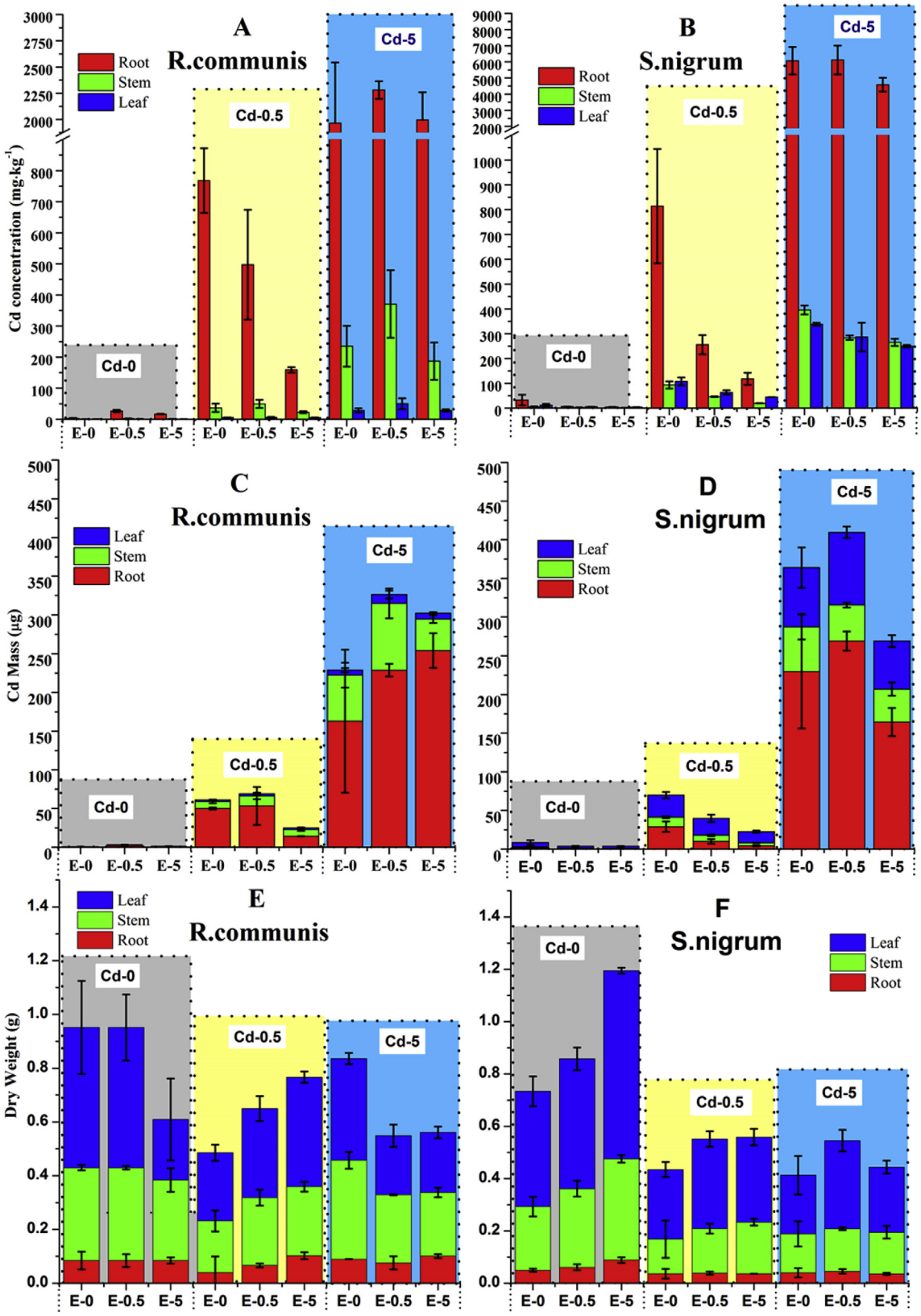

Fig. 2. Cd concentration (A, B), Cd mass (C, D) and Dry weight (E, F) of root, stem and leaf of *R. communis* and *S. nigrum* in the Cd and EDTA solution conditions. Error bars show standard error (SE) of the four replicates.

Table 1
The Cd isotope discrimination during different tissues of *R. communis* and *S. nigrum* during the Cd and EDTA solution conditions.

$\triangle^{114/110}$Cd	Cd Treatments	EDTA Treatments	Root-solution	Stem-solution	Leaf-solution	Plant-solution	Root-plant	Shoot-plant	Shoot-root
R.communis	0.5 mg L^{-1}	0 mg L^{-1}	−0.32	−0.12	−0.28	−0.29	−0.03	0.14	0.18
		0.5 mg L^{-1}	−0.57	−0.38	−0.56	−0.54	−0.04	0.13	0.16
		5 mg L^{-1}	−0.70	−0.51	−0.75	−0.64	−0.06	0.09	0.15
	5 mg L^{-1}	0 mg L^{-1}	−0.36	−0.16	−0.34	−0.30	−0.05	0.13	0.18
		0.5 mg L^{-1}	−0.37	−0.17	−0.28	−0.31	−0.05	0.13	0.18
		5 mg L^{-1}	−0.36	−0.10	−0.34	−0.32	−0.04	0.19	0.22
S. nigrum	0.5 mg L^{-1}	0 mg L^{-1}	−0.60	−0.45	−0.49	−0.53	−0.07	0.05	0.13
		0.5 mg L^{-1}	−0.71	−0.61	−0.54	−0.60	−0.11	0.04	0.15
		5 mg L^{-1}	−0.97	−0.94	−0.77	−0.84	−0.13	0.03	0.16
	5 mg L^{-1}	0 mg L^{-1}	−0.37	−0.3	−0.15	−0.31	−0.06	0.10	0.16
		0.5 mg L^{-1}	−0.43	−0.29	−0.27	−0.37	−0.05	0.10	0.15
		5 mg L^{-1}	−0.49	−0.40	−0.33	−0.43	−0.05	0.08	0.13

$$\triangle^{114/110}Cd_{root\text{-}plant} = \delta^{114/110}Cd_{root} - \delta^{114/110}Cd_{plant} = \triangle^{114/110}Cd_{translocation} \ln F_{root} \quad (4)$$

$$\triangle^{114/110}Cd_{shoot\text{-}plant} = \delta^{114/110}Cd_{shoot} - \delta^{114/110}Cd_{plant} = \triangle^{114/110}Cd_{translocation} \ln F_{shoot} \quad (5)$$

