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Graphical and textual abstract

Various stages of an analytical method for high-precision cadmium (Cd) isotope ratio measurements by MC-ICP-MS (sample preparation, matrix separation, instrumental analysis and data evaluation) were critically evaluated and optimized for the processing of carbon-rich environmental samples. The method was used in a pilot study focusing on the assessment of factors affecting Cd isotope composition in birch leaves.

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Cadmium isotope ratio measurements in environmental matrices by MC-ICP-MS

Cite this: DOI: 10.1039/x0xx00000x

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Received 00th June 2014,
Accepted 00th June 2014

DOI: 10.1039/x0xx00000x

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Various stages of an analytical method for high-precision cadmium (Cd) isotope ratio measurements by MC-ICP-MS (sample preparation, matrix separation, instrumental analysis and data evaluation) were critically evaluated and optimized for the processing of carbon-rich environmental samples. Overall reproducibility of the method was assessed by replicate preparation and Cd isotope ratio measurements in various environmental matrices (soil, sediment, Fe-Mn nodules, sludge, kidney, liver, leaves) and was found to be better than 0.1 ‰ (2σ for $\delta^{114}Cd/^{110}Cd$) for the majority of samples. Cd isotope ratio data for several commercially-available reference materials are presented and compared with previously published results where available. The method was used in a pilot study focusing on the assessment of factors affecting Cd isotope composition in tree leaves. A summary of results obtained for a large number (n>80) of birch (*Betula pubescenes*) leaves collected from different locations in Sweden and through the entire growing season is presented and potential reasons for observed variability in Cd isotope composition are discussed. Seasonal dynamics of element concentrations and isotope compositions in leaves were also compared for Os, Pb, Zn and Cd.

Introduction

Cadmium is present in terrestrial materials as a trace element with average concentrations in nature at the μg kg^{-1} level and having eight isotopes covering a 10 *amu* range from ^{106}Cd to ^{116}Cd. Human activities such as ore mining[1], smelting/refining[2,3] including the industrial production of nickel-cadmium batteries[4], waste incineration and coal combustion, one of the major anthropogenic Cd sources[5], contribute to the anthropogenic burden of this element. In living beings Cd accumulates in vital organs with toxic and carcinogenic effects[6]. For example, it has been reported that accumulation can take place in vertebrate's kidneys with serious consequences such as irreversible renal tubular damage[1].

Pilot studies on Cd isotopic system date back to the 1970s, when large variations were measured in meteoritic and extraterrestrial materials[7]. This started a discussion involving different laboratories, interested in exploring the potential of Cd isotopic variations in environmental source tracing.

The field of science focusing on the use of Cd isotopes is still in its infancy compared with other more commonly usedstable heavy isotopic systems such as Fe, Zn, Cu or Mo. Insufficient measurement precision, typically about 0.5‰ to 1.0‰, limited by both the lack of appropriate analytical technology and the low natural Cd abundance in many matrices, have, until relatively recently, hindered advancement in the area, primarily in terrestrial samples[8]. Instrumental developments and the refining of analytical methods during the last decade have resulted in significantly improved precision in Cd isotopic analyses[3] as well as the capability to analyze samples with lower analyte contents[9,10]. In their pioneering work, Wombacher et al.[6] reported on the first isotopic analyses of Cd in terrestrial materials by multiple collector inductively coupled plasma mass spectrometry (MC-ICP-MS) achieving good long term reproducibility and high precision allowing the resolution of minor isotopic variations of ca. 0.1‰.

Measured isotopic variations in samples of different nature, *viz.* meteorites, sea water, geological and environmental matrices, highlighted the possibility of directly linking Cd isotopic composition with source[6]. Two main mechanisms were reported to influence Cd isotopic fractionation: biological activity and partial evaporation/condensation[6]. Although most Cd isotope ratio studies performed to date have focused on cosmological and geological samples, recent research shows how Cd isotopic composition can be a valuable aid in distinguishing potential pollution sources in a wide range of environmental applications [2–4]. Overall, even though reported Cd isotopic variations, deriving from both natural processes and anthropogenic activity, exceed 0.5‰ for $^{114}Cd/^{110}Cd$ ratio, several major terrestrial environments, e.g. silicate Earth, are characterized by very stable isotope composition with variability within 0.05‰[6,8].

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Table 1 MC-ICP-MS operating parameters for Cd isotope ratio measurements

Rf power (W)	1450
Ion lens settings	Optmized for maximum sensitivity and signal stability
Coolant gas (l min^{-1})	14
Sample gas (l min^{-1})	0,9-1,2
Auxiliary gas (l min^{-1})	1,1
Sample Uptake rate (ml min-1)	0,04-0,1

Variable	Specification
Isotopes (cup)	^{107}Ag(L4), ^{108}Cd(L3), ^{109}Ag(L1), ^{110}Cd(Centre), ^{112}Cd+^{112}Sn(H1), ^{114}Cd+^{114}Sn(H2), ^{116}Cd+^{116}Sn(H3), ^{117}Sn(H4)
Integration time, s	1.049
Number of integrations	5
Number of blocks	5
Cycles per block	5
Amplifier rotation	Left
ICP parameters	Adjusted daily for highest sensitivity and signal stability
Zoom optic parameters	Adjusted daily for highest sensitivity and peak shape

Further investigations have proven Cd isotopic variation in biological matrices to be influenced by both natural and anthropogenic processes [9–11]. Ripperger et al.[11] for example were able to demonstrate the influence of biological activity on Cd isotopic fractionation of $\delta^{114}Cd/^{110}Cd \approx +0.3‰$ to 3.8‰ in oceans. Correlations between Cd concentrations and isotopic composition in near-surface waters were attributed to the processes associated with the uptake of dissolved Cd by phytoplankton.

On the other hand several contemporary studies have assessed the environmental impact of smelting and refining processes with the help of Cd isotopic abundances and the results suggest that, even though isotope fractionation for a single industrial process generally does not exceed 1‰ ($\delta^{114}Cd/^{110}Cd$), the anthropogenic signature in exposed objects is still clearly distinguishable[2–4,6,12]. There is also a growing number of environmental applications relying upon information for several isotopes[2,3]. For example, in a multi-tracer study, Cd, Zn and Pb isotopic compositions and elemental concentrations were used to distinguish between natural and anthropogenic sources of these metals in bivalves[9,10].

Interest in bio-monitoring using natural accumulator organisms has grown in the past few decades. Analyzing different type of samples of plant origin can provide detailed spatial and temporal records of pollution[13,14]. Trees growing in urban or industrial proximities constitute the easiest and least expensive tool for trace metal contamination assessment[14,15] while surplus isotopic information (e.g. Pb, U, Os) can be useful in identification of pollution sources[16,17]. Birch (*Betula pubescenes*) can be utilized as bio indicators, being particularly useful for such studies owing to their tolerance to high levels of heavy metals and fast growing rate[15].

The aim of this work was to evaluate and where required modify existing analytical methodology for reproducible Cd isotope ratio measurement in various biological matrices suitable for bio-monitoring programs and to assess the natural variability of Cd isotope composition of birch leaves.

Experimental

Instrumentation

Cd and Pb isotope ratio measurements were performed by a NEPTUNE PLUS (Thermo Scientific, Bremen, Germany) MC-ICP-MS instrument operated in low resolution mode. Two sample introduction configurations were used, namely standard (consisting of PFA nebulizer with approximately 50 µl min^{-1} sample uptake, cyclonic/Scott double spray chamber arrangement and H-skimmer cone) and high sensitivity set ups (comprising an Aridus II desolvating nebulizer system from Cetac, Omaha, NE, USA and MC-ICP-MS interface equipped with either standard cones or 'Jet' sampler and 'X-type' skimmer cone). The cup configuration and operating conditions are given in **Table 1.**

All measurements of element concentrations were performed by an ELEMENT XR (Thermo Scientific) double-focusing sector field ICP-MS instrument equipped with a nickel sampler cone, a high sensitivity 'X-type' skimmer cone, a demountable quartz torch with 1.5 mm i.d. sapphire injector and a platinum capacitively de-coupling shield using combination of internal standardization (In) and external calibration. The sample introduction system consisted of a PFA spray chamber with two gas inlet ports (Cetac), a micro-concentric PolyPro nebulizer and a FAST SD2 auto-sampler (ESI, Perkin-Elmer, Santa Clara, CA, USA) equipped with a six-port valve and a 2-ml sample loop filled and rinsed by vacuum suction. Methane addition to the plasma was used to decrease formation of oxide-based spectral interferences, improve sensitivity for elements with high first ionization potentials, and to minimize matrix effects[18]**.** Operating conditions and measurement parameters for concentration measurements were as in previous studies[19].

Ashing of samples was performed at 550^{o}C in a laboratory oven (Nabertherm GmbH, Lilienthal, Germany). A laboratory microwave (MW) oven (MARS 5, CEM Corporation, Matthews, USA), a high pressure asher (HPA-S, Anton Paar, Malmö, Sweden) and an UltraWave single reaction chamber MW digestion system (Milestone, Sovisole, Italy) were used for sample digestions.

Chemicals and reagents

Nitric acid (HNO_3) and hydrochloric acid (HCl), (both from Sigma-Aldrich Chemie Gmbh, Munich, Germany) and hydrogen fluoride (HF, 48%, Merck, Darmstadt, Germany) used in this work were all of analytical grade. Water used in all experimental procedures was de-ionized Milli-Q water (Millipore, Bedford, MA, USA) purified by reverse osmosis followed by ion-exchange

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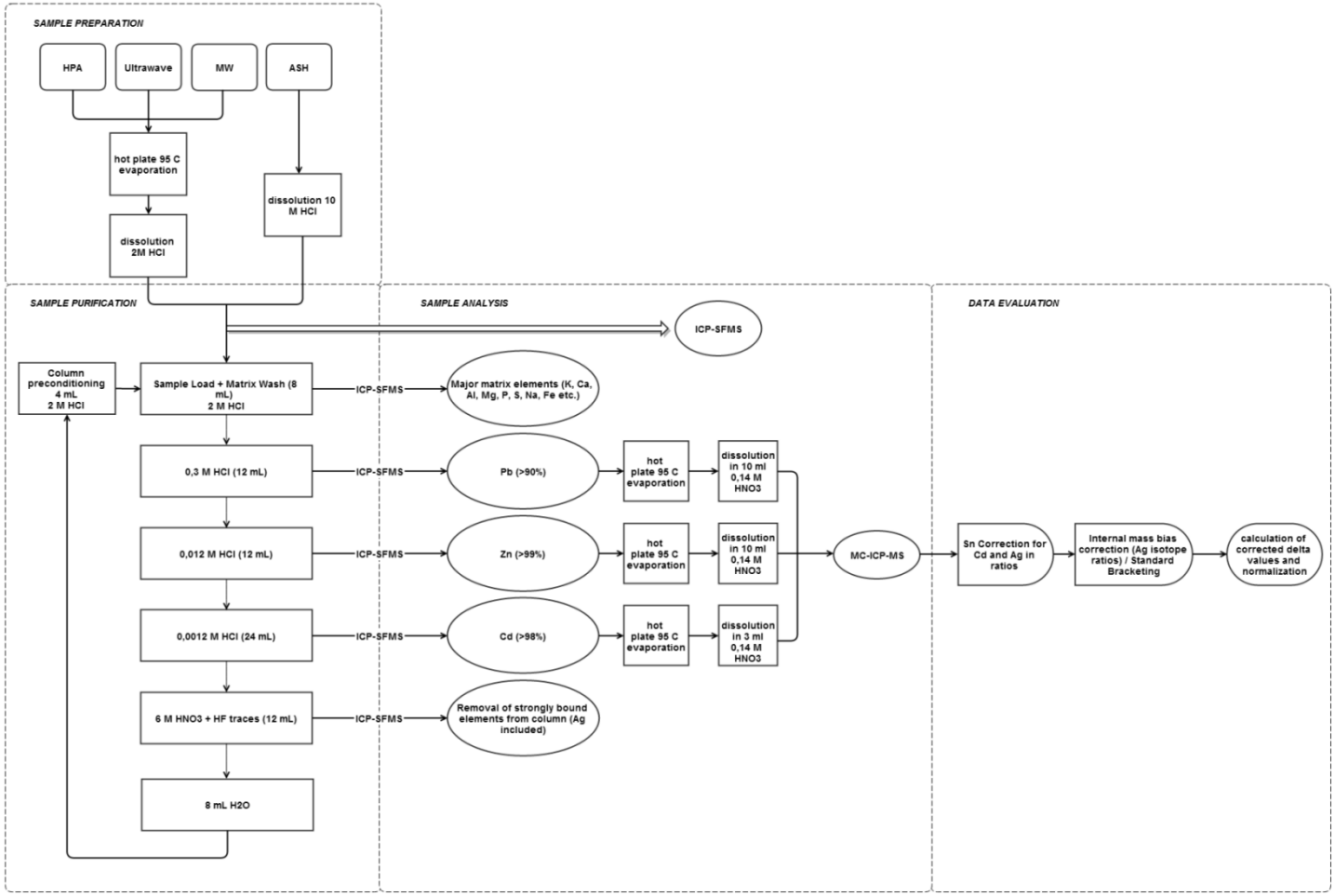

Figure 1 Flow chart of the analytical method.

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cartridges. AG-MP-1M ion-exchange resin (macroporous, 100-200 dry mesh size, 75-150 µm wet bead size, Bio-Rad Laboratories AB, Solna, Sweden) was cleaned by overnight soaking in 0.7 M HNO_3 followed by multiple rinses with Milli-Q water and loading as slurry into 2 ml columns.

NIST SRM3108 Cd solution Lot 130116 (National Institute of Standards and Technology, Gaithersburg, MD, USA) was used as 'δ-zero' Cd isotope bracketing standard, as suggested by Abouchami et al.[20], in all measurement sessions. Three commercially available 1000 mg l^{-1} Cd standards were used to prepare three quality control samples (QCS), namely QCS A (Spectrapure Standards, Oslo, Norway, Lot 111 issued February 2012, with Cd metal as starting material), QCS B (Ultra Scientific, North Kingstown, USA, Lot K00951 issued September 2009, with Cd nitrate hydrate Lot BH01228 as starting material) and QCS C (Ultra Scientific, Lot M00538A issued May 2011, with Cd nitrate hydrate Lot BH01820 as starting material). The first solution was analyzed at the beginning and at the end of every analytical session, while the remaining two were analyzed in random order through the duration of the study. An aliquot of so called 'UM-Münster-Cd' standard solution (20,6 mg l^{-1}) was provided by the Institute for Geology and Mineralogy of the University of Cologne. Two solid Cd chemicals, [illegible] d acetate dehydrate (Riedel De Haën AG, Hannover, Germany, Lot 32308), dissolved in concentrated HNO_3 were used to test the range of Cd isotope composition in chemicals used in the laboratory.

Working solutions of the abovementioned standard or chemicals with Cd concentrations of 5, 20 or 200 µg l^{-1}, depending on configuration of MC-ICP-MS introduction system used, were prepared daily by serial dilution in 0.14 M HNO_3. Internal standard Ag, prepared from 1000 mg l^{-1} stock standard from Ultra Scientific, was added to all measurement solutions at half of the respective Cd concentration.

Samples

For method optimization and testing, a range of certified reference materials (CRM), ERM BB186 Pig Kidney (Institute for Reference Materials and Measurements, Geel, Belgium), NIST SRM 2711 Montana Soil (provided by CRPG – Centre de Recherches Pétrographiques et Géochimiques, Vandœuvre les Nancy, France), SRM 2709 San Joaquin Soil (NIST), VKI-QC municipal sludge (Eurofins A/S, Vallensbæk Strand, Denmark), LGC6187 river sediment (LGC, Teddington, Middlesex, UK), GBW07311 stream sediment (National Research Centre for Certified Reference Materials, Beijing, PR China), PACS-2 marine sediment and TORT-1 Lobster Hepatopancreas (National Research Council of Canada, Ottawa, Canada), NOD-P-1 and NOD-A-1 manganese nodules (United States Geological Survey, Denver, CO, USA), as well as in-house control samples, namely freeze-dried kidney collected from moose (*Alces alces*) hunted in Northeast Sweden, freeze-dried herring (*Clupea harengus* from Bothnian Bay) liver, pooled dried mushroom (*Boletus edulis*) and two pooled samples of birch (*Betula pubescenes*) leaves were used. Note that none of the materials mentioned above has a certified Cd isotopic composition.

After optimization, the analytical methodology was applied to analyses of more than 80 birch leave samples collected during 2005-2013 from different locations in Sweden. Most of these samples were taken within an area of less than 0.5 km^2 north of the town of Luleå (22°8'25''E, 65°37'37''N) in Northeast Sweden during the 2012 and 2013 growing seasons (mid-May to October). Either leaves from single trees (not more than two leaves from a single branch on each sampling occasion), or from a group of trees in the same location (2-3 leaves from each tree, hereafter referred to as pooled samples) were collected by hand wearing powder-free laboratory gloves into Zip-Lock plastic bags marked with tree size, location and sampling date. Almost all samples were collected from the ground limiting the maximum sampling height to approximately 2.5 m. Hence only lower branches were sampled for large trees. On one occasion, it was possible to collect leaves representing different heights from birch freshly felled in a storm. The amount of collected material was chosen to provide approximately 2 g of dried leaves for individual or 6-8 g for pooled samples. Samples of litter, consisting from fall-out and partly decomposed birch leaves, mushrooms (*Boletus edulis*) and large pendulous lichens (*Alectoria sarmentosa*) were sampled in 2012-2013 at the Luleå location. Within 24 h of collection, samples were transported to the laboratory, dried at 50°C overnight, homogenized and stored in closed bags at room temperature prior to analysis.

A flow chart depicting all steps of the analytical method is shown in **Fig. 1.**

Sample preparation

All laboratory ware coming into contact with samples or sample digests was soaked in 0.7M HNO_3 (>24 h at room temperature) and rinsed with MQ water prior use. All sample manipulations were performed in clean laboratory areas (Class 10000) by personnel wearing clean room gear and following all general precautions to reduce contamination[21]

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Table 2 Details of sample preparation procedures

Variable	Procedure A	Procedure B	Procedure C	Procedure D	Procedure E
Max. sample weight, g	0.05	0.5	0.5	0.5	5
Vessel volume and material	25ml Teflon	60ml Teflon	60ml Teflon	15ml Teflon 70ml Quartz	20 ml porcelain
Number of vials per rack	40	40	40	15/4	NA
Acid mixture	HNO_3/HF	HCl/HNO_3/HF	HCl/HNO_3/HF	HNO_3	HCl
v/v ratio	99/1	6/2/2	6/2/2	NA	NA
Volume, ml	2	10	10	10	1
Digestion system	MARS 5	MARS 5	MARS 5	UltraWave/HPA-S	Nabertherm
Max. Temp., ^oC	170	170	170	250/300	550
Ramp time, min	15	15	15	25/30	120
Hold time, min	25	25	25	10/40	300

Sample digestion

To bring solid samples into solution, several preparation schemes were tested (**Table 2**):

- MW-assisted digestion in closed Teflon vials using a HNO_3/HF acid mixture (Procedure A)[22];
- MW-assisted digestion in closed Teflon vials using a HCl/HNO_3/HF acid mixture (Procedure B);
- MW-assisted digestion in closed Teflon vials using a HCl/HNO_3/HF acid mixture preceded by ashing of the sample at 550^o C (Procedure C);
- HPA or UltraWave digestion using only HNO_3 (Procedure D);
- Ashing at 550^{o}C followed by solubilization of ash in concentrated HCl (Procedure E).

The first procedure was used exclusively for preparation of carbon-rich matrices for determination of element concentrations. Procedures B and C were used for soil, sludge and sediment CRMs. Procedures D and E were used for preparation of carbon-rich matrices for isotope ratio measurements.

MW-assisted digestion is a mature and broadly available technique for digestion of various environmental matrices using acid or acid mixtures[23,24]. Up to 40 samples can be prepared simultaneously in less than 3 hours, including sample weighing, adding reagents, temperature ramping, holding and cooling times, as well as transferring digests to storage or evaporation vessels. However, for dried organic matrices the maximum recommended sample size per digestion vessel is limited to 0.3-0.5 g. Therefore it may require several parallel digestions in order to obtain a representative sample or to prepare a sufficient amount of analyte needed for isotope ratio measurements. Moreover, incomplete oxidation of carbon may interfere with ion-exchange separation at later stages and preceding ashing of organic-rich matrices might be necessary. HPA and UltraWave digestions occur at much higher pressures and temperatures thus ensuring more efficient oxidation, though the cost of instrumentation is significantly higher and sample throughput (especially for the HPA) is somewhat lower than for MW oven-based methods.

Ashing of samples provides efficient mineralization of organic-rich matrices with the ability to handle large initial weights, has the lowest equipment costs and allows high throughput. Simple room temperature leaching of the solid sample residue in a low volume of mineral acids can be sufficient for quantitative analyte recovery thus limiting blank contributions from reagents and non-disposable digestion vessels.[21] Potential volatilization losses of analytes having significant vapour pressures can, however, limit the applicability of the latter approach and needs to be carefully checked.

An aliquot of digests, including those intended primarily for isotope ratio measurements, was diluted and analyzed by ICP-SFMS. Cd recoveries for all CRMs tested in this study and using all digestion approaches were in 94-102% range with no statistically significant differences between procedures with or without ashing step. Hence, volatilization losses of Cd during ashing procedure (if any) are below 6% confirming similar observations from studies employing Cd radioisotopes[25–27].It should be noted that significant losses of Hg, Te, Tl, Sb and Se were observed during ashing. The rest of sample solutions were evaporated to dryness in 25 ml Teflon beaker at 95^{o}C on ceramic-top hot plate, followed by dissolution in 4 ml of 2M HCl thus ready for Cd purification. For samples prepared by Procedure E, ashed material was transferred to 50 ml polyethylene, conical-bottom vial following by addition of 1 ml 10 M HCl, mechanical agitation at room temperature for 1 hour and addition of 4 ml H_2O. Remaining undissolved phases (residual carbon particles and silicates) were allowed to settle and upper 4 ml of clear phase was used for Cd purification step.

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Cd purification

A number of procedures have been proposed for the separation and purification of Cd, varying in complexity from a single pass through one column to very elaborative, multiple-stage schemes[28–31], depending on the sample matrix. The majority of recent studies on high-precision Cd isotope ratio measurements in environmental samples rely upon the separation procedure using AG-MP-1M ion-exchange resin originally proposed by Cloquet et al.[8], in some cases with minor modifications according to Gao et al.[3]. The applicability of this procedure for the matrices analyzed in the present study was evaluated for mushroom (prepared using Procedures A, D and E) and soil (prepared using Procedures B and C) digests separated using columns filled with approximately 2 ml of resin slurry. This was done by sampling load, matrix wash and all elution fractions with 1 ml resolution followed by ICP-SFMS analysis, thus obtaining detailed elution profiles for practically all elements present in these samples. Very similar elution profiles for major matrix elements and for Cd were obtained regardless of sample matrix and preparation procedure, except that much broader elution peaks and therefore significantly higher elution volumes needed to be collected for complete Cd recovery from the separation of digests prepared by Procedure A. Interference from high concentrations of residual non-oxidized carbon, the presence of which was obvious from the distinctly deep-brown colour of load solutions prepared by this procedure as opposed to the transparent or light-yellow tinged digests typical of other procedures, affecting the column separation process is the most probable explanation for this effect. Consequently sample preparation by Procedure A was not used for Cd isotopic analyses. It is possible that an undigested residue of organic matrix contributes to matrix effects previously attributed solely to resin-derived organic compounds and inorganic elements[20,32].

For digests prepared by all other procedures and in both tested matrices, >98% of major matrix elements (K, Ca, Al, Mg, P, S, Na, Fe, Si, Mn, etc.) pass through the column during sample loading (4 ml) and the subsequent 2 M HCl matrix wash (8 ml). The next fraction (12 ml of 0.3 M HCl) contains >90% of the initial Pb and can be used for Pb isotope ratio measurements by ICP-SFMS or MC-ICP-MS if needed, with a minor fraction, approximately 0.5-1.0% of the matrix elements initially present remaining. Zn is quantitatively (>99%) recovered in 12 ml of 0.012 M HCl that can be used for Zn isotope ratio measurements by MC-ICP-MS if needed, followed by Cd elution (>98% recovery) in 0.0012M HCl (24 ml). Finally, 12 ml of 6 M HNO_3 containing traces of HF remove the remaining Ag, Bi, Tl, Nb, Sb, Sn, Hg, Zr and U that are strongly bound to the resin. The fact that almost all Ag elutes in this last fraction contradicts the findings of Gao et al.[3] who reported that Ag was recoved during sample load and matrix wash. The entire separation procedure, including column pre-conditioning and passing 10 ml of H_2O at the end of separation, takes 5-6 h and up to 40 columns have been used in parallel. The performance of the columns was unchanged after five consecutive separation cycles.

Apart from efficient Cd separation from the sample matrix, manifest by < 0.02% of the total dissolved solids load present in the original digests eluting in the Cd fraction, and quantitative recovery, contamination by elements that could result in spectral interferences on Cd and Ag isotopes to be monitored by MC-ICP-MS[6] need to be assessed. Concentrