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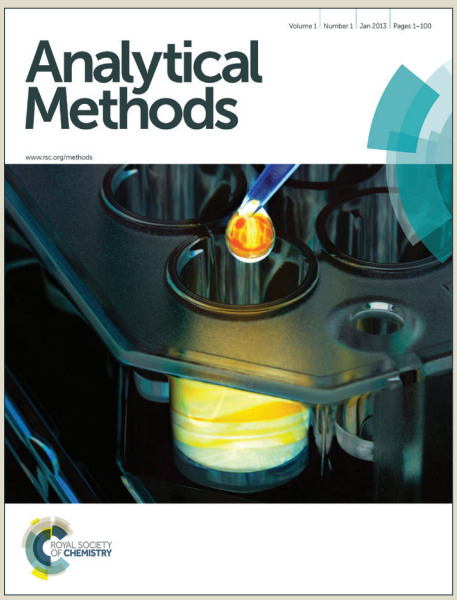

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Analytical Method for Precise Determination of Cadmium Isotopic Composition in Plant Samples using Multiple Collector Inductively Coupled Plasma Mass Spectrometry

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ABSTRACT

Isotope techniques can be applied to discover the migration and transformation of metal elements in plants. However, only few studies on Cd isotope in plants have been carried out so far. In this study, an optimized analytical method of digestion, purification and determination of Cd isotope in plants was developed. Three Cd standard solutions as well as four plant species (*Solanum nigrum*, *Ricinus communis*, *Cyperus alternifolius* and *Pteris vittata)*, which were grown in soil or hydroponic cultures, were repeatedly analyzed for Cd isotope using Multiple Collector Inductively Coupled Plasma Mass Spectrometry (MC-ICPMS). The factors that affected the accuracy of Cd isotopic determination, such as isobaric interferences and instrumental mass fractionation, have been carefully evaluated and corrected. The purification procedure yielded a Cd recovery of not less than 95% and effectively eliminated the spectral interference of Pd, In and Sn as well. The analysis of pure Cd standard materials showed accurate isotope values, which matched with the results of previously published methods. This technique provided an average long-term external reproducibility of ±0.09‰ for $\delta^{114/110}$Cd (2SD). The overall $\delta^{114/110}$Cd values of four plant species ranged from -0.39‰ to -0.08‰ and provided direct evidence for Cd isotopic fractionation in herbaceous plants.

Key word: Cd isotope; Analytical method; plant; Standard-Sample Bracketing; Anion-exchange

1 Introduction

Cadmium (Cd) is a heavy metal that causes particular concern for environmental quality and

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human health, due to its high toxicity and carcinogenic properties[1]. Soil Cd contents have dramatically increased in recent decades worldwide through mining, smelting, electroplating, wastewater irrigation, and abuse of chemical fertilizers and pesticides[2, 3]. Given this widespread use and rapid urbanization, soil pollution by Cd becomes an urgent environmental issue that could adversely affect plant growth and human health by the increasing concentrations of Cd within the food chain[4]. Consequently, there is an imperative need to identify the major Cd sources and develop effective approaches to remediate Cd polluted soils[5].

Phytoremediation is a novel, cost-effective, efficient and environment friendly remediation method of heavy metal-polluted soils[6]. Recently, a number of studies have been carried out in order to identify enrichment and tolerance mechanisms and transfer processes of heavy metals in hyperaccumulators[4, 5, 7]. Isotope techniques can be applied to trace the migration and transformation of metals in plants[8]. Combined with plant physiology research, isotope techniques can also explore accumulation and tolerance mechanisms of metals in plants. At present, some fractionation processes of non-traditional stable isotopes (e.g. Fe, Zn, Ca, Mg, Cu) in plants have been reported[9-15]. Plant uptake plays an important role in the variation of metal isotopic compositions in plants[9, 16]. The metal isotope composition in different parts of plants can be used to identify the translocation and accumulation processes of metals. However, up to date, only very few studies[16] on Cd isotopes in plants have been carried out.

The advent of multi-collector inductively coupled plasma mass spectrometry (MC-ICPMS) has dramatically extended applications of stable Cd isotopes as tracers in natural systems. Besides studies on mass-dependent fractionations in cosmochemistry, Cd isotopes have also been applied to

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trace biogeochemical cycling of Cd in the oceans[17-19]. Moreover, coupled with the Zn and Pb isotopic systems, Cd isotopes have been also used to trace pollution sources[20-22]. The accuracy and precision of Cd isotope ratios measured by MC-ICPMS depends on three analytical requirements: 1) accurate corrections for isobaric interferences (i.e. ^{110}Pd on ^{110}Cd, ^{112}Sn on ^{112}Cd, ^{114}Sn on ^{114}Cd); 2) the quantitative removal of polyatomic species from the recombination of argon, atmospheric gases, solvents and sample matrix (e.g. $^{70}Zn^{40}Ar^{+}$ on ^{110}Cd, $^{98}Mo^{16}O^{+}$ on ^{114}Cd); 3) precise correction of the instrumental mass bias that strongly depends on the matrix effects and overall purity in which elements are presented[23]. Isobaric interferences are principally reduced through chemical separation procedures prior to determination. Isobaric interferences can be monitored and corrected if not too large. It is not possible to monitor polyatomic interferences during determination[24]. Furthermore, matrix effects on the plasma will affect the transmission efficiency of ions and the instrument response to determination[25]. At present, some efforts have been made to evaluate matrix effects in the analysis of Cd isotope by MC-ICPMS. Shiel *et al*.[26] found that residual resin-derived organics were a significant source of column-induced matrix effects. Therefore, it was important to eliminate the organic compounds and metallic elements for precise determination of Cd stable isotopic composition in plants.

In this study, we chose four plant species including *Solanum nigrum* (Cd hyperaccumulator), *Ricinus communis* (Cd-tolerant), *Cyperus alternifolius* (high Cd concentration) and *Pteris vittata* (high Cd concentration). The *Solanum nigrum* and the *Ricinus communis* were cultivated as hydroponic cultures while *Cyperus alternifolius* and *Pteris vittata* were grown in polluted soil. All the plants were digested with the mixed acids of HNO_3 and HF while the organic matter were

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removed by $HClO_4$. Cd fractions were separated from the digestion solutions by an anion-exchange purification protocol. The four plant species and three Cd standard materials were analyzed for Cd isotopes using MC-ICPMS and instrumental mass bias were corrected with standard-sample bracketing. The objectives of this study are: 1) to test an anion-exchange purification technique of Cd extraction from samples with various matrices; 2) to assess the accuracy and the external reproducibility of Cd isotope ratios in plants with organic matrices; 3) to develop a routine analytical protocol of Cd purification and Cd isotope analysis in plant samples that allows high confidence in long-term reproducibility and accuracy.

2 Experimental Procedures

2.1 Sample Preparation

2.1.1 Reagents and Materials

All critical sample preparation work was carried out in a class 100 clean room facility. The mineral acids were purified twice by sub-boiling distillation in Teflon stills. The water was 18.2 MΩ·cm grade from a Milli-Q water purification system (Millipore, Bedford, MA, USA). HNO_3, HF, $HClO_4$ and HCl were purchased from Beijing Institution of Chemical Reagents (Beijing, China) and were prepared freshly on the day of use. AG-MP-1M ion-exchange resin (100−200 mesh, chloride form, Bio-Rad Laboratories) was used throughout this study.