The Cd isotope fractionation of the root and shoot depended on two factors, viz: ① the isotope discrimination during the translocation process in the root and shoot ($\triangle^{114/110}Cd_{translocation}$), and ② Cd proportion remaining in the root and shoot after translocation (F_{root} and F_{shoot}). The data generated good fits between $\triangle^{114/110}Cd_{root\text{-}plant}$ and ln F_{root} ($R^2 = 0.9759$), and between $\triangle^{114/110}Cd_{shoot\text{-}plant}$ and ln F_{shoot} ($R^2 = 0.9533$) (Fig. 4). Shown in Fig. 4, the enrichment of light isotopes was enhanced with the decrease of the Cd mass fraction stored in the root while the opposite relationship was observed in the shoot. These results indicate that the magnitude of Cd isotope fractionation between plant tissues correlates with the Cd redistribution in tissues.

Our results were similar to those of Wiggenhauser et al. (2016), who proposed that wheat plants were slightly enriched in the light Cd isotope relative to the Cd $(NO_3)_2$-extractable Cd or did not significantly differ in Cd isotopic composition. In contrast, Pallavicini et al. (2014) suggested that birch leaf was enriched in heavy Cd isotopes relative to soils. The distinct Cd fractionation in wheat or birch plants may be attributed to the growing conditions of the plants (e.g. solution and soil culture, or field study), or the nature of plants (the Cd-sensitive or Cd-tolerant plant). Indeed, Tang et al. (2016) found that Zn isotope fractionation of same plants showed great inconsistency between hydroponic and field conditions. Moreover, Verbruggen et al. (2009) reported that a hyperaccumulator works differently than a Cd non tolerant plant like a grass. Noticeably, the Cd isotopic compositions in leaf of *S. nigrum*

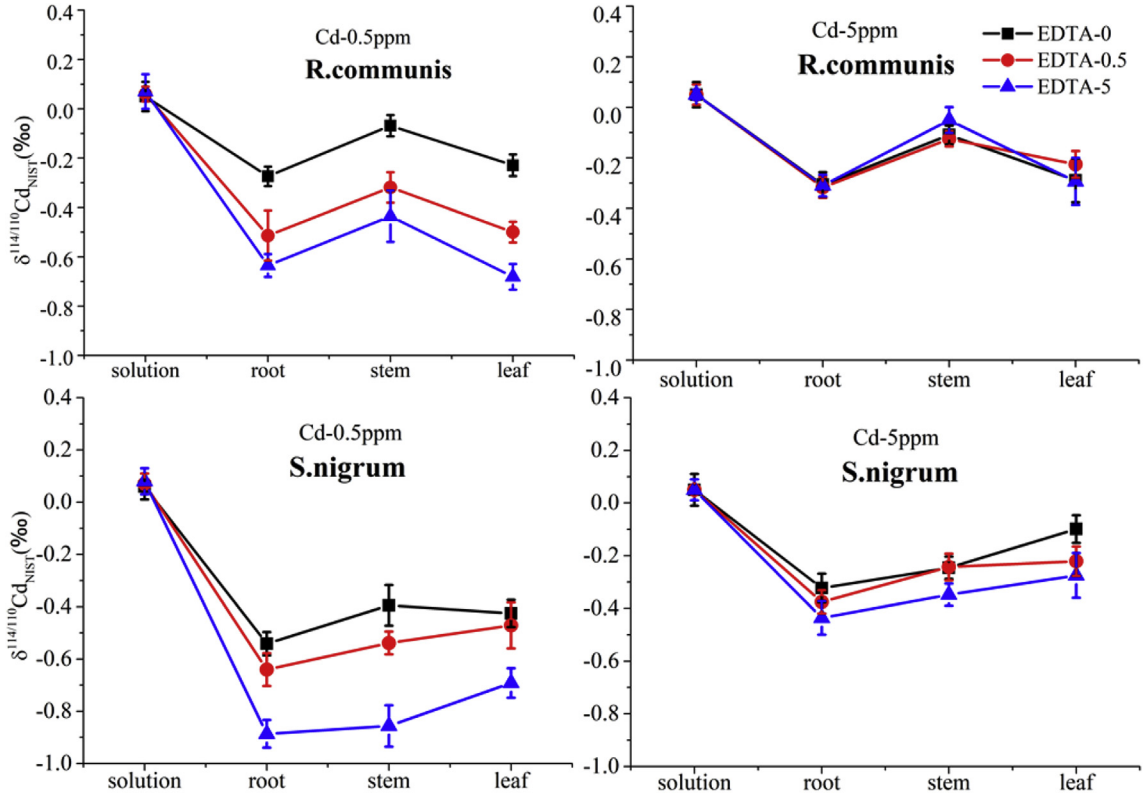

Fig. 3. Cd isotope compositions (reported as $\delta^{114/110}Cd_{NIST}$) in the final solution, root, stem and leaf of *R. communis* and *S. nigrum* in the Cd and EDTA solution conditions. Error bars show standard error (SE) of the four replicates.

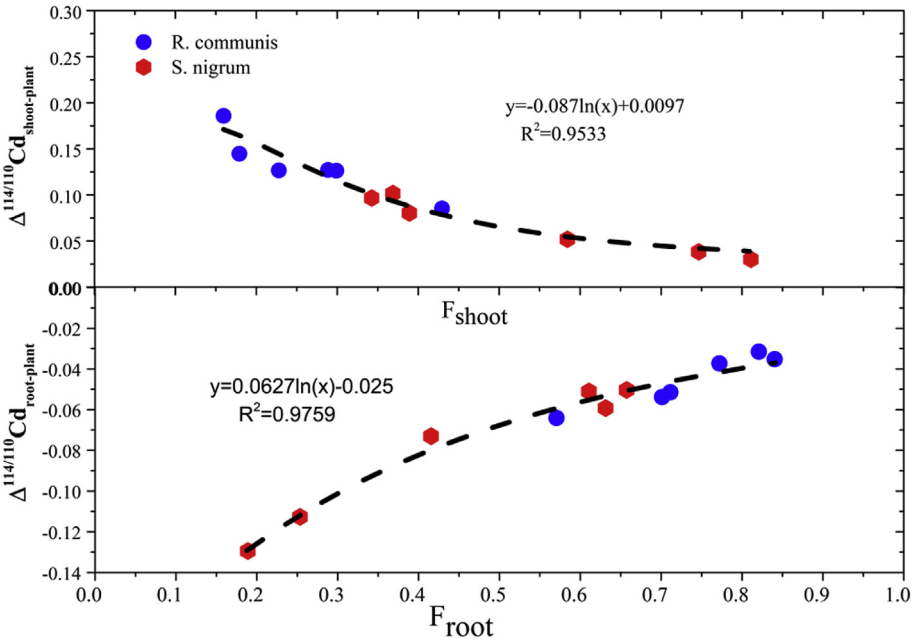

Fig. 4. Cd isotopic compositions between root (shoot) and whole plant ($\Delta^{114/110}Cd_{root\text{-}plant}$ and $\Delta^{114/110}Cd_{shoot\text{-}plant}$) as a function of F_{root} (F_{shoot}) in *R. communis* and *S. nigrum*. F_{root} is given as the ratio of Cd mass in the root to Cd mass in the whole plant: $F_{root} = [Cd]_{root}/[Cd]_{whole}$ while F_{shoot} is given as the ratio of Cd mass in the shoot to Cd mass in the whole plant: $F_{shoot} = [Cd]_{shoot}/[Cd]_{whole}$.

were heavier than those in the stem, whereas in *R. communis*, the opposite was noted, which was consistent with the result of previous studies (Wei et al., 2016). Isotope fractionation between the stem and leaf could be caused by combination of many processes, such as ion speciation/adsorption/assimilation in the stem or leaf (Wiggenhauser et al., 2016). The different Cd isotope fractionation patterns from stem to leaf may indicate the distinct transportation and enrichment mechanisms between the Cd tolerant and hyperaccumulator species. Correspondingly, the processes causing Cd isotope fractionation from stem to leaf can be further clarified with more elaborate experimental designs, thereby the relationships between the Cd isotope fractionation and translocation within plants will also be stated.

4.2. Cadmium isotope fractionation during Cd transfer from nutrient solution to root

The results clearly demonstrated that the Cd taken up by root was always enriched in the light Cd isotope compared to the nutrient solution ($\Delta^{114/110}Cd_{root\text{-}solution} = -0.70$ to -0.32‰ for *R. communis* and -0.97 to -0.37‰ for *S. nigrum*) (Shown as Table 1), irrespective of the considered experiments. This result was in accord with those obtained in previous studies which the root of wheat was enriched in light isotope (Wiggenhauser et al., 2016). Similar to Cd, root of plant was enriched in the light Cu (Jouvin et al., 2012), Ca (Cobert et al., 2011), Ni (Deng et al., 2014) and Fe isotopes (Guelke and Von Blanckenburg, 2007) compared to solutions or soils. In contrast, Mg (Bolou-Bi et al., 2010) and Zn (Weiss et al., 2005; Aucour et al., 2011; Jouvin et al., 2012; Tang et al., 2012; Deng et al., 2014) isotopes in root of plant were enriched in the heavy isotopes compared to the nutrient solutions.

During Cd uptake by root, Cd is first adsorbed on the root surface and then crosses the membrane through ion channels, electrogenic pumps (Fig. 5c), or carrier-proteins (Fig. 5b) (Verbruggen et al., 2009; Lux et al., 2011). In the root cell, the Cd either is chelated with glutathione synthetase (Cd-GS_2) and metallothioneins (MT) in the cytoplasm or is transferred into the vacuoles by phytochelatin (PC) before being further translocated to the xylem (Verbruggen et al., 2009). Thus, in the root, three main processes which could induce the Cd isotope fractionation will be discussed in this session.

Cd diffusion in rhizospheric solution may cause Cd isotope fractionation. The concentration gradient usually leads to ion diffusion in the rhizospheric solution (Lux et al., 2011). The concentration gradient at the solution-root interface is expected to be steeper at lower Cd concentrations. The enlarged concentration gradients will accelerate the ion diffusion on root surface, thereby causing kinetic isotope fractionation. Usually, the light isotopes move faster than heavy ones (Rodushkin et al., 2004). Rodushkin et al. (2004) provide direct evidence that ion diffusion in solution resulted in light isotope enrichment. Deng et al. (2014) demonstrated that ion diffusion in solution resulted in light isotope enrichment ($\Delta^{66}Zn_{plant\text{-}solution} = -0.16$ to -0.13‰) in low Zn treatments. Jouvin et al. (2012) also proposed that the diffusion processes would lead to light Zn and Cu isotope enrichment in higher plants. Cd was thought to take a similar route within plants to that of element Zn (Yamaji and Ma, 2014; Page and Feller, 2015; Wiggenhauser et al., 2016). In our metal element experiments, it has been confirmed that the Zn and Cu uptake in *S. nigrum* was significantly associated with Cd treatments (Table S1). Indeed, the effect of ion diffusion could explain the Cd isotope fractionation of *R. communis* and *S. nigrum* in our study. Shown as Table 1, the isotope shifts in the low Cd treatments ($\Delta^{114/110}Cd_{root\text{-}solution} = -0.70$ to -0.32‰ for *R. communis* and -0.97 to -0.60‰ for *S. nigrum*) (Table 1) were greater than those in the high Cd treatments ($\Delta^{114/110}Cd_{root\text{-}solution} = -0.37$ to -0.36‰ for *R. communis* and -0.49 to -0.36‰ for *S. nigrum*) (Table 1). It is attributed to the fact that the ion diffusion in the rhizosphere has more effect in the low Cd treatments than that in high Cd treatments.

In addition, ion transport across root cell membrane could generate the Cd isotope fractionation. The previous studies (Weiss et al., 2005; John et al., 2007; Deng et al., 2014; Caldelas and Weiss, 2017) supposed that the enrichment of light isotopes occurred during the low-affinity transport (e.g., ion channel and electrogenic pumps) (Fig. 5c), whereas the reverse is true for high-affinity

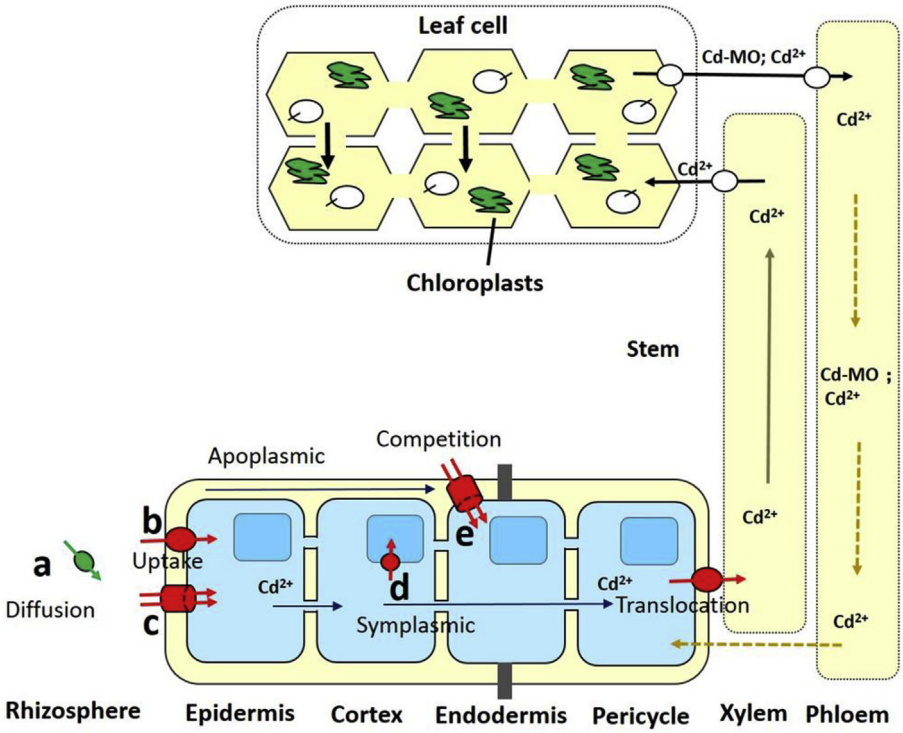

Fig. 5. Schematic of the Cd translocation within plants. The processes of Cd translocation in the rhizospheres and root cells usually include (a) ion diffusion, (b) carrier-proteins, such as ZIP (Zinc-regulated transporter or Iron-regulated transporter-like protein) transporters, (c) ion channels, electrogenic pumps, (d) vacuole sequestration, and (e) ion competition. In the root, Cd will follow symplasmic or apoplasmic pathway to the xylem. Then Cd is transferred with the transpiration stream via the xylem to the leaf as Cd^{2+} ions. In the leaf, some Cd will be sequestrated in the leaf cells through the chelation of organic molecule (e.g. chlorophyll, Cd-adenosine triphosphate, Cd-proteins), whereas parts of Cd associated with organic molecules will be translocated through the phloem toward root organs (Verbruggen et al., 2009; Lux et al., 2011).

transport (e.g. ion carrier) (Fig. 5b). Usually, a high-affinity transport will be triggered at extremely low concentrations, while above the critical concentration, a low-affinity transport will dominate (Deng et al., 2014). Gault-Ringold and Stirling (2012) proposed that phytoplankton could switch isotope fractionation pattern as Cd concentration changed from the deficient to the sufficient level. Furthermore, for other metal isotopes, Hacisalihoglu et al. (2001) suggested 10 nM of Zn^{2+} as the threshold for bread wheat with switch from high-to low-affinity transports. Deng et al. (2014) also proposed that larger negative isotopic shift in low Ni treatments compared with high Ni treatments caused lighter isotope enrichment in plants. Indeed, our results conformed to this conclusion. It was evident in our study that *R. communis* and *S. nigrum* grown in low Cd treatments had the greater negative isotopic shift ($\Delta^{114/110}Cd_{\text{root-solution}} = -0.70$ to $-0.31‰$ and -0.97 to $-0.60‰$) (Table 1), whereas the isotopic shift became less pronounced in the high Cd treatments ($\Delta^{114/110}Cd_{\text{root-solution}} = -0.37$ to $-0.36‰$ and -0.49 to $-0.37‰$) (Table 1).

The threshold for triggering low-affinity transport differed with plant species and it is usually high in hyperaccumulators compared with other plants (Deng et al., 2014). Indeed, the impact of low-or high-affinity transport on isotopic fractionation could explain the findings observed in our experiments. In our case, two investigated species presented light Cd isotope enrichment and those grown in low Cd treatments were enriched with lighter Cd isotopes than those in high Cd treatments, which might reflect the functioning of low-affinity transport systems. This was corroborated with Wiggenhauser et al. (2016), who inferred that Cd was transported through a low-affinity transport system from uptake kinetic studies. Moreover, the range of isotopic fractionation in hyperaccumulators *S. nigrum* is larger than that in the tolerant *R. communis* during all the treatments (-0.84 to $-0.31‰$ vs -0.64 to $-0.29‰$) (Table 1). It indicates that low-affinity transport systems play a more significant role on the hyperaccumulators.

Besides the two processes mentioned above (diffusion and transport), the chelation of organic compounds may also lead to the Cd isotope fractionation in the rhizosphere (Lux et al., 2011). In our experiments, EDTA was added in the nutrient solution as major organic ligand. From Fig. 3A and C, *R. communis* and *S. nigrum* grown in high EDTA treatments showed a greater negative isotopic shift than that in low or no EDTA treatments when the plants grew in low Cd stress solutions. It indicates that the concentration of EDTA has an impact on the Cd isotope fractionations and the chelation process has caused lighter Cd isotope enrichment in *R. communis* and *S. nigrum*. This conclusion was supported by the observation of Cd isotopic fractionation in previous studies. For example, Yang et al. (2015) suggested that S-containing ligands in hydrothermal fluids might preferentially complex light Cd isotopes compared with other inorganic ligands. Moreover, Horner et al. (2013) proposed that Cd sequestration onto membrane thiols could lead to light Cd isotope enrichment in the Cd complexes by organisms of *Escherichia coli*, unlike other elements such as Cu (Bigalke et al., 2010; Ryan et al., 2014) and Fe (Dideriksen et al., 2008; Morgan et al., 2010). Also, our finding is in agreement with fractionation profiles of other metal isotopes reported in literature. For instance, Hg bound to thiols favored light isotopes compared with chloride and hydroxide forms of the elements (Wiederhold et al., 2010). Besides the chelation in the media, previous studies showed that the chelate of cellular sequestration could lead to the enrichment of heavy metal isotopes (Aucour et al., 2011). However, how Cd complexation of cellular sequestration affects the Cd isotope fractionation is unknown and it needs to be further studied

by measuring Cd isotope composition in the cytoplasm and vacuoles.

Based on the above discussion, the Cd isotope fractionation in root could be affected by the Cd and EDTA concentration, plant species through the processes of ion transport across root cell membrane, ion chelation in media or cell, and ion diffusion in the rhizospheric solution.

4.3. Cadmium isotope fractionation during Cd transfer from root to shoot

Although all the tissues of two studied plants were enriched with light Cd isotope compared with the initial solution, the Cd exported to shoot was isotopically heavier ($\Delta^{114/110}Cd_{shoot\text{-}root}$ = +0.13 to +0.22‰) relative to Cd pools in the root (Table 1). This was consistent with the results of Wiggenhauser et al. (2016) that the shoots of wheat were enriched with heavier isotopes compared to root. In contrast, the shoots were enriched with the light isotopes compared to the roots for Cu (Jouvin et al., 2012), Zn (Weiss et al., 2005; Aucour et al., 2011; Jouvin et al., 2012; Tang et al., 2012; Deng et al., 2014), Mg (Bolou-Bi et al., 2010), Ca (Cobert et al., 2011; Schmitt et al., 2013). In particular, the $\Delta^{114/110}Cd_{shoot\text{-}root}$ values were larger for the *R. communis* (+0.15 to +0.22‰) than *S. nigrum* (+0.13 to +0.16‰). It might be associated with plant height because the *R. communis* is obviously higher than *S. nigrum*. Furthermore, the $\Delta^{114/110}Cd_{shoot\text{-}root}$ values for the two plants were similar in the low Cd or EDTA treatments and high Cd or EDTA treatments, indicating that the Cd isotope fractionation associated with root-shoot translocation was independent of the Cd and EDTA concentrations in the solution.

Once Cd enters the xylem, it will migrate with the transpiration stream to leaf via the xylem (Mendoza-Cozatl et al., 2011). Wiggenhauser et al. (2016) inferred that the isotope fractionation between shoot and root was mainly influenced by vacuolar sequestration and xylem loading processes. Moreover, previous studies also revealed that, the majority of Cd was loaded as free ion in the xylem sap, whereas Cd was mostly transported through complexation with thiols and specific proteins in the phloem sap (Ueno et al., 2008; Kato et al., 2010; Alvarez-Fernandez et al., 2014; Hazama et al., 2015). Thus, vacuolar sequestration, as discussed by Wiggenhauser et al. (2016), usually leads to heavy isotope enrichment whereas xylem loading processes are likely preferentially choose light isotopes (Deng et al., 2014). Therefore, the counterbalance of two processes will generate the heavy or light Cd isotope enrichment from the root to shoot. In our case, the vacuoles sequestration has been demonstrated as important process for Cd storage in the shoot of Cd tolerant and hyperaccumulator plants (Verbruggen et al., 2009; Wiggenhauser et al., 2016). Consequently, the enrichment of heavy Cd isotope in the shoots of *R. communis* and *S. nigrum* relative to the roots may be attributed to vacuolar sequestration of Cd in the shoots.

5. Conclusions

Firstly, the two investigated plants were more enriched in light isotopes under low Cd and high EDTA solutions than that under high Cd and low EDTA solutions. Moreover, Cd-hyperaccumulator *S. nigrum* was more enriched in light isotopes than Cd-tolerant *R. communis*. This suggested that the intensity of fractionation was likely mediated by the Cd content, EDTA concentration of the nutrient solutions, and species-specific differences, indicating a potential for utilizing isotopic signatures to illuminate the role of Cd in plant physiological processes.

Secondly, the presence of EDTA increased Cd uptake into hyperaccumulator plants at low Cd supply, which could be used for remediation purposes. Furthermore, the chelation process has caused lighter Cd isotope enrichment in *R. communis* and *S. nigrum*.

Thirdly, the fits between $\triangle^{114/110}Cd_{root\text{-}plant}$ and ln F_{root} (or between $\triangle^{114/110}Cd_{shoot\text{-}plant}$ and ln F_{shoot}) suggest that Cd isotopic signatures can be used to the study of Cd transportation during the metabolic process in plants. This also indicates the potential to infer the transfer of Cd into the aboveground plant parts from the knowledge of the Cd isotope ratio in the root and the whole plant, which might help in identifying Cd-avoiding crop cultivars.

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Author contributions

Q.J.G., G.R.Y and S.L.L proposed and organized the project. R.F.W., Q.J.G., and S.L.L. discussed and designed the experiment. R.F.W., Q.J.G., J.K., L.Y.T and X.K.H. carried out the experiments. R.F.W., Q.J.G., Z.L.S. and J.H. analyzed and interpreted the data together. Q.J.G. and R.F.W wrote the main manuscript text. C.P.O revised the manuscript. All the authors participated in discussions of the research.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2018.01.103.

References

Abouchami, W., Galer, S.J.G., de Baar, H.J.W., Middag, R., Vance, D., Zhao, Y., Klunder, M., Mezger, K., Feldmann, H., Andreae, M.O., 2014. Biogeochemical cycling of cadmium isotopes in the southern ocean along the zero meridian. Geochem. Cosmochim. Acta 127, 348–367.

Ali, H., Khan, E., Sajad, M.A., 2013. Phytoremediation of heavy metals-concepts and applications. Chemosphere 91, 869–881.

Alvarez-Fernandez, A., Diaz-Benito, P., Abadia, A., Lopez-Millan, A.F., Abadia, J., 2014. Metal species involved in long distance metal transport in plants. Front. Plant Sci. 5, 105–124.

Arnold, T., Kirk, G.J., Wissuwa, M., Frei, M., Zhao, F.J., Mason, T.F., Weiss, D.J., 2010. Evidence for the mechanisms of zinc uptake by rice using isotope fractionation. Plant Cell Environ. 33, 370–381.

Aucour, A.M., Pichat, S., Macnair, M.R., Oger, P., 2011. Fractionation of stable zinc isotopes in the zinc hyperaccumulator *Arabidopsis halleri* and nonaccumulator *Arabidopsis petraea*. Environ. Sci. Technol. 45, 9212–9217.

Bigalke, M., Weyer, S., Wilcke, W., 2010. Copper isotope fractionation during complexation with insolubilized humic acid. Environ. Sci. Technol. 44, 5496–5502.

Black, J.R., Epstein, E., Rains, W.D., Yin, Q.Z., Casey, W.H., 2008. Magnesium-isotope fractionation during plant growth. Environ. Sci. Technol. 42, 7831–7836.

Bolou-Bi, E.B., Poszwa, A., Leyval, C., Vigier, N., 2010. Experimental determination of magnesium isotope fractionation during higher plant growth. Geochem. Cosmochim. Acta 74, 2523–2537.

Caldelas, C., Weiss, D.J., 2017. Zinc Homeostasis and isotopic fractionation in plants: a review. Plant Soil 411, 17–46.

Chrastný, V., Čadková, E., Vaněk, A., Teper, L., Cabala, J., Komárek, M., 2015. Cadmium isotope fractionation within the soil profile complicates source identification in relation to Pb–Zn mining and smelting processes. Chem. Geol. 405, 1–9.

Cloquet, C., Carignan, J., Libourel, G., Sterckeman, T., Perdrix, E., 2006. Tracing source pollution in soils using cadmium and lead isotopes. Environ. Sci. Technol. 40, 2525–2530.

Cloquet, C., Rouxel, O., Carignan, J., Libourel, G., 2005. Natural cadmium isotopic variations in eight geological reference materials (NIST SRM 2711, BCR 176, GSS-1, GXR-1, GXR-2, GSD-12, Nod-P-1, Nod-A-1) and anthropogenic samples,

measured by MC-ICP-MS. Geostand. Geoanal. Res. 29, 95–106.
Cobert, F., Schmitt, A.-D., Bourgeade, P., Labolle, F., Badot, P.-M., Chabaux, F., Stille, P., 2011. Experimental identification of Ca isotopic fractionations in higher plants. Geochem. Cosmochim. Acta 75, 5467–5482.
Conway, T.M., John, S.G., 2015. Biogeochemical cycling of cadmium isotopes along a high-resolution section through the North Atlantic Ocean. Geochem. Cosmochim. Acta 148, 269–283.
Deng, T.H., Cloquet, C., Tang, Y.T., Sterckeman, T., Echevarria, G., Estrade, N., Morel, J.L., Qiu, R.L., 2014. Nickel and zinc isotope fractionation in hyperaccumulating and nonaccumulating plants. Environ. Sci. Technol. 48, 11926–11933.
Dideriksen, K., Baker, J.A., Stipp, S.L.S., 2008. Equilibrium Fe isotope fractionation between inorganic aqueous Fe(III) and the siderophore complex, Fe(III)-desferrioxamine B. Earth Planet Sci. Lett. 269, 280–290.
Evangelou, M.W., Ebel, M., Schaeffer, A., 2007. Chelate assisted phytoextraction of heavy metals from soil. Effect, mechanism, toxicity, and fate of chelating agents. Chemosphere 68, 989–1003.
Gao, B., Zhou, H.D., Liang, X.R., Tu, X.L., 2013. Cd isotopes as a potential source tracer of metal pollution in river sediments. Environ. Pollut. 181, 340–343.
Gault-Ringold, M., Stirling, C.H., 2012. Anomalous isotopic shifts associated with organic resin residues during cadmium isotopic analysis by double spike MC-ICPMS. J. Anal. Atomic Spectrom. 27, 449–459.
Guelke, M., Von Blanckenburg, F., 2007. Fractionation of stable iron isotopes in higher plants. Environ. Sci. Technol. 41, 1896–1901.
Hacisalihoglu, G., Hart, J.J., Kochian, L.V., 2001. High- and low-affinity zinc transport systems and their possible role in zinc efficiency in bread wheat. Plant Physiol. 125, 456–463.
Hazama, K., Nagata, S., Fujimori, T., Yanagisawa, S., Yoneyama, T., 2015. Concentrations of metals and potential metal-binding compounds and speciation of Cd, Zn and Cu in phloem and xylem saps from castor bean plants (*Ricinus communis*) treated with four levels of cadmium. Physiol. Plant. 154, 243–255.
Hindshaw, R.S., Reynolds, B.C., Wiederhold, J.G., Kiczka, M., Kretzschmar, R., Bourdon, B., 2012. Calcium isotope fractionation in alpine plants. Biogeochemistry 112, 373–388.
Hohl, S.V., Galer, S.J.G., Gamper, A., Becker, H., 2017. Cadmium isotope variations in Neoproterozoic carbonates - a tracer of biologic production? Geochem. Perspect. Lett. 3, 32–44.
Horner, T.J., Lee, R.B., Henderson, G.M., Rickaby, R.E., 2013. Nonspecific uptake and homeostasis drive the oceanic cadmium cycle. Proc. Natl. Acad. Sci. U. S. A. 110, 2500–2505.
Huang, H., Yu, N., Wang, L., Gupta, D.K., He, Z., Wang, K., Zhu, Z., Yan, X., Li, T., Yang, X.E., 2011. The phytoremediation potential of bioenergy crop *Ricinus communis* for DDTs and cadmium co-contaminated soil. Bioresour. Technol. 102, 11034–11038.
John, S.G., Geis, R.W., Saito, M.A., Boyle, E.A., 2007. Zinc isotope fractionation during high-affinity and low-affinity zinc transport by the marine diatom *Thalassiosira oceanica*. Limnol. Oceanogr. 52, 2710–2714.
Jouvin, D., Weiss, D.J., Mason, T.F.M., Bravin, M.N., Louvat, P., Zhao, F., Ferec, F., Hinsinger, P., Benedetti, M.F., 2012. Stable isotopes of Cu and Zn in higher plants: evidence for Cu reduction at the root surface and two conceptual models for isotopic fractionation processes. Environ. Sci. Technol. 46, 2652–2660.
Kato, M., Ishikawa, S., Inagaki, K., Chiba, K., Hayashi, H., Yanagisawa, S., Yoneyama, T., 2010. Possible chemical forms of cadmium and varietal differences in cadmium concentrations in the phloem sap of rice plants (*Oryza sativaL.*). Soil Sci. Plant Nutr. 56, 839–847.
Kiczka, M., Wiederhold, J.G., Kraemer, S.M., Bourdon, B., Kretzschmar, R., 2010. Iron isotope fractionation during Fe uptake and translocation in alpine plants. Environ. Sci. Technol. 44, 6144–6150.
Lacan, F., Francois, R., Ji, Y.C., Sherrell, R.M., 2006. Cadmium isotopic composition in the ocean. Geochem. Cosmochim. Acta 70, 5104–5118.
Lambelet, M., Rehkamper, M., de Flierdt, T.V., Xue, Z.C., Kreissig, K., Coles, B., Porcelli, D., Andersson, P., 2013. Isotopic analysis of Cd in the mixing zone of Siberian rivers with the Arctic Ocean-New constraints on marine Cd cycling and the isotope composition of riverine Cd. Earth Planet Sci. Lett. 361, 64–73.
Leleyter, L., Rousseau, C., Biree, L., Baraud, F., 2012. Comparison of EDTA, HCl and sequential extraction procedures, for selected metals (Cu, Mn, Pb, Zn), in soils, riverine and marine sediments. J. Geochem. Explor. 116–117, 51–59.
Lux, A., Martinka, M., Vaculik, M., White, P.J., 2011. Root responses to cadmium in the rhizosphere: a review. J. Exp. Bot. 62, 21–37.
Martinkova, E., Chrastny, V., Francova, M., Sipkova, A., Curik, J., Myska, O., Mizic, L., 2016. Cadmium isotope fractionation of materials derived from various industrial processes. J. Hazard Mater. 302, 114–119.
Mendoza-Cozatl, D.G., Jobe, T.O., Hauser, F., Schroeder, J.I., 2011. Long-distance transport, vacuolar sequestration, tolerance, and transcriptional responses induced by cadmium and arsenic. Curr. Opin. Plant Biol. 14, 554–562.
Morgan, J.L., Wasylenki, L.E., Nuester, J., Anbar, A.D., 2010. Fe isotope fractionation during equilibration of Fe-organic complexes. Environ. Sci. Technol. 44, 6095–6101.
Moynier, F., Pichat, S., Pons, M.-L., Fike, D., Balter, V., Albarède, F., 2009. Isotopic fractionation and transport mechanisms of Zn in plants. Chem. Geol. 267, 125–130.
Page, B.D., Bullen, T.D., Mitchell, M.J., 2008. Influences of calcium availability and tree species on Ca isotope fractionation in soil and vegetation. Biogeochemistry 88, 1–13.
Page, V., Feller, U., 2015. Heavy Metals in Crop Plants: transport and redistribution processes on the whole plant level. Agronomy 5, 447–463.
Pallavicini, N., Engstrom, E., Baxter, D.C., Ohlander, B., Ingri, J., Rodushkin, I., 2014. Cadmium isotope ratio measurements in environmental matrices by MC-ICP-MS. J. Anal. Atomic Spectrom. 29, 1570–1584.
Ripperger, S., Rehkamper, M., 2007. Precise determination of cadmium isotope fractionation in seawater by double spike MC-ICPMS. Geochem. Cosmochim. Acta 71, 631–642.
Ripperger, S., Rehkamper, M., Porcelli, D., Halliday, A.N., 2007. Cadmium isotope fractionation in seawater - a signature of biological activity. Earth Planet Sci. Lett. 261, 670–684.
Rodushkin, I., Stenberg, A., Andren, H., Malinovsky, D., Baxter, D.C., 2004. Isotopic fractionation during diffusion of transition metal ions in solution. Anal. Chem. 76, 2148–2151.
Ryan, B.M., Kirby, J.K., Degryse, F., Scheiderich, K., McLaughlin, M.J., 2014. Copper isotope fractionation during equilibration with natural and synthetic ligands. Environ. Sci. Technol. 48, 8620–8626.
Schmitt, A.D., Cobert, F., Bourgeade, P., Ertlen, D., Labolle, F., Gangloff, S., Badot, P.M., Chabaux, F., Stille, P., 2013. Calcium isotope fractionation during plant growth under a limited nutrient supply. Geochem. Cosmochim. Acta 110, 70–83.
Shahid, M., Austruy, A., Echevarria, G., Arshad, M., Sanaullah, M., Aslam, M., Nadeem, M., Nasim, W., Dumat, C., 2013. EDTA-enhanced phytoremediation of heavy metals: a review. Soil Sediment Contam. Int. J. 23, 389–416.
Shiel, A.E., Weis, D., Orians, K.J., 2010. Evaluation of zinc, cadmium and lead isotope fractionation during smelting and refining. Sci. Total Environ. 408, 2357–2368.
Staessen, J.A., Roels, H.A., Emelianov, D., Kuznetsova, T., Thijs, L., Vangronsveld, J., Fagard, R., 1999. Environmental exposure to cadmium, forearm bone density, and risk of fractures: prospective population study. Lancet 353, 1140–1144.
Tang, Y.T., Cloquet, C., Deng, T.H., Sterckeman, T., Echevarria, G., Yang, W.J., Morel, J.L., Qiu, R.L., 2016. Zinc isotope fractionation in the hyperaccumulator *Noccaea caerulescens* and the nonaccumulating plant *Thlaspi arvense* at low and high Zn supply. Environ. Sci. Technol. 50, 8020–8027.
Tang, Y.T., Cloquet, C., Sterckeman, T., Echevarria, G., Carignan, J., Qiu, R.L., Morel, J.L., 2012. Fractionation of stable zinc isotopes in the field-grown zinc hyperaccumulator *Noccaea caerulescens* and the zinc-tolerant plant *Silene vulgaris*. Environ. Sci. Technol. 46, 9972–9979.
Ueno, D., Iwashita, T., Zhao, F.J., Ma, J.F., 2008. Characterization of Cd translocation and identification of the Cd form in xylem sap of the Cd-hyperaccumulator *Arabidopsis halleri*. Plant Cell Physiol. 49, 540–548.
Verbruggen, N., Hermans, C., Schat, H., 2009. Mechanisms to cope with arsenic or cadmium excess in plants. Curr. Opin. Plant Biol. 12, 364–372.
von Blanckenburg, F., von Wiren, N., Guelke, M., Weiss, D.J., Bullen, T.D., 2009. Fractionation of metal stable isotopes by higher plants. Elements 5, 375–380.
Wei, R., Guo, Q., Wen, H., Liu, C., Yang, J., Peters, M., Hu, J., Zhu, G., Zhang, H., Tian, L., Han, X., Ma, J., Zhu, C., Wan, Y., 2016. Fractionation of stable cadmium isotopes in the cadmium tolerant *Ricinus communis* and hyperaccumulator *Solanum nigrum*. Sci. Rep. 6, 24309.
Wei, R., Guo, Q., Wen, H., Yang, J., Peters, M., Zhu, C., Ma, J., Zhu, G., Zhang, H., Tian, L., Wang, C., Wan, Y., 2015. An analytical method for precise determination of the cadmium isotopic composition in plant samples using multiple collector inductively coupled plasma mass spectrometry. Anal. Methods 7, 2479–2487.
Wei, S.H., Zhou, Q.X., Koval, P.V., 2006. Flowering stage characteristics of cadmium hyperaccumulator *Solanum nigrum L.* and their significance to phytoremediation. Sci. Total Environ. 369, 441–446.
Weinstein, C., Moynier, F., Wang, K., Paniello, R., Foriel, J., Catalano, J., Pichat, S., 2011. Isotopic fractionation of Cu in plants. Chem. Geol. 286, 266–271.
Weiss, D.J., Mason, T.F., Zhao, F.J., Kirk, G.J., Coles, B.J., Horstwood, M.S., 2005. Isotopic discrimination of zinc in higher plants. New Phytol. 165, 703–710.
Wen, H., Zhang, Y., Cloquet, C., Zhu, C., Fan, H., Luo, C., 2015. Tracing sources of pollution in soils from the Jinding Pb-Zn mining district in China using cadmium and lead isotopes. Appl. Geochem. 52, 147–154.
Wiederhold, J.G., 2015. Metal stable isotope signatures as tracers in environmental geochemistry. Environ. Sci. Technol. 49, 2606–2624.
Wiederhold, J.G., Cramer, C.J., Daniel, K., Infante, I., Bourdon, B., Kretzschmar, R., 2010. Equilibrium mercury isotope fractionation between dissolved Hg(II) species and thiol-bound Hg. Environ. Sci. Technol. 44, 4191–4197.
Wiggenhauser, M., Bigalke, M., Imseng, M., Muller, M., Keller, A., Murphy, K., Kreissig, K., Rehkamper, M., Wilcke, W., Frossard, E., 2016. Cadmium isotope fractionation in soil-wheat systems. Environ. Sci. Technol. 50, 9223–9231.
Wombacher, F., Rehkämper, M., 2004. Problems and suggestions concerning the notation of cadmium stable isotope compositions and the use of reference materials. Geostand. Geoanal. Res. 28, 173–178.
Wombacher, F., Rehkämper, M., Mezger, K., Bischoff, A., Münker, C., 2008. Cadmium stable isotope cosmochemistry. Geochem. Cosmochim. Acta 72, 646–667.
Wombacher, F., Rehkamper, M., Mezger, K., Munker, C., 2003. Stable isotope compositions of cadmium in geological materials and meteorites determined by multiple-collector ICPMS. Geochem. Cosmochim. Acta 67, 4639–4654.
Xue, Z., Rehkamper, M., Schonbachler, M., Statham, P.J., Coles, B.J., 2012. A new methodology for precise cadmium isotope analyses of seawater. Anal. Bioanal. Chem. 402, 883–893.
Yamaji, N., Ma, J.F., 2014. The node, a hub for mineral nutrient distribution in graminaceous plants. Trends Plant Sci. 19, 556–563.
Yang, J., Li, Y., Liu, S., Tian, H., Chen, C., Liu, J., Shi, Y., 2015. Theoretical calculations of Cd isotope fractionation in hydrothermal fluids. Chem. Geol. 391, 74–82.
Yang, S.C., Lee, D.C., Ho, T.Y., 2012. The isotopic composition of cadmium in the

water column of the South China Sea. Geochem. Cosmochim. Acta 98, 66–77.
Yin, R., Feng, X., Chen, B., Zhang, J., Wang, W., Li, X., 2015. Identifying the sources and processes of mercury in subtropical estuarine and ocean sediments using Hg isotopic composition. Environ. Sci. Technol. 49, 1347–1355.
Zhang, H., Guo, Q., Yang, J., Chen, T., Zhu, G., Peters, M., Wei, R., Tian, L., Wang, C., Tan, D., Ma, J., Wang, G., Wan, Y., 2014. Cadmium accumulation and tolerance of two castor cultivars in relation to antioxidant systems. J. Environ. Sci. (China) 26, 2048–2055.
Zhang, Y., Wen, H., Zhu, C., Fan, H., Luo, C., Liu, J., Cloquet, C., 2016. Cd isotope fractionation during simulated and natural weathering. Environ. Pollut. 216, 9–17.
Zhu, C., Wen, H., Zhang, Y., Fan, H., 2016. Cadmium and sulfur isotopic compositions of the Tianbaoshan Zn-Pb-Cd deposit, Sichuan Province, China. Ore Geol. Rev. 76, 152–162.