Chromatographic Separation of Cd from Plants *via* Anion-Exchange Resin for an Isotope Determination by Multiple Collector ICP-MS

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In this study, key factors affecting the chromatographic separation of Cd from plants, such as the resin column, digestion and purification procedures, were experimentally investigated. A technique for separating Cd from plant samples based on single ion-exchange chromatography has been developed, which is suitable for the high-precision analysis of Cd isotopes by multiple-collector inductively coupled plasma mass spectrometry (MC-ICP-MS). The robustness of the technique was assessed by replicate analyses of Cd standard solutions and plant samples. The Cd yields of the whole separation process were higher than 95%, and the $^{114/110}$Cd values of three Cd second standard solutions (Münster Cd, Spex Cd, Spex-1 Cd solutions) relative to the NIST SRM 3108 were measured accurately, which enabled the comparisons of Cd isotope results obtained in other laboratories. Hence, stable Cd isotope analyses represent a powerful tool for fingerprinting specific Cd sources and/or examining biogeochemical reactions in ecological and environmental systems.

Keywords Cd isotopes, anion exchange, plants, MC-ICP-MS, non-traditional isotopes, Cd standard solutions

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Introduction

The hazard of cadmium (Cd) from environmental exposure has drawn global attentions since "itai-itai" disease appeared in Japan in the 1950's.[1] Cadmium possesses eight stable isotopes (^{106}Cd, ^{108}Cd, ^{110}Cd, ^{111}Cd, ^{112}Cd, ^{113}Cd, ^{114}Cd and ^{116}Cd) with natural abundances of 1.3, 0.9, 12.5, 12.8, 24.1, 12.2, 28.7 and 7.5%, respectively.[2] The mass difference of ~27.8% makes Cd isotope variations a potential tool to resolve mechanisms of mass dependent fractionation processes, and to trace Cd sources in the environment.[3-8] Especially, the recent advent in multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS) has extended dramatically the range of applications of stable Cd isotopes as tracers in natural systems.[9,10] Cd isotope analyses have been applied in order to investigate the evolution of celestial bodies,[11-13] mineral genesis and material source,[7,14] paleo-ocean environments,[15-19] and environmental cadmium pollution sources.[3,4,8,20-22] Furthermore, Wei *et al.*[6] suggested that Cd isotope fractionation in Cd hyperaccumulator plants could apply new insights in the enrichment mechanisms.

Despite these advancements, the Cd isotope applications in the environment have been hindered by two issues, which are the low efficiency of Cd separation from the matrix and the choices of Cd isotope reference materials. Moreover, the low efficiency of Cd separation is usually attributed to: 1) low Cd recovery from the matrix and 2) matrix effects or spectral interferences. As a rule, Cd recovery is required to be above 95% when sample standard bracketing (SSB) is used to correct the instrumental mass fractionation.[20,23,24] In addition, accurate measurements of the Cd isotope ratios are influenced by the isobaric interferences (*i.e.* $^{106/108/110}$Pd on $^{106/108/110}$Cd, $^{112/114}$Sn on $^{112/114}$Cd, ^{113}In on ^{113}Cd), polyatomic interferences (*e.g.* $^{70}Zn^{40}Ar^{+}$ on ^{110}Cd, $^{70}Ge^{40}Ar^{+}$ on ^{110}Cd, $^{76}Se^{40}Ar^{+}$ on ^{116}Cd, $^{98}Mo^{16}O^{+}$ on ^{114}Cd, $^{98}Ru^{16}O^{+}$ on ^{114}Cd)[25] and contaminant-prone elements (*e.g.* K, Ca, Na, Mg).[20] Therefore, the interference ions, such as Pd, Sn, In, Zn, Ge, Se, Ru, Mo, K, Ca Na, and Mg in the Cd fraction prepare for MC-ICP-MS, should be removed from the matrix as completely as possible in order to achieve accurate Cd isotope ratios.

Recently, new chemical separation methods for Cd have been presented.[22,25-29] Most protocols for the chemical separation of Cd from matrix elements included isolation *via* ion exchange chromatography.[25,28,29] Building a large body of earlier work,[26,27] Wombacher *et al.*[28] proposed a two-stage column chemistry procedure, involving a strongly basic anion-exchange resin and hydrochloric acid media, to separate Cd from geological matrices. This method achieved a more efficient separation of Cd by adding a HNO_3–HBr acid mixture to elute the Zn fractions.[28] Based on a method of Wombacher *et al.*,[28] Ripperger and Rehkamper[25] proposed a three-stage column chemistry procedure to isolate Cd from seawater samples. Even if the Cd concentration of seawater was low, Cd could be separated

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sufficiently from the samples.

However, these procedures were insufficient for Cd separation from complex matrices, and could not provide the required Cd purity for isotopic analysis. Therefore, a precolumn precipitation step or oxidation step using a mixture of hydrogen peroxide and nitric acid (H_2O_2–HNO_3) to remove residual organic matrix derived from the resin has been carried out in order to sufficiently purify Cd.[18,30] Clearly, the more often a sample is processed, the less likely it is to achieve 100% recovery of all fractions. Cloquet *et al.*[23] developed a one-stage purification procedure to separate Cd from soil samples using HCl and a macroreticular strong base anion exchanger (AG-MP-1 resin) resulting in a Cd recovery higher than 95%. Based on the procedures of Cloquet *et al.*[23] and Gao *et al.*,[31] Zhu *et al.*[24] exchanged the added 0.06 mol L^{-1} HCl by 0.012 mol L^{-1} HCl leading to Cd yields of up to 99.82%.

Until now, only a few studies have been performed on the Cd isotopic compositions of plant samples,[5,6,20] which are rich in organic matter and metallic elements. Incomplete oxidation of carbon may disturb ion-exchange separation at later stages. Metallic elements and residual resin-derived organic compounds lead to inaccurate Cd isotope results.[32] Pallavicicini *et al.*[20] suggest ashing of organic-rich matrices. Wei *et al.*[5,6] removed the organic matter of plants by $HClO_4$. In this study, key factors affecting the chromatographic separation of Cd from plants, such as the resin column, digestion and purification procedures, were experimentally and systematically investigated. Furthermore, four Cd standard solutions (NIST SRM 3108, Münster Cd, Spex Cd, Spex-1 Cd solutions) as well as plant samples have been repeatedly analyzed. The final goals of this study are: i) to optimize the Cd purification procedure as well as a digestion method for plant samples, ii) to establish a widely used chromatographic technique for quantitative Cd separation and iii) to enable comparisons of Cd isotope results obtained in other laboratories by the Cd isotope values analysis of second standard solutions.

Experimental

All of the reagents and materials were prepared according to method described by Wei *et al.*[5] The sample handling and chemistry was performed in a class-100 clean laboratory. The acids (HNO_3 and HCl) were purified in-house prior to use by sub-boiling distillation of reagent grade feedstock in a quartz still. The water was of 18.2 MΩ·cm grade from a Milli-Q water purification system (Millipore, Bedford, MA, USA). All materials, including columns, sample bottles, test tubes and pipette tips, were washed in a heated bath of 10% HCl and rinsed with purified water prior to use.[29] AG-MP-1M ion-exchange resin (100 – 200 mesh, chloride form, Bio-Rad Laboratories) was used throughout this study.

Anion-exchange purification procedures

A mixture of the certified reference materials "GSB single element solutions" (K, Ca, Na, Mg, Al, Fe, Cd, Pb, Zn, Pd, In, Cu, Cr, Mn, Ni, Sn) was prepared. Then, 1 mL of each single standard solution with a concentration of 100 mg kg^{-1}, was taken for the GSB mixture solution. All GSB single standard solutions were purchased from the testing center of national iron and steel materials (Iron and Steel Research Institute, Beijing, China). The solutions were equilibrated for 3 days prior to further processing. Then, the mixtures were dried and redissolved in 2 mL of 2 mol L^{-1} HCl to convert the residue into the chloride form. Three replicates were prepared. The Cd fractions were separated from the matrix utilizing three anionic exchange chromatographic procedures adopted from the studies of Cloquet *et al.*,[23] Gao *et al.*[31] and Zhu *et al.*,[24] which were termed as Procedure 1, Procedure 2 and Procedure 3, respectively. Each eluate (2 mL) was dried and redissolved by 2 mL of 2% HNO_3 before the elements concentrations were measured by inductively coupled plasma mass spectrometry (ICP-MS) (Elan DRC-e, Perkin Elmer, USA).

Table 1 Column diameter, resin volume and Cd recovery applied for the elution of GSB mixtures (SD, $N = 3$)

Resin height/ mm	Column diameter/ mm	Resin volume/ mL	Cd recovery, %
78	6	2.2	115.96 ± 2.41
110	6	3.1	98.88 ± 3.95
140	6	4.0	110.14 ± 4.56
80	7	3.0	112.5 ± 2.97
103	7	4.0	114.26 ± 4.56
115	7	4.4	100.16 ± 3.28
60	8	3.0	101.18 ± 4.36
70	8	3.5	113.98 ± 3.89
80	8	4.0	91.66 ± 2.84
90	8	4.5	111.9 ± 4.21

Diameter of resin columns and volume of resin

The diameter of the resin columns and the volume of the resins were set according to Table 1. The GSB mixture solutions were eluted according to Procedure 3. The element concentrations of each elute (5 mL) were measured by inductively coupled plasma mass spectrometry (ICP-MS) (Elan DRC-e, Perkin Elmer, USA) as all samples analyzed for this study.

Plant digestion

To bring solid samples into solution, two preparation procedures were tested: 0.2 g of plant species *Cyperus alternifolius* (underground and overground parts) were digested using mixtures of HNO_3–HF–H_2O_2 and HNO_3–HF–$HClO_4$, respectively. Firstly, the samples were digested in concentrated aristar-grade HNO_3 (5 mL) and HF (1 mL) for 48 h in acid-cleaned teflon beakers. The closed breaker were placed on a hot plate for 8 h at 80°C and then at 160°C until the plants were completely digested. Secondly, 2 – 3 mL of H_2O_2 or $HClO_4$ were added to the digested solutions to remove any organic materials. After evaporation at 165 – 180°C, the samples were dried and redissolved in 5 mL 1%(v/v) HNO_3. Then, 2 mL of supernatants were transferred into pre-cleaned polyethylene bottles for determinating the metal content. The rest of the fractions were evaportated to dryness, redissolved in 10 mol L^{-1} HCl (convert the residue into Cl^- form), dried again, taken up by 2 mL of 2 mol L^{-1} HCl for loading on the columns, and subsequently purified according to Procedure 3. The Cd elutes were dried at 120°C, and then redissolved in 14 mol L^{-1} HNO_3 for storage. Just prior to use, the solutions were evaporated to nearly complete dryness at 120°C and taken up by an appropriate volume of 1% HNO_3 to obtain the desired Cd concentration for mass spectrometric analysis.[25] The concentrations of metals in *Cyperus alternifolius* (underground and overground parts) are given in Table 2. The Cd concentrations of the underground and overground parts of *Cyperus alternifolius* were 1.79 ± 0.09 and 17.88 ± 1.40 mg kg^{-1}, respectively.

Table 2 Concentration of metal elements (mg kg^{-1}) in *Cyperus alternifolius* (underground and overground) (SD, $N = 3$)

Metal	Overground	Underground
Cd	1.79 ± 0.09	17.88 ± 1.40
Cr	14.83 ± 1.23	37.66 ± 2.54
Cu	18.34 ± 1.52	48.03 ± 2.85
Mg	4347.63 ± 245.21	3277.67 ± 253.14
Mn	49.80 ± 3.56	568.99 ± 45.21
Mo	2.66 ± 0.15	3.13 ± 0.12
Ni	2.78 ± 0.14	9.25 ± 0.45
Pb	18.65 ± 1.24	298.27 ± 21.30
Zn	53.45 ± 3.25	118.97 ± 9.52
As	6.02 ± 0.25	43.15 ± 2.54

Cd isotope analysis

To evaluate the Cd separation method developed in this study, Cd isotope ratios were measured using a Neptune multi collector inductively coupled plasma mass spectrometer (MC-ICP-MS) at Nanjing University. A Cd standard reference material (NIST SRM 3108 from National Institute of Standards and Technology) was used as an internal reference standard. Three other standard solutions (Münster Cd solution, Spex Cd solution and Spex-1 Cd solution) were used as the second reference materials. The Spex-1 Cd solution (Lot No. CL6-30CDY) was from Spex CertiPrep company while Münster Cd solution and Spex Cd solution were from Münster University and Nancy University, respectively. The Cd concentrations of the sample solution and standard solution were 0.4 mg L^{-1}, resulting in ion beam intensities of 6 V on ^{114}Cd. The concentrations in the samples and the standard Cd solution matched within 10%. The Cd isotope ratios were measured by 30 cycles for each sample with an internal precision of ±0.01 – 0.02‰ (RSD). The ion currents of ^{105}Pd, ^{110}Cd, ^{111}Cd, ^{112}Cd, ^{114}Cd and ^{117}Sn were measured simultaneously with the faraday cups. The ion beams of ^{105}Pd and ^{117}Sn were monitored to correct isobaric interferences from ^{110}Pd, ^{112}Sn, and ^{114}Sn.[27] The standard-sample bracketing method was used to calculate delta values. All concentration values were corrected for the procedural blank, which ranged from 128 to 375 pg during the course of this study. At this level, the total procedure blank has a negligible effect on the measured Cd isotopic compositions, because it constitutes to less than 0.1% of the indigenous Cd present in the plant samples. The instrumental reproducibility based on repetitive $\delta^{114/110}$Cd measurements of the NIST SRM 3108 Cd standard solution was 0.08‰ (2SD, $N = 97$).

Results and Discussion

As required for precise measurements of Cd isotopes, Cd was extracted from the sample matrix, with 1) nearly 100% recovery, 2) complete removal of isobars and the interference elements (mainly Pd, Sn, In, Zn, Ge, Se, Ru, Mo, K, Ca Na, and Mg), 3) no isotope fractionation[5,15,33] We will discuss the chromatographic separation efficiency based on these three factors mentioned above.

Different purification procedures

As mentioned in the introduction, the purification procedures of the solids samples mainly include Wombacher *et al.*,[28] Cloquet *et al.*,[23] Gao *et al.*[31] and Zhu *et al.*[24] up to our experiement. Wombacher *et al.*[28] proposed a two-stage column chemistry procedure and used three types acid (*i.e.* HCl, HNO_3, HBr) as elution, while three other procedures developed the one-stage purification to separate Cd from the samples only using a type acid (*i.e.* HCl). Overall, the three procedures of Cloquet,[23] Gao[31] and Zhu[24] were relatively simple compared with the procedure of Wombacher.[28] Therefore, the latter three procedures were selected as our experimental subjects. The elution curves of the anion exchange chemistry for the GSB mixture according to Procedures 1, 2 and 3 with the 110 × 6 column are shown in Figs. 1a, 1b and 1c, respectively.

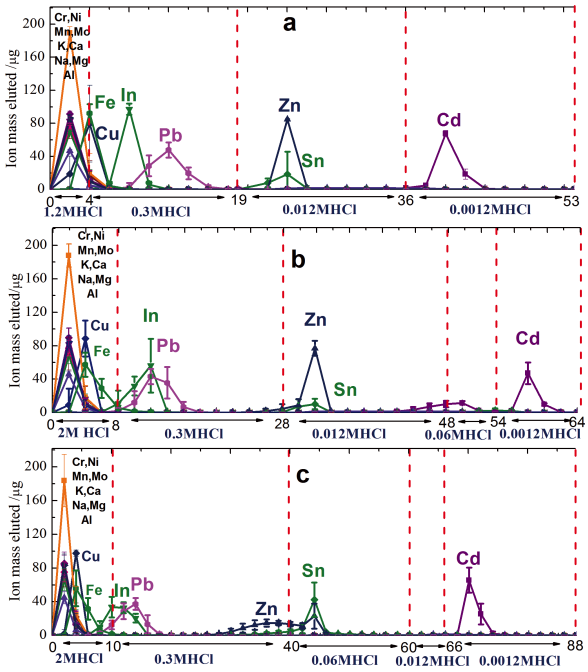

Fig. 1 Elution curve of the anion exchange chemistry for GSB mixtures with application of Procedure 1 (a), Procedure 2 (b) and Procedure 3 (c).[5]

As shown in Fig. 1a, Procedure 1 comprised four stages. The elution of the matrix started with 4 mL of 1.2 mol L^{-1} HCl and 15 mL of 0.3 mol L^{-1} HCl. Then, the elution continued with 17 mL of 0.012 mol L^{-1} HCl and the Cd fractions were finally eluted with 17 mL of 0.0012 mol L^{-1} HCl. At the first stage (using 1.2 mol L^{-1} HCl), besides parts of Fe and Cu, most matrix elements (such as K, Ca, Na, Mg, Al, Ni, Cr, Mo and Mn) were eluted. At the second stage (using 0.3 mol L^{-1} HCl), In and Pb were mostly eluted in addition to the rest of Fe and Cu. At the third stage (using 0.012 mol L^{-1} HCl), Zn and Sn were eluted. At the last stage (using 0.0012 mol L^{-1} HCl), Cd was eluted.

As shown in Fig. 1b, Procedure 2 comprised five stages. The elution of matrix started with 8 mL of 2 mol L^{-1} HCl and 20 mL of 0.3 mol L^{-1} HCl. Then, the elution continued with 20 mL of 0.012 mol L^{-1} HCl, followed by 6 mL of 0.06 mol L^{-1} HCl. Finally, the Cd fractions were eluted with 10 mL of 0.0012 mol L^{-1} HCl. At the first stage (using 2 mol L^{-1} HCl), besides the matrix elements (such as K, Ca, Na, Mg, Al, Ni, Cr, Mo and Mn), Fe and Cu were also eluted. At the second stage (using 0.3 mol L^{-1} HCl), In and Pb were eluted. At the third stage (using 0.012 mol L^{-1} HCl), most of Zn and Sn were eluted. It was thus clear that parts of Cd were eluted at the third and fourth stages (using 0.012 and 0.06 mol L^{-1} HCl) before the last stage (using 0.0012 mol L^{-1} HCl).

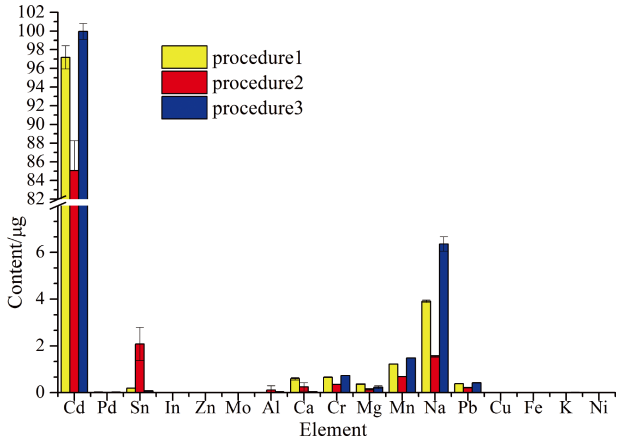

Fig. 2 Element contents of Cd elution in GSB mixtures with application of three different purification procedures.

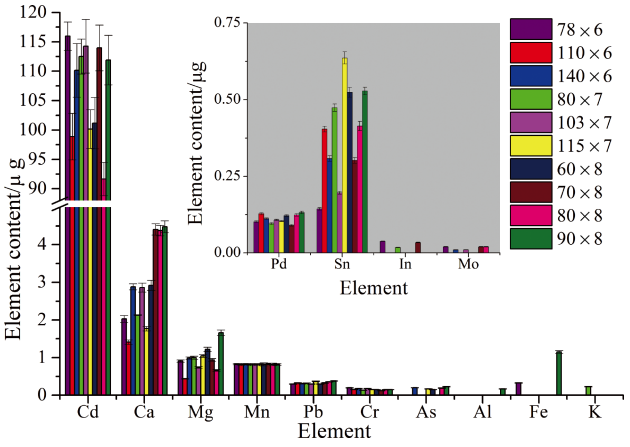

Fig. 3 Element contents of Cd elution utilizing different diameters of the resin columns and different resin volumes.

As shown in Fig. 1c, Procedure 3 also comprised five stages. The elution of matrix started with 10 mL of 2 mol L^{-1} HCl and 30 mL of 0.3 mol L^{-1} HCl. Then, the elution continued with 20 mL of 0.06 mol L^{-1} HCl, followed by 6 mL of 0.012 mol L^{-1} HCl. Finally, the Cd fractions were eluted with 22 mL of 0.0012 mol L^{-1} HCl. At the first stage (using 2 mol L^{-1} HCl), besides the matrix elements (such as K, Ca, Na, Mg, Al, Ni, Cr, Mo and Mn), all Fe and Cu as well as parts of In and Pb were eluted. At the second stage (using 0.3 mol L^{-1} HCl), besides In and Pb, parts of Zn and Sn were eluted. At the third stage (using 0.06 mol L^{-1} HCl), most of the Zn and Sn were eluted. The rest of Zn and Sn were eluted at the fourth stage (using 0.012 mol L^{-1} HCl). At the last stage (using 0.0012 mol L^{-1} HCl), Cd was eluted largely.

The sequences of the eluted ions during the three purification procedures were essentially consistent. Matrix elements (such as K, Ca, Na, Mg, Al, Fe, Ni, Cr, Cu, Mo and Mn) were eluted firstly, followed by In, Pb, Zn and Sn. Finally, the Cd fractions were eluted. The volumes of the eluents differed in each procedure, so some elements (such as Zn and Sn) were eluted at different elution stages during the three procedures. At the first stage, matrix elements (such as K, Ca, Na, Mg, Al, Fe, Ni, Cr, Cu and Mn) were mainly eluted during Procedures 2 and 3, but they were not eluted completely during Procedure 1. This can be explained by the addition of a lower volume of 1.2 mol L^{-1} HCl during Procedure 1. Moreover, except for the matrix elements, parts of In and Pb were also eluted by 10 mL of 2 mol L^{-1} HCl in Procedure 3. At the second stage (using 0.3 mol L^{-1} HCl), In and Pb were mainly eluted during Procedure 2. Except for In and Pb, the rest of the matrix elements were also eluted mainly during Procedure 1, while parts of Zn and a small part of Sn were eluted during Procedure 3. Based on Procedure 1, the stage using 0.06 mol L^{-1} HCl was added during Procedures 2 and 3. Therefore, Zn and Sn was mainly eluted at the stage using 0.012 mol L^{-1} HCl during Procedure 1, whereas these elements were also eluted at the stage using 0.06 mol L^{-1} HCl during Procedure 2 and 3. This was due to the fact that the volumes and sequence of elution differed in the three procedures. Cd was finally eluted at the last stage using 0.0012 mol L^{-1} HCl.

The yields of Cd generated by Procedures 1, 2 and 3 were 97.18 ± 1.24% (SD, $N = 3$), 85.07 ± 3.21% (SD, $N = 3$) and 99.95 ± 0.85% (SD, $N = 3$), respectively.[5] During Procedure 2, Cd was partially eluted by 0.06 mol L^{-1} HCl and 0.012 mol L^{-1} HCl followed by 0.0012 mol L^{-1} HCl. Consequently, the Cd yields of Procedure 2 were lower compared with the other two procedures.

Isobaric interferences (such as Pd, Sn and In), molecular interferences (such as $^{70}Zn^{40}Ar^{+}$, $^{70}Ge^{40}Ar^{+}$, $^{98}Mo^{16}O^{+}$ and $^{98}Ru^{16}O^{+}$) and contaminant-prone elements (such as K, Ca, Na and Mg) usually influenced the measurement of Cd isotope ratios. As shown in Fig. 2, a fraction of Sn occurred in all of the Cd eluates produced by the three procedures. Moreover, the content of Sn in the Cd eluate produced during Procedure 3 was the lowest, whereas the eluates of Procedure 2 showed the highest Sn contents. In addition, in all eluates relatively high amounts of Na and Mn, as well as only small amounts of Pb, Mg, Cr and Ca could be observed. Generally, in view of the Cd yields, isobaric, molecular and other ion interferences, the purification of Procedure 3 was the most suitable in this study.

Chromatographic separation efficiency influenced by volume of resin

Both the diameter of the resin columns and the volume of the resin affected the chromatographic separation efficiency. The Cd yields of ten distinct columns differed (as shown in Table 1). The minimum yield (91.66 ± 2.41%) was observed for 80 × 8 (Height × Diameter) columns, whereas the maximum yield (115.96 ± 2.83%) was detected for 78 × 6 columns. Moreover, Cloquet *et al.*[29] suggested that possible Cd isotopic fractionation during purification could be ignored, when the Cd yields were between 95 and 105%. In this study, only three columns exhibited Cd yields within this range: 115 × 7 (100.16 ± 3.24%), 110 × 6 (98.88 ± 3.43%) and 60 × 8 (101.18 ± 4.32%), respectively.

During measurements, the Cd isotopic ratios can be affected by isobaric (*i.e.* Pd, In and Sn) and molecular (*e.g.* $^{70}Zn^{40}Ar^{+}$, $^{70}Ge^{40}Ar^{+}$, $^{98}Mo^{16}O^{+}$ and $^{98}Ru^{16}O^{+}$) interferences.[28] Pd, Sn and Mo, but no Ge, Ru, In and Zn, were present in the Cd eluate after application of the three above-mentioned columns (115 × 7, 110 × 6 and 60 × 8) (Fig. 3). The Pd contents in the eluates were in the order of 110 × 6 > 60 × 8 > 115 × 7 while the Sn contents were in the order of 115 × 7 > 60 × 8 > 110 × 6. Contents of other ions were lowest in the Cd eluate after utilizing the 110 × 6 column. Generally, in view of Cd yields, isobaric, molecular and other ion interferences, the 110 × 6 column was the most suitable in this study. Resin volume (3 mL) added to the 110 × 6 column as well as the resin type (AG-MP-1) was the same as used for the study of Gao *et al.*[31]

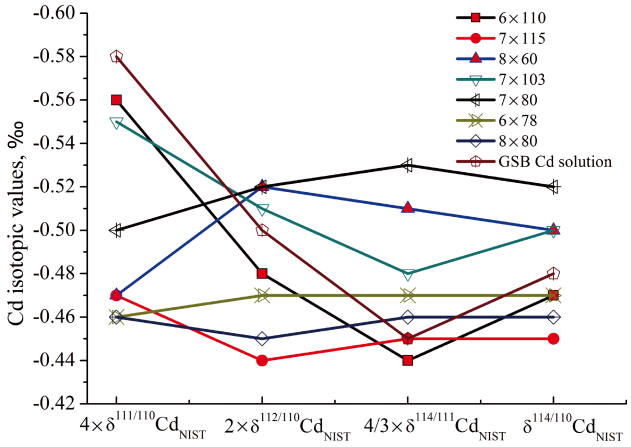

Fig. 4 Cd isotopic compositions of eluted GSB mixtures with application of different ion columns.

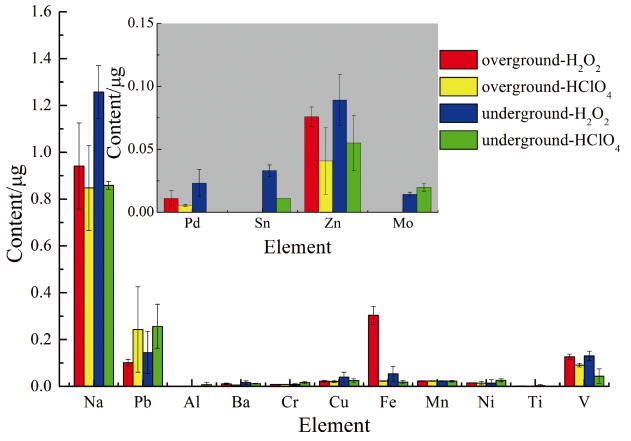

Fig. 5 Element contents of Cd elution in *Cyperus alternifolius* digested by HNO_3–HF–H_2O_2 and HNO_3–HF–$HClO_4$.

Significant mass fractionation may occur during ion-exchange chromatography.[34,35] Thus, it is of crucial importance to avoid mass fractionation during the Cd purification procedure. As shown in Fig. 4, the Cd isotope values of the eluted GSB mixture using the 110 × 6 column were consistent with those of the initial GSB Cd solution, which was termed as "theoretical value", indicating no significant Cd isotope fractionation during digestion and purification. Hence, we conclude that the technique developed in this study is robust and the obtained Cd isotopes values are reliable.

Chromatographic separation efficiency influenced by organic matters

The designed separation technique was tested using plant samples of *Cyperus alternifolius* as a representative plant. The Cd concentrations of underground and overground parts of *Cyperus alternifolius* were 1.79 ± 0.09 and 17.88 ± 1.40 mg kg^{-1}, respectively (Table 2). In the study of Wei *et al.*,[5] the Cd recovery (131%) was anomalously high, when the *Cyperus alternifolius* samples were only digested by HNO_3 and HF. The organic matter, which remained in the digested solution, might have influenced the Cd purification process. In this study, H_2O_2 and $HClO_4$ were used to eliminate the organic matter during digestion.

The Cd yields have been calculated according to the Cd contents before and after purification. The Cd yields in the eluates of overground and underground parts digested by HNO_3–HF–H_2O_2 were 93.78 ± 0.06 and 98.48 ± 10.16%, respectively. Similarly, the Cd yields of overground and underground parts digested by HNO_3–HF–$HClO_4$ were 93.83 ± 0.24 and 97.09 ± 2.71%, respectively. As a result, the Cd yields of the eluates produced by these two methods were similar.

The Cd contents eluted from overground and underground parts were 0.41 – 0.52 and 3.94 – 4.09 μg, respectively. As shown in Fig. 5, except for Pb and Mo, the concentration of the interference ions (*e.g.* Pd, Sn, In and $^{70}Zn^{40}Ar^{+}$) in the eluates after HNO_3–HF–$HClO_4$ digestion were lower than after HNO_3–HF–H_2O_2 digestion. Moreover, the concentrations of the interference ions in the eluates of the overground parts were lower compared to those of the underground parts. Only low contents of Pd and Zn in the eluates of the overground parts were detected. Furthermore, besides Pd and Zn, only low concentrations of Sn and Mo could be observed in the eluates of the underground parts. Moreover, high contents of Na and Pb, but low concentrations of Fe, Cu, Mn, Ni and V could be detected in the Cd eluates of both underground and overground parts.

Table 3 Ratios of Cd and interference ions in the eluates from *Cyperus alternifolius* digested by a mixture of HNO_3–HF–H_2O_2 and HNO_3–HF–$HClO_4$ (SD, $N = 3$)

	Overground-H_2O_2	Overground-$HClO_4$	Underground-H_2O_2	Underground-$HClO_4$
	%			
Pd/Cd	2.66 ± 0.12	1.05 ± 0.08	0.59 ± 0.04	0.00 ± 0.02
Sn/Cd	0.00 ± 0.01	0.00 ± 0.01	0.84 ± 0.06	0.27 ± 0.01
Zn/Cd	18.35 ± 0.87	7.77 ± 0.58	2.26 ± 0.16	1.34 ± 0.12
Mo/Cd	0.00 ± 0.01	0.00 ± 0.01	0.36 ± 0.02	0.48 ± 0.02

Previous studies[28,29] have shown that the presence of Zn, Sn and Pd did not influence the measurement of Cd isotope ratios when the ratios of Zn/Cd, $^{118}Sn/^{114}Cd$ and $^{105}Pd/^{110}Cd$ were below 10, 18 and 0.8%, respectively. As shown in Table 3, the ratios of Pd/Cd and Zn/Cd in the eluates of the overground parts digested by HNO_3–HF–H_2O_2 were higher than the respective values, whereas only the ratios of Pd/Cd in the eluates of the overground parts digested by HF–HNO_3–$HClO_4$ were higher than the respective values. Consequently, the measurements of the Cd isotopes from the overground parts digested by HNO_3–HF–H_2O_2 might be interfered by Pd and Zn ions while the ones for Cd isotopes from the overground parts digested by HNO_3–HF–$HClO_4$ might only be interfered by Pd ions. In addition, the ions in the eluate of the underground parts digested by those two methods did not show any influence on the Cd isotope measurements. Consequently, the digestion method using HNO_3–HF–$HClO_4$ is more suitable than the method using HNO_3–HF–H_2O_2.

As shown in Fig. 6, the $\delta^{114/110}Cd_{NIST}$ of the overground and underground parts of *Cyperus alternifolius* was 0.07 and –0.29‰, respectively. The linear relationship of $\delta^{111/110}Cd$, $\delta^{112/110}Cd$, $\delta^{114/111}Cd$ and $\delta^{114/110}Cd$ after HF–HNO_3–$HClO_4$ digestion, was better than after HNO_3–HF–H_2O_2 digestion. It is, thus, clear that all of the polyatomic interferences were better resolved by adding $HClO_4$ rather than H_2O_2. The inaccurate Cd isotope ratios might be caused by the incomplete oxidation of

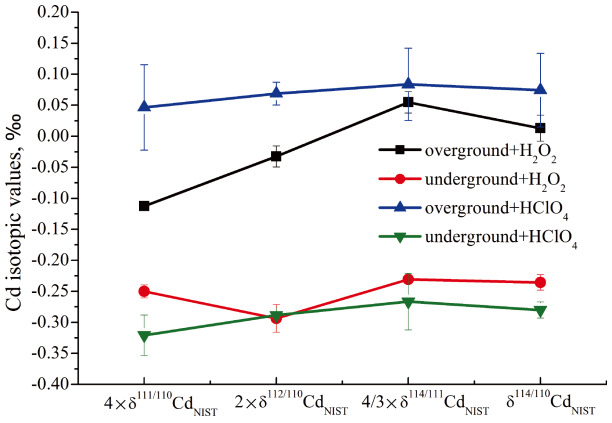

Fig. 6 Cd isotope values of *Cyperus alternifolius* digested by HNO_3–HF–H_2O_2 and HNO_3–HF–$HClO_4$.

organic compounds. Shiel *et al.*[32] demonstrated that residual resin-derived organic compounds could affect the accuracy of the Cd isotope ratios significantly. Some chemical treatments with refluxed HNO_3 or $HClO_4$/HNO_3 have been used to remove the resin-derived organic components. Gault-Ringold *et al.*[36] added a mixture of H_2O_2–7 M HNO_3 (50% v/v) to decompose residual organic material, which remained in the samples. Moreover, anomalous shifts in the Cd isotope ratios could be observed for samples with low Cd concentrations, when the reaction with the decomposing agents was incomplete. Pallavicini *et al.*[20] suggested that incomplete oxidation of carbon might interfere during ion-exchange separation and suggested to remove organic-rich matrices through ashing.

The Cd isotopic values of Cd standard solutions

The other serious issue of hindering the development of Cd isotope analyses was the right choice of the "zero-delta" reference material.[10,37] Most laboratories used their own and in-house Cd reference materials for "zero-delta", such as BAM 1012, JMC Cd Münster, Alfa Cd Zürich, JMC Cd Mainz, NIST SRM 3108, Münster Cd, and so on.[18,19,25,36] It is clear that the Cd isotopic composition was different if the laboratories used their own Cd reference solutions as "zero-delta". Based on this situation, Abouchami *et al.*[10] calibrated the Cd isotope values of the Cd reference materials in different laboratories and advocated NIST SRM 3108 as the Cd isotope standard for "zero-delta" in future studies firstly. In this study, four second Cd standard solutions were measured and the $^{114/110}$Cd of Münster Cd, Spex Cd, Spex-1 Cd solutions relative to the NIST SRM 3108 were +4.45 ± 0.08‰ (2SD, N = 12), –0.09 ± 0.01‰ (2SD, N = 2), –1.25 ± 0.06‰ (2SD, N = 3), respectively. The $^{114/110}$Cd of Münster Cd solutions relative to the NIST SRM 3108 was similar to the values of previous studies, which ranged from 4.46 - 4.55‰.[18,38] The $^{114/110}$Cd values in this study were measured accurately. This enabled our comparison with the Cd isotope results obtained in other laboratories.

Conclusions

In this study, an appropriate ion-exchange chromatography protocol for the quantitative separation of Cd in organic- and metal-rich plants was elaborated, which can be applied prior to Cd isotope measurements *via* MC-ICP-MS. After complete digestion of the plant samples using a HNO_3–HF–$HClO_4$ mixture, the chemical separation of Cd from the interference and matrix elements was achieved by a one-step protocol yielding high Cd recoveries. In addition, the obtained Cd isotope results confirmed that the digestion and separation processes were not accompanied by Cd isotopic fractionation. The $^{114/110}$Cd of Münster Cd, Spex Cd, Spex-1 Cd solutions relative to NIST SRM 3108 were +4.45 ± 0.08‰ (2SD, N = 12), –0.09 ± 0.01‰ (2SD, N = 2), –1.25 ± 0.06‰ (2SD, N = 3), which enabled the comparison of Cd isotope results obtained in other laboratories. Consequently, this digestion and purification procedure can be used to separate Cd from plant samples. Moreover, the proposed method is much simpler and faster compared with the procedures from previous studies. Hence, the Cd isotope extraction, purification and measurement technology introduced in this study is a powerful tool for fingerprinting specific Cd sources and/or examining biogeochemical reactions in ecological and environmental systems

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