Our study focused on developing a method suitable for Cd isotopic determination in plant samples. However, at present no international standard material for plants was available. To assess the accuracy and precision of Cd isotopic measurements, three different Cd standard materials were

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utilized: 1) A 1000 mg•L^{-1} Spex Cd solution (Lot No: 74-075219K) was used as "zero delta" Cd standard[27]. 2) Another 1001 mg•L^{-1} Cd solution (Lot No: CL6-30CDY) was purchased from Spex (Metuchen, NJ, USA) and termed "spex-1 Cd solution". 3) A fractionation Cd isotope standard material termed "Münster Cd" was provided by Zhu *et al*.[27]

All GSB single element standard solutions were obtained from the national testing center of iron and steel materials (Iron and Steel Research Institute, Beijing, China). A mixture of the certified reference material "GSB single element solutions" (K, Ca, Na, Mg, Al, Fe, Cd, Pb, Zn, Pd, In, Cu, Cr, Mn, Ni, Sn) was prepared by taking 1.0 mL aliquots of 1000 mg•L^{-1} single standard solutions.

2.1.2 Plant Culture

Four different plant species (*Solanum nigrum, Ricinus communis, Cyperus alternifolius* and *Pteris vittata)* were utilized in this study. The *Solanum nigrum* and the *Ricinus communis* plants were cultivated as hydroponic cultures in a greenhouse of the Institute of Geographic Sciences and Natural Resources Research (CAS, Beijing, China). The *Cyperus alternifolius* and *Pteris vittata* plants were grown for two years in polluted soil collected in the Yunnan province.

Seeds of *Ricinus communis* and *Solanum nigrum* were obtained from the Zibo Academy of Agricultural Sciences (Shandong, China) and the Institute of Applied Ecology, Chinese Academy of Sciences (Shenyang, China), respectively. All seeds were washed in running deionized water before germination in the substrate for 14 d. The seedlings were then transferred into polycarbonate pots containing 400 mL of nutrient solution with 2 plants per pot. After 7 d, $CdCl_2$•$2.5H_2O$ were diluted in deionized water to adjust their Cd concentration up to 2 mg•L^{-1} and 5 mg•L^{-1}. Four replicates were prepared for each nutrient solution. Plants were cultured for 30 d under controlled conditions (16 h

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photoperiod with a white light intensity of 350 μ mol photons m^{-2} s^{-1}; day: night temperatures 25 ℃: 18 ℃; relative humidity 60%–70%) [9]. In the first 21 d, nutrient solutions were changed weekly. Afterwards, they were changed every 3-4 d to avoid nutrient depletion. Water was sprinkled on the plants daily to compensate the water loss by transpiration. The total amount of Cd in the nutrient solution was always far in excess of the amount of Cd taken up by plants to avoid reservoir depletion.

Upon harvest, all plant materials were washed by running deionized water to remove superficial nutrient solution and soil. Root materials were submerged sequentially in a bath of 20 mol •L^{-1} EDTA-Na_2 for 15 min to remove apoplastically bound Cd [9].

2.1.3 Sample Digestion

A mixture of acids was utilized in the sample digestion according to methods of Shiel *et al.*[26] and Weinstein *et al.*[10] 0.2 g of plant samples were then digested in concentrated Aristar grade HNO_3 (5 mL) and HF (1 mL) for 48h in acid-cleaned Teflon beakers. The closed beakers were placed on a hot plate for 8 h at 80 ℃ and then at 160 ℃ until the plants were completely digested. Then 2-3 mL of $HClO_4$ was added to the digested solutions to remove organic materials. After evaporation at 165 ℃, the samples were dried and redissolved in 5 mL 1% (v/v) HNO_3 (to convert the residue into the nitrate form). 2 mL of supernatant were transferred into pre-cleaned polyethylene bottles for the determinations of Cd content. The rest fractions were evaporated to dryness, redissolved in 10 mol•L^{-1} HCl (to convert the residue into the chloride form), dried again, and finally taken up in 2 ml 2 mol•L^{-1} HCl for loading of columns.

2.1.4 Chemical Separation

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In this study, three anionic exchange chromatographic methods were adopted to separate Cd from the GSB mixture matrix. The different purification procedures with the respective elution stages carried out for previous studies [20, 21 27]are shown in Table 1. One method adopted from Zhu *et al.* [27] was used to separate Cd from the plant samples. The details of this ion-exchange elution method are as follows:2 mL of the sample solution (in 2 mol•L^{-1} HCl) was loaded onto the resin, which was previously treated by 15 mL of 0.0012 mol•L^{-1} HCl and conditioned by 10 mL of 2 mol•L^{-1} HCl in order to remove all non-sample Cd. The elution of the matrix started with 10 mL of 2 mol •L^{-1} HCl and 30 mL of 0.3 mol•L^{-1} HCl. The elution continued with 20 mL of 0.06 mol•L^{-1} HCl, which removed most of Zn and Sn, followed by a small fraction of Sn in 6 mL of 0.012 mol•L^{-1} HCl. The Cd fractions were finally eluted with 22 mL of 0.0012 mol•L^{-1} HCl, evaporated to dryness and dried again with a few drops of 14 mol•L^{-1} HNO_3 to remove any residual chloride, and redissolved in 5 mL 14 mol•L^{-1} HNO_3 for storage. Just prior to use, the solutions were evaporated to near complete dryness and taken up in an appropriate volume of 1% HNO_3 to obtain the desired Cd concentration for mass spectrometric analysis[28].

The element concentrations were measured by inductively coupled plasma quadrupole mass spectrometry (ICP-QMS) (Elan DRC-e, America). A reference material GBW07603 (GSV-2) was used to monitor the Cd recovery of the plant samples in digestion. The matrix and Cd concentration of the analyzed plant samples and GSV-2 are shown in Table 2. The certified Cd concentration of GSV-2 was 0.38 mg·kg^{-1} and the Cd digestion recovery of GSV-2 was 97 ±3 % (SD, N=3).

2.2 Mass Spectrometry

2.2.1 Instrumentation and Measurement Protocols

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The isotope measurements were performed with a Nu Plasma HR MC-ICPMS equipped with nine Faraday cups. A Teflon nebulizer with an uptake rate of 100 $\mu L \cdot min^{-1}$ was used for sample introduction. Each isotopic value corresponded to a measurement of 30 cycles and bracketed by the Cd standard solution. Prior to each block, the baseline signals were monitored for 20s and the background values of the instrument were automatically deducted by the computer. Each analysis was followed by a thorough washout (~5min), whereby the sample introduction system was flushed first with 5% HNO_3 and then with 1% HNO_3. Cd concentrations for sample solution and standard solution were 0.4 $mg \cdot L^{-1}$, resulting in ion beam intensities of 6 V on ^{114}Cd. The concentrations of samples and standard solutions were matched to be better than 10%. Cd isotope ratios were measured by 30 cycles for each sample with an internal precision of ±0.01‰～±0.02‰ (RSD).

2.2.2 Interference Corrections and Matrix Effects

To avoid isobaric interferences and matrix effects, a chemical separation of Cd from the sample materials had to be carried out. Direct isobaric interferences with Cd isotopes were generated by the elements of Pd, In and Sn. In this study, some Sn and traces of Pd, but no In was found in the Cd eluents. 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 for isobaric interferences from ^{110}Pd and ^{112}Sn, ^{114}Sn, respectively[28].

2.2.3 Mass Bias Correction

There are mainly three mass bias correction procedures to obtain accurate and precise measurement by MC-ICPMS: 1) standard-sample bracketing[20, 21, 27], 2) internal normalization [29, 30] alone, or combined with SSB[26], 3) double spiking[28, 31-34]. All those procedures have both advantages

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and inconveniences, which have been described in previous literatures[24, 29]. Wombacher *et al*.[29] found that Cd isotope ratios obtained from standard-sample bracketing and internal normalization were consistent and accurate. In addition, the results of other studies[20, 21, 26] showed that the technique of standard-sample bracketing generated accurate Cd isotope values in soil, sediments and smelter samples. Compared to internal normalization, this correction technique took shorter analysis time and was more straightforward to correct instrumental mass bias[23]. Therefore, as a more convenient technique[20], it was applied to the corrections of all samples and standard solutions in this study.

The efficiency of "standard-sample bracketing" approach depended on two requirements[21]: 1) the mass spectrometer had to be relatively stable during the daily analytical section, 2) the mass bias behavior of standards and samples had to be the same. To monitor the stability of the mass spectrometer, long-time measurements of the Spex Cd standard solution were performed during different analytical sessions over half a year. The long-term reproducibility achieved for the Cd isotopic compositions of the Spex Cd standard solution (Fig.1) revealed a precision of 0.09‰ for $\delta^{114/110}$Cd（2SD, N=214）and suggested that standard-sample bracketing was a reliable correction method to achieve accurate Cd isotope ratios.

2.3 Data Analysis

All results were expressed as the deviation relative to the Spex Cd reference solution and were given in per mil (Delta) notation according to following the equation:

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

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where i and j represent Cd isotopes of 110,111,112 or 114, Standard 1 and Standard 2 represent the standard solution measured before and after the sample [20].

The Cd isotope values of the samples relative to distinct Cd standards in the references are different. Thus the δ values of each sample need to be recalculated. The conversion of the δ value relative to two different Cd standards is as follows:

$$\delta^{114/110}Cd_{X\text{-}A} = \delta^{114/110}Cd_{X\text{-}B} + \delta^{114/110}Cd_{B\text{-}A} + (\delta^{114/110}Cd_{X\text{-}B})(\delta^{114/110}Cd_{B\text{-}A})/1000 \quad (2)$$

where X represents the sample, A and B are distinct Cd standards [21].

3 Results and Discussion

3.1 Chemical Pretreatment of Samples

The measurement of the Cd isotopic composition in the samples required the extraction of Cd from the sample matrix, with 1) nearly 100% recovery, 2) complete removal of isobars (mainly Pd, In, Sn, $^{70}Zn^{40}Ar^{+}$ and $^{98}Mo^{16}O^{+}$), 3) high sample/blank ratios [30, 35]

3.1.1 The different procedures of the anion exchange chemistry

Fig.2 shows the elution curve of the anion exchange procedure for the GSB mixture adopted from the method of Zhu *et al*. [27]. Most matrix elements(such as K, Ca, Na, Mg, Al, Fe, Ni, Cr, Cu and Mn) were removed by 2 mol•L^{-1} HCl. In addition, most of the In and Pd as well as parts of the Zn and Sn were eluted by 0.3 mol•L^{-1} HCl. Most of Zn and Sn were eluted in 0.06 mol•L^{-1} HCl. The remaining Sn was removed by 0.012 mol•L^{-1} HCl. In the last step, Cd was completely eluted by 0.0012 mol•L^{-1} HCl. In our study, part of Zn and Sn have been eluted by 0.3 mol•L^{-1} HCl, as opposed to the procedures adopted by Gao et al.[21] and Cloquet et al.[20], in which Zn and Sn were

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primarily collected in the 0.012 and 0.06 mol•L^{-1} HCl fractions. Such behavior can be attributed to a higher volume of eluent utilized (30 mL in this study in contrast to 20 mL and 15 mL in Gao et al.[21] and Cloquet et al.[20]). Moreover, Zn was mostly eluted in 0.06 mol•L^{-1} HCl fraction but not in 0.012 mol•L^{-1} HCl as in Gao et al.[21]. This can be explained by the exchange of the steps of 0.012 mol•L^{-1} HCl and 0.06 mol•L^{-1} HCl.

The Cd recoveries based on the three procedures in this study were 97±1% (SD, N=3), 85±3% (SD, N=3) and 99.95±0.85% (SD, N=3), while the previously published values were 95%, 90% and around 96%, respectively[20, 21, 27]. The Cd recovery in this work was higher than the other two procedures. It can be attributed to a lower volume of 0.012 mol•L^{-1} HCl fraction utilized (5 mL in this study in contrast to 20 mL and 16mL in Gao et al.[21] and Cloquet et al.[20]). In addition, the Cd elution using the procedure of Zhu *et al*.[27] had fewer isobaric interference (such as Pd and Sn) compared with the other two procedures. Consequently, the anion exchange chemistry method of Zhu *et al*.[27] was the most suitable one for plants in this study.

The isotopic fractionation during column separation was assessed since Cd recovery was still lower than 100%, though it was higher compared to the recovery of 96% reported by Zhang *et al*.[36]. This was carried out by doping the "low Cd and high matrix" plant samples with small amounts of the Spex Cd standard solution, which were then analyzed following the normal procedure. The obtained $\delta^{114/110}$Cd value was +0.02±0.02‰, which indicated there was no systematic Cd isotopic bias. The concentration data correction for the procedural blank revealed that procedural blank constituted to less than 0.01‰ of the indigenous Cd present in the GSB mixture. At this level, the blank had a negligible effect on the measured isotopic compositions.

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3.1.2 The digestion and Cd purification of plant samples

In contrast to the GSB mixture, the analyzed plant samples were rich in organic matter. It was unclear whether the described methods for the determination of stable Cd isotopic compositions were well suitable for samples with high amounts of organic matter. *Cyperus alternifolius* showed relatively high metal concentrations as shown in Table 2 and a Cd concentration of 17.9 $mg \cdot kg^{-1}$. So the digestion and purification procedure of *Cyperus alternifolius* was investigated as a representative plant in this study.

Cyperus alternifolius were digested using two methods. The method which applied HNO_3 and HF, yielded an anomalously high Cd recovery of 131%. This unexpectedly high recovery value might have emanated from organic matter remaining in the digested solutions which affected the Cd purification. In contrast, the method that simultaneously utilized HF, HNO_3 and $HClO_4$ for digestion, yielded approximately 100% recovery (97%) (Table 3). In addition, most trace metals in *Cyperus alternifolius* could be separated from Cd digestion solutions as shown in Fig.3. Yang *et al.*[31] found that the Cd isotopic composition of the original standard was not affected at sodium concentrations below 100 $\mu g \cdot L^{-1}$. Sodium (Na) concentrations of the purified sample solutions analyzed in this study were generally lower than 20 $\mu g \cdot L^{-1}$, suggesting no significant influence on the accuracy and precision of the Cd isotopic analyses.

Isotopic measurements revealed $\delta^{111/110}Cd$, $\delta^{112/110}Cd$, $\delta^{114/111}Cd$ and $\delta^{114/110}Cd$ values of -0.10‰,-0.19‰,-0.27‰ and -0.37‰, respectively, as well as a correlation coefficent of 0.9982 (Fig. 4). This value indicated that Cd isotope ratios were not affected by isobaric interferences, polyatomic species or matrix elements.

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Overall, after the samples were digested utilizing the acids of HF, HNO_3 and $HClO_4$ simultaneously, the chemical separation yielded a recovery of nearly 100% and eliminated most of the elements interfering with Cd. The Cd isotope ratios had a well linear relationship, suggesting that Cd isotope ratios were not affected by isobaric interferences, polyatomic species or matrix elements. Hence, the applied methods of digestion and Cd purification were suitable for plant samples.

3.2 The precision and accuracy of Standard Solutions

The accuracy tests for Cd isotope ratios have not been feasible due to paucity of information on Cd isotope reference materials matching with the matrix of environmental samples[16]. In order to assess the reliability of measurements, Cd isotopic compositions of four Cd standard materials (Spex Cd, Spex-1 Cd, Münster Cd and GSB Cd) were measured repeatedly. Though the four Cd standard materials did not match with the matrix of plants completely, it was convenient for comparing with previously published results. The $\delta^{114/110}$Cd value obtained for Spex Cd solution against itself was 0.00±0.09‰ while the $\delta^{114/110}$Cd value of Spex-1 Cd relative to the Spex Cd solution was -1.07±0.06‰. In a previous study[21], the $\delta^{114/110}$Cd value of the Spex-1 Cd solution relative to the Spex Cd solution was +0.54±0.11‰, which was much larger than the $\delta^{114/110}$Cd value of Spex-1 Cd solution in this study. Therefore, the Spex-1 Cd solutions produced in different stages showed major isotope fractionation though this may form a subject for further study. In addition, the $\delta^{114/110}$Cd value of the GSB Cd solution relative to the Spex Cd solution was -0.56±0.03‰. Though there were no previously published $\delta^{114/110}$Cd values for the GSB Cd solution in literature, data generated from this study could serve as reference values for future studies. As the secondary isotopic reference, the $\delta^{114/110}$Cd value measured for the Münster Cd solution was +4.53±0.08‰. This value matched the

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theoretical values (+4.48±0.04‰～+4.60±0.13‰)[20, 21] and confirmed that Cd isotope values of standard solutions in this study were accurate.

3.3 The precision and accuracy of Cd isotopic measurement in plants

3.3.1 Evaluation of matrix effect, isobaric and molecular interferences

The Cd yields were calculated by the Cd concentrations before and after the chemical separation procedure. The total yields of all samples (Table 4) were higher than 95%. Considering Cd isotopes monitored in this study (^{110}Cd, ^{111}Cd, ^{112}Cd, and ^{114}Cd), the isobaric interferences from ^{110}Pd, ^{112}Sn and ^{114}Sn were expected to be problematic for precise Cd isotopic ratios measurement. In previous studies, the interference corrections for Sn and Pd became unreliable when the ^{118}Sn/^{114}Cd and ^{105}Pd/^{110}Cd ratios exceeded 18% and 0.8%, respectively[24]. Pallavicini *et al.*[16] reported that an extra measurement session for Sn isotopes was advisable when dealing with matrices containing a Cd/Sn concentration ratio below 10 in order to assess potential natural or artificially-introduced isotopic fractionations of the Sn eluted in the Cd fractions. In this study, the ratio of Sn/Cd was less than 1% and the ratio of Pd/Cd was less than 0.76% (Table 4). Hence, the accuracy of the Cd isotopic ratio measurements was not significantly affected by isobaric interferences.

Also, it has been established that the polyatomic species from the recombination of argon, atmospheric gases, solvents and sample matrix (e.g. ^{70}Zn^{40}Ar^{+}, ^{98}Mo^{16}O^{+}) could interfere with Cd isotopes during analyses[24]. Consequently, after chemical separation, the Cd eluents have been analyzed on elements or compounds that might interfere with Cd. Only traces of Pb, Zn, Cu, Mn, Fe, Cr and Mo were detected. Of these, ^{70}Zn^{40}Ar^{+} and ^{98}Mo^{16}O^{+} interfered with ^{110}Cd and ^{114}Cd respectively. Cloquet *et al.*[20] found no detectable mass bias at Zn/Cd ratios lower than 10%. In this

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study, Zn/Cd ratios in most Cd eluents were lower than 0.5% with a maximum ratio of 9.63%. In addition, some tests also demonstrated that a few ratios ($^{112}Cd/^{110}Cd$, $^{114}Cd/^{110}Cd$ and $^{116}Cd/^{111}Cd$) were not significantly affected if the concentration ratios of Cd/Mo were 4 or above[16]. In this study, Mo/Cd ratios in most Cd eluents were lower than 0.1%, which were too low to affect the Cd isotopic ratio measurements.

The measured isotopic composition of the Cd standard solutions and samples were plotted on a single theoretical mass fractionation line in a three-isotope diagram (Fig.5). The three-isotope graph showed experimental mass-dependent fractionation with a slope of 1.9992 (99.99% confidence interval), which agreed well with the theoretical equilibrium fractionation slope. The excellent agreement of $\delta^{114/110}Cd$ and $\delta^{112/110}Cd$ values of the samples gave us confidence that all polyatomic interferences were well-resolved for all tested instrumental set-ups.

3.3.2 Reproducibility and Accuracy of Cd Isotope Data for Plants

The reproducibility of the Cd isotope ratios was tested by three Cd standard materials (Münster Cd solution, Spex-1 Cd solution and GSB Cd solution) and different plants with rich organic matter. The reproducibility of the Münster Cd solution was $\pm 0.08‰$ in $\delta^{114/110}Cd$ (2SD), while the reproducibility of the plant samples ranged from $\pm 0.01‰$ to $\pm 0.10‰$ in $\delta^{114/110}Cd$ (2SD) as shown in Table 4. In comparison, Pallavicini *et al.*[16] reported a reproducibility of $\pm 0.03‰$ to $\pm 0.10‰$ in $\delta^{114/110}Cd$ (2SD) for various carbon-rich environmental samples (soil, sediment, Fe-Mn modules, sludge, kidney, liver, leaves) normalized to Ag. Wombacher *et al.*[29] achieved a precision of $\pm 0.4‰$ to $\pm 0.6‰$ for $\delta^{114/110}Cd$ (2SD) in geological samples normalized to Ag. Cloquet *et al.*[20] reported a $\delta^{114/110}Cd$ reproducibility of about $\pm 0.12‰$ (2SD) in various geological and

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anthropogenic samples using standard-sample bracketing. Ripperger and Rehkamper[28] displayed external $\delta^{114/110}$Cd reproducibility between ±0.1 ‰ and ±0.16 ‰ (2SD) in seawater samples with very low Cd concentrations of around 8 ng using a ^{110}Cd-^{111}Cd double spike. Nonetheless, the results confirmed that this analysis technique yielded very precise Cd isotope data and was appropriate for Cd isotopic measurements of organic-rich samples, such as plants. In this study, only four plant species were investigated, however, the elemental compositions in plants were broadly similar, so the analytical method also was suitable for other plants.

3.4 Cadmium Isotopic Composition

3.4.1 Cadmium Isotopic Composition in Plant Samples

The Cd isotopic composition of the plant samples are illustrated in Fig.6. The overall $\delta^{114/110}$Cd values ranged from -0.39‰ to -0.08‰, which were lower relative to the Spex Cd solution. Three *Ricinus communis* plants yielded Cd isotopic compositions ($\delta^{114/110}$Cd) of -0.12‰, -0.14‰ and -0.08‰, respectively, and did not show any significant Cd isotopic variation compared to the Cd Spex solution. On the other hand, three *Solanum nigrum* plants yielded $\delta^{114/110}$Cd values of -0.39‰, -0.37‰ and -0.34‰, which were significantly lower relative to the Cd Spex solution and the *Ricinus communis* plants. Additionally, *Cyperus alternifolius* and *Pteris vittata* showed $\delta^{114/110}$Cd value of -0.37‰ and -0.34‰, respectively. Such a difference among plants strongly suggested that the degree of Cd isotopic fractionation was related to plant species. Furthermore, it suggested that the plants preferred the light Cd isotopes. Lacan *et al.*[30] also confirmed that phytoplankton preferentially took up light Cd isotopes. However, Pallavicini *et al.*[16] reported that the $\delta^{114/110}$Cd value of birch leaves ranged from +0.30‰ to +1.3‰, therefore favoring enrichment in heavier Cd isotopes. The different

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Cd isotopic compositions in the different plant samples might result from a distinct mechanism of Cd accumulation in plants or different source Cd (from soil or nutrient solution), as well as potential differences in various plant parts should be considered. Therefore, further studies are required to confirm this conclusion.

3.4.2 Cadmium Isotopic Fractionation in Nature

As shown in Fig.6, Cd isotope investigations have been applied on a wide range of natural materials, including seawater[37], extraterrestrial matter[38, 39], industrial samples[22, 40], sediments[41], minerals and silicate rocks[42]. Natural mass-dependent fractionation (MDF) of cadmium isotopes was a promising new tool for investigating Cd pathways and cycling in geological and biological materials[43]. One interesting new application was as a proxy for nutrient utilization in paleoceanography[37]. The $\delta^{114/110}$ Cd value of seawater varied from -0.86‰ to +3.54‰ relative to the "JMC Cd" reference standard (Johnson Matthey Company). The large isotope fractionation mainly resulted from the preferential biological uptake of light Cd by plankton[30]. The largest isotope variations were found in extraterrestrial materials (meteorite and lunar samples), which were affected by thermal processes. Reported isotope variations for these samples ranged from -9.2‰ to +16‰ ($\delta^{114/110}$Cd). Samples from Pb-Zn smelting and refining plants and soil polluted by the emissions of those plants featured $\delta^{114/110}$Cd values from -1.61‰ to +0.52‰[40], which was probably the result of Cd isotopic fractionation during partial evaporation/condensation. Compared with those materials, the variations of Cd isotope ratios of plants in this study ranging from -0.39‰ to -0.08‰ were rather small. However, the results provided an evidence for the fractionation of Cd isotopes in four

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herbaceous plants. The herbaceous plant species had a predominant light Cd isotopic composition and could represent a reservoir for light Cd isotopes in nature.

4 Conclusions

1) An optimized and accurate analytical method of digestion, purification and determination of Cd isotope in plants has been developed by investigating the four plant species (*Solanum nigrum*, *Ricinus communis*, *Cyperus alternifolius* and *Pteris vittata)*, grown in soil or hydroponic cultures.

2) The developed method characterized by high accuracy and recovery rates, turned out to be suitable for the investigation of Cd isotopic compositions in plants. The purification procedure yielded a Cd recovery higher than 95% and effectively eliminated the spectral interference of Pd, In and Sn as well. The Münster Cd standard material showed an accurate Cd isotopic value of +4.53±0.08‰ and matched the isotope results of previously published methods (+4.48±0.04‰～+4.60±0.13‰). The method provided an average long-term external reproducibility of ±0.09‰ for $\delta^{114/110}$Cd (2SD).

3) The overall $\delta^{114/110}$Cd values of the four analyzed plant species ranged from -0.39‰ to -0.08‰, indicating Cd isotopic fractionation in four herbaceous species. The herbaceous plant species could represent a reservoir for light Cd isotopes in nature. It is necessary to clarify whether similar isotopic fractionations are present in other herbaceous plant species such as *Sedum alfredii* and paddy rice polluted by Cd.

In summary, the results presented in this study indicated the applicability of the developed methods for Cd extraction and Cd isotope analyses in herbaceous plants. The established methods

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have also exhibited wide perspective of precise Cd isotope analyses on other plants in the future. Cd isotope represented an important and promising tool of elucidating the mechanisms of Cd uptake and accumulation in plants. Moreover, Cd isotope would be also applied to trace Cd source in many fields, such as environmental engineering, agricultural science and ecology.

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Figure legends

Fig.1 Typical long-term reproducibility achieved for isotopic composition of $\delta^{114/110}$Cd determined by repeated measurements of Spex Cd reference by MC-ICPMS

Fig.2 Elution curve of the anion exchange chemistry for GSB mixture

Fig.3 Ratios of metal elements and Cadmium in solution of *Cyperus alternifolius* sample (B: before the chemical separation; A: after the chemical separation)

Fig.4 Cd isotope ratios of *Cyperus alternifolius* digested by HF, HNO_3 and $HClO_4$ simultaneously

Fig.5 Three-isotope plots of Cd isotope ratios of plant samples and Cd standard materials presented in this study

Fig.6 Cd isotopic composition in plants and in nature materials (reported as $\delta^{114/110}$Cd relative to the Spex Cd solution) [20, 22,29-37, 40-41, 44-45, this study]

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Table 1 The purification procedures and the eluted elements with the elution stages in the different studies

Eluent	Volume (mL)			Eluted Elements		
	Zhu *et al*[27]	Gao *et al*[21]	Cloquet *et al*[20]	Zhu *et al*[27]	Gao *et al*[21]	Cloquet *et al*[20]
AG-MP-1M	3	2-3	2			
Sample	2	2	1			
2 mol•L^{-1} HCl	10	8	N.A	Matrix (including Pb, In)		
1.2 mol•L^{-1} HCl	N.A.	N.A	4			Matrix
0.3 mol•L^{-1} HCl	30	20	15	Pb, In, Zn, Sn	Pb	Pb, In
0.012 mol•L^{-1} HCl	N.A	20	16		Zn, Sn	Zn, Sn
0.06 mol•L^{-1} HCl	20	6	N.A	Zn, Sn	Sn	
0.012 mol•L^{-1} HCl	5	N.A	N.A	Sn		
0.0012 mol•L^{-1} HCl	20	10	17	Cd	Cd	Cd

Not-added (N.A.)

Table 2 Type and metal concentration of the analyzed samples and GSV-2

Name	Type	Cd	Cu	In	Pb	Sn	Zn	Pd	Mo
		mg kg-^{1}							
Ricinus communis-1	root	1325.4	14.2	0.78	10.9	9.7	57.2	0.17	19.7
Ricinus communis-2	stem	216.2	10.5	0.31	6.6	6.8	33.5	0.32	27.4
Ricinus communis-3	leaf	35.7	13.6	0.12	6.1	7.4	42.7	0.41	28.5
Solanum nigrum L-1	root	1931.6	38.2	0.67	42	32.3	23.7	0.87	160
Solanum nigrum L-2	stem	155.6	5.7	0.43	7.8	12.4	20.8	0.53	5.4
Solanum nigrum L-3	leaf	114.5	2.8	0.14	4.1	7.6	23.1	0.47	3.7
Cyperus alternifolius	root	17.9	48.0	0.94	298.3	8.82	119	0.91	3.1
Pteris vittata	root	11.7	11.5	0.78	20.5	5.5	55.5	0.84	15.1
GSV-2	plant	0.37	5.95	---	43.6	--	19.5	--	0.25

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Table 3 Cd total recovery of *Cyperus alternifolius* digested by HNO_3+HF and HF+HNO_3+$HClO_4$

Digestion	Cd introduced (μg)	Cd found (μg)	Cd recovery (%)	Cd average recovery (%)
HNO_3+HF	3.53	4.74	134	131
	3.55	4.56	129	
HF+HNO_3	3.57	3.53	99	97
+$HClO_4$	4.90	4.66	95	

Table 4 Metal/Cd ratios and total recovery of Cd elution, Cd isotopic compositions of plant samples analyzed in this study

Name	**Pd/Cd**	**Sn/Cd**	**In/Cd**	**Zn/Cd**	**Mo/Cd**	**Cd Recovery**	**$\delta^{114/110}$Cd**	**±2SD (N=3)**
				%			‰	
Ricinus communis-1	0.01	0.00	0.00	0.24	0.01	99	-0.08	0.04
Ricinus communis-2	0.00	0.00	0.00	0.15	0.03	96	-0.14	0.01
Ricinus communis-3	0.01	0.00	0.00	0.17	0.01	97	-0.12	0.05
Solanum nigrum-1	0.04	0.00	0.00	0.33	0.04	96	-0.37	0.03
Solanum nigrum-2	0.02	0.00	0.00	0.18	0.02	98	-0.39	0.10
Solanum nigrum-3	0.02	0.02	0.00	1.57	0.03	100	-0.34	0.09
Cyperus alternifolius	0.25	0.20	0.00	8.46	0.09	97	-0.37	0.01
Pteris vittata	0.76	0.82	0.00	9.63	0.32	97	-0.34	0.02

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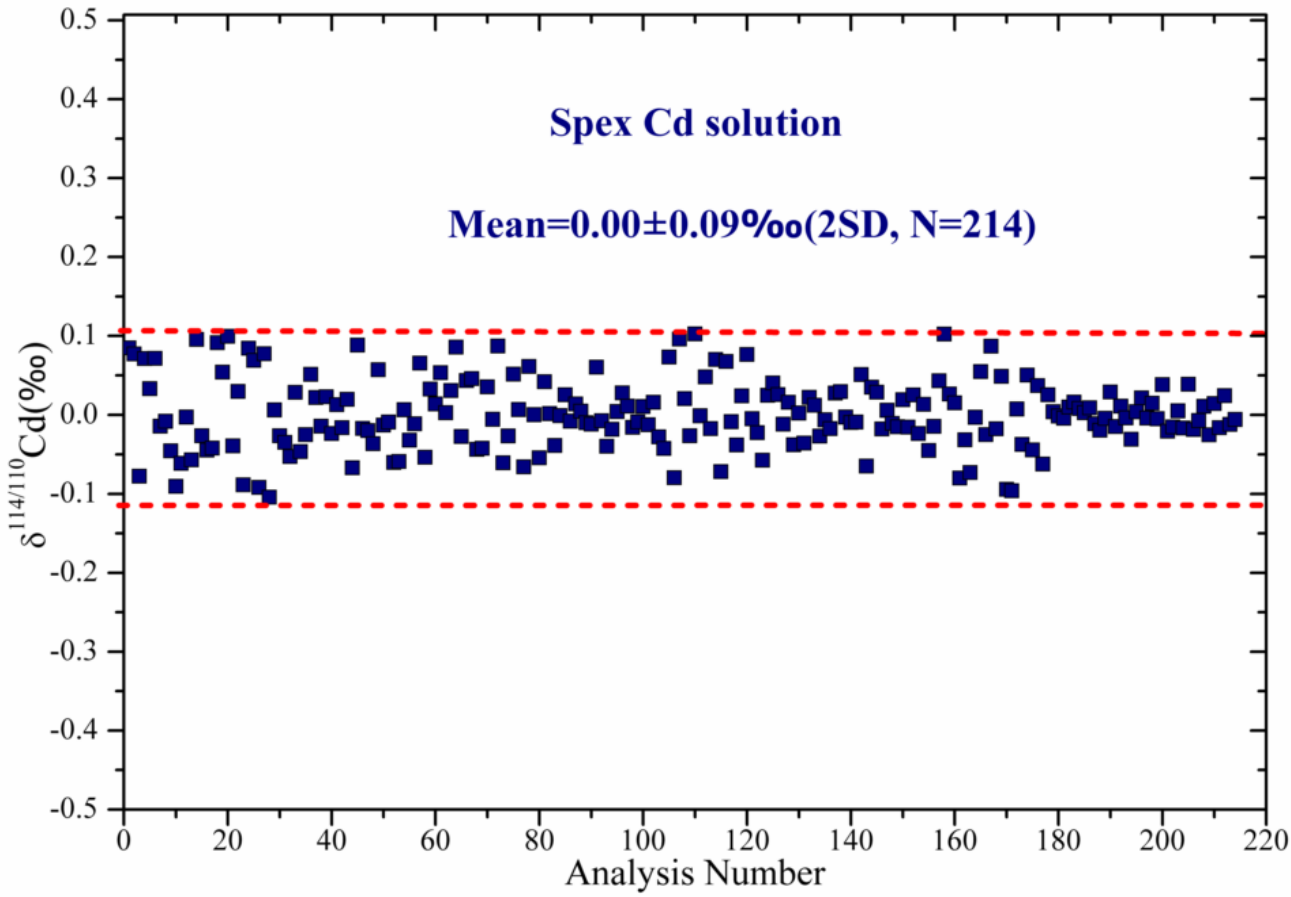

Fig.1

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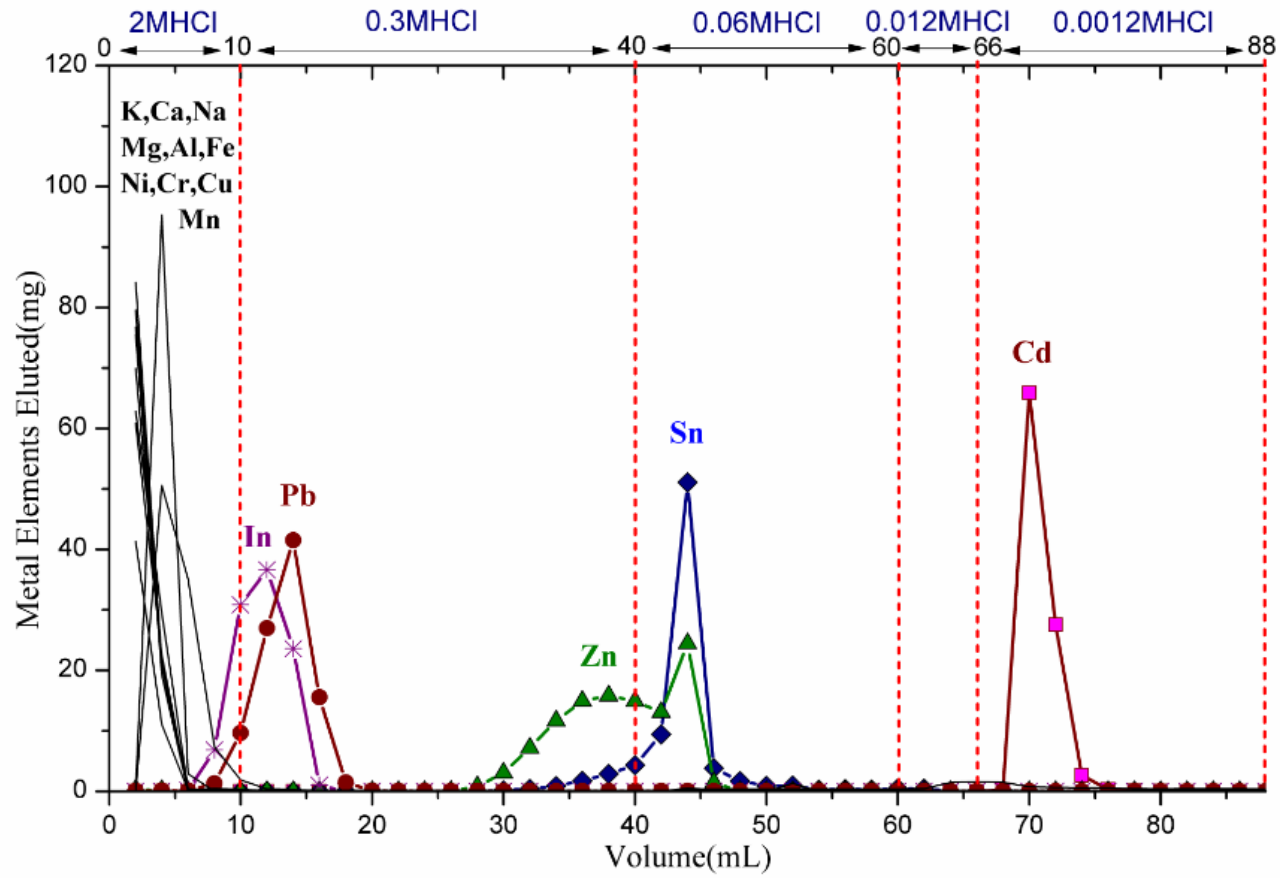

Fig.2

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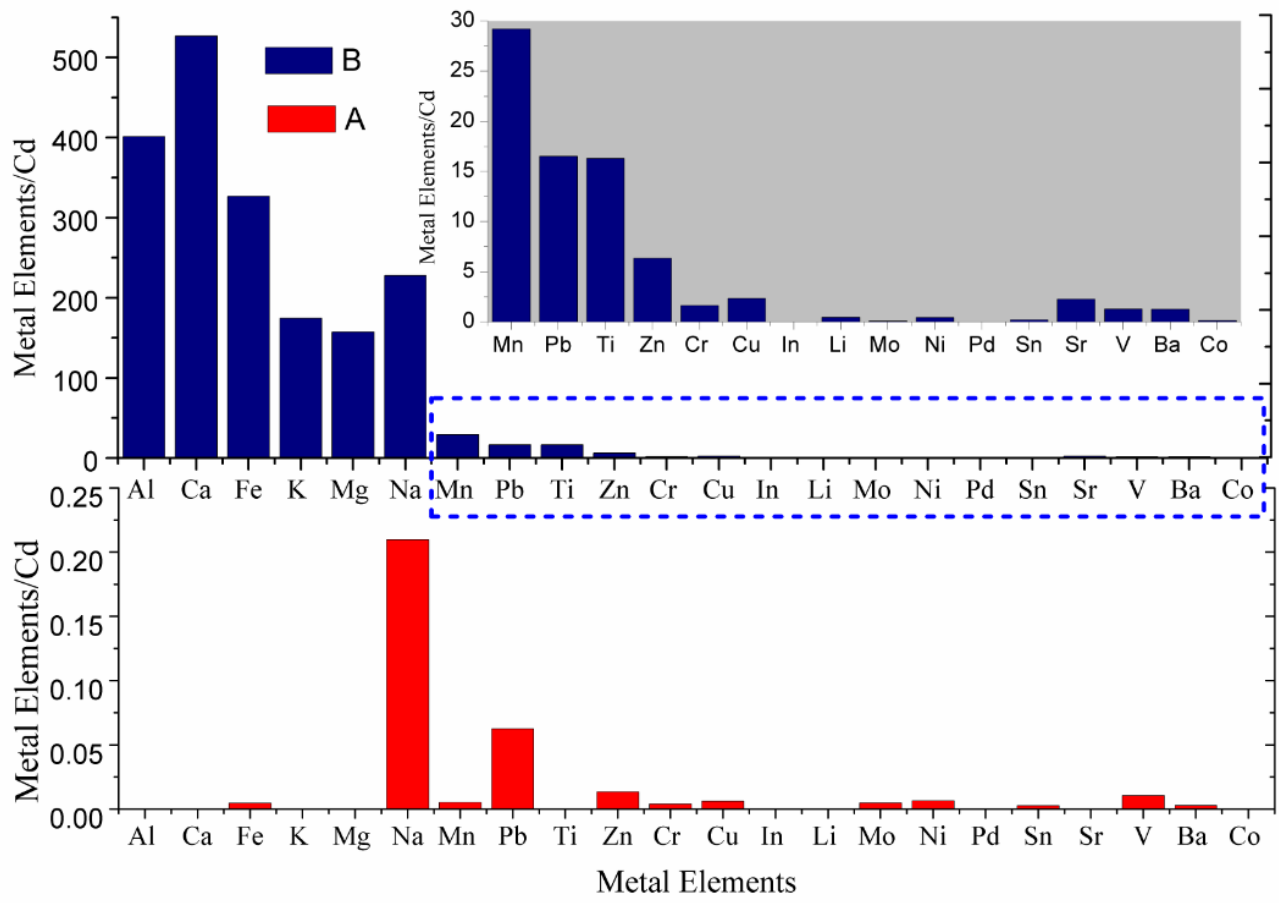

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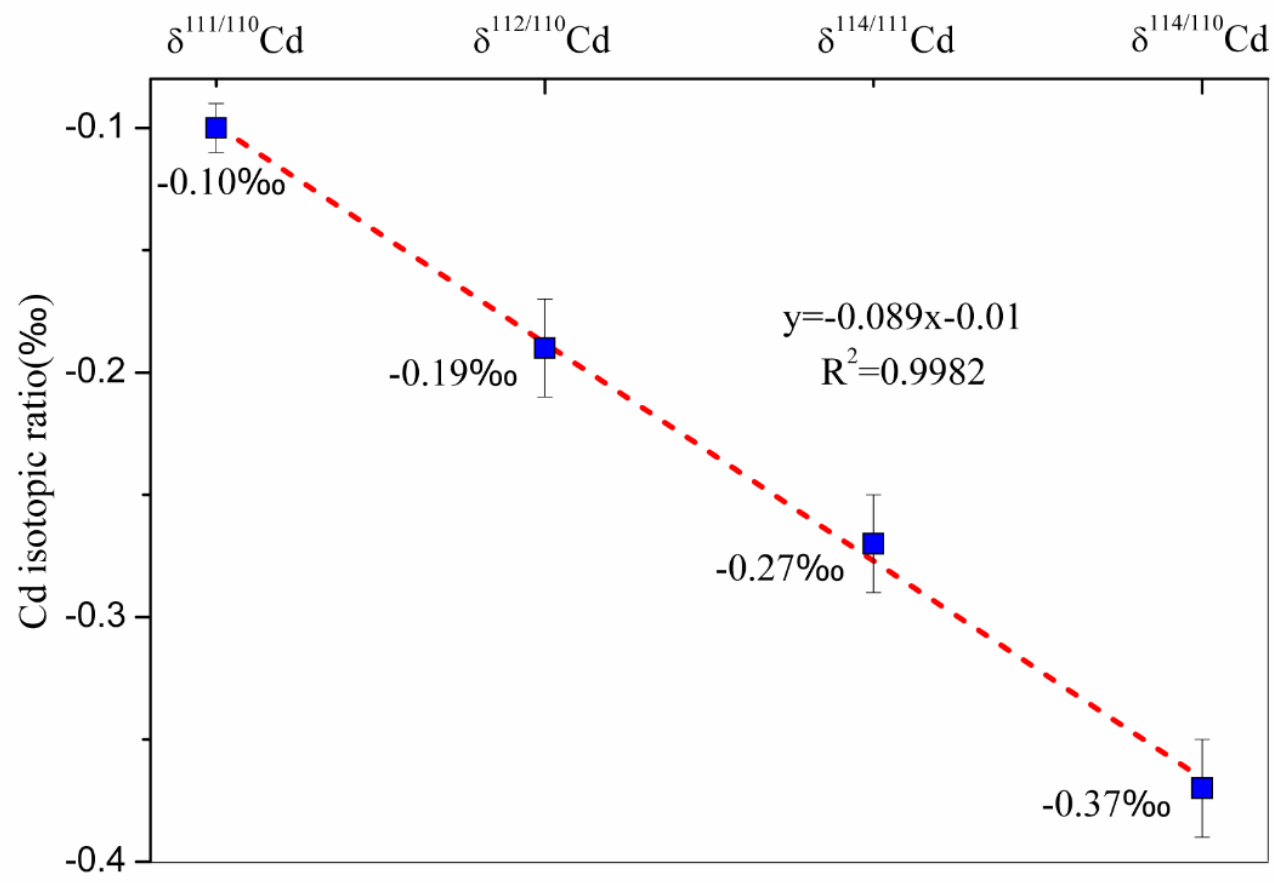

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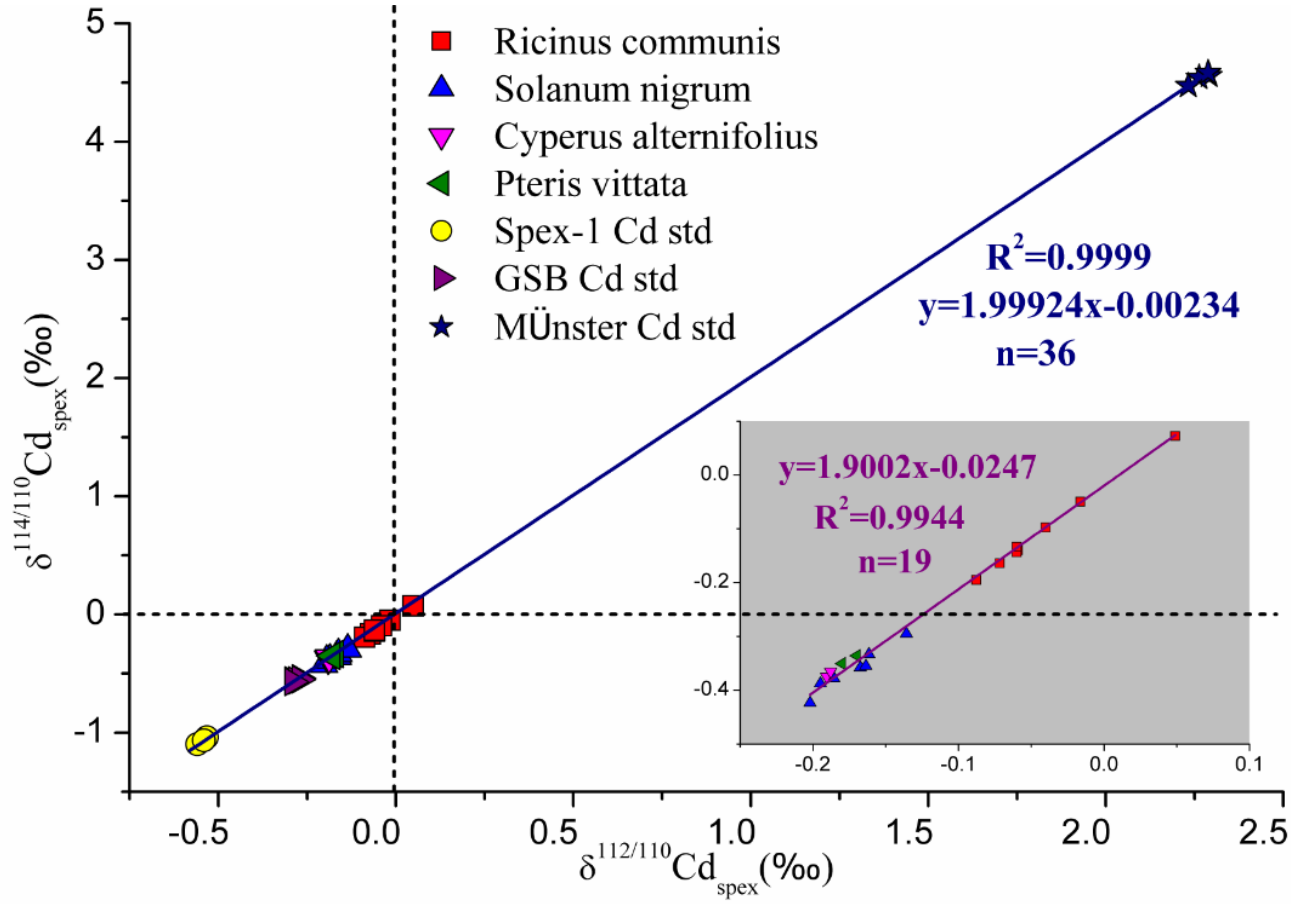

Fig.5

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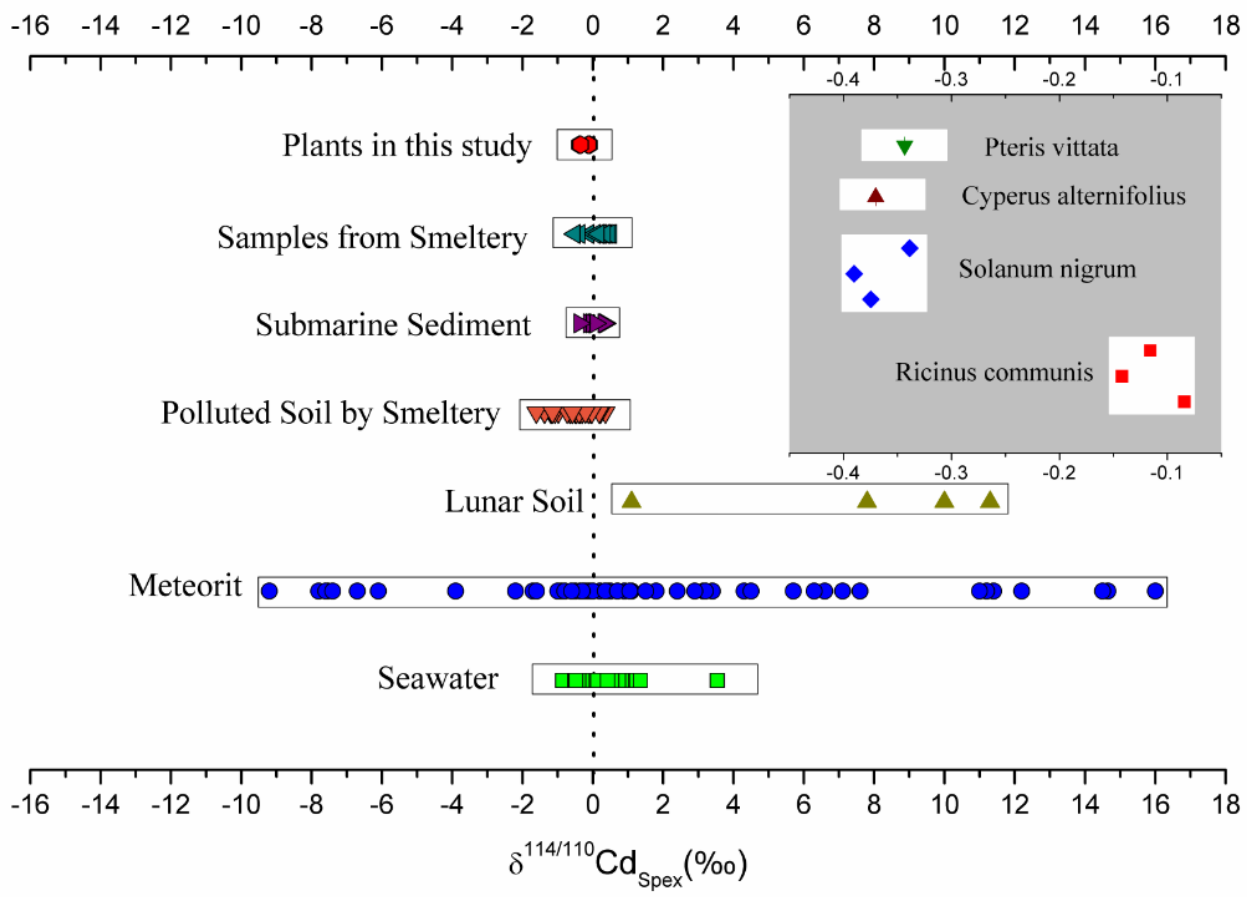

Fig.6

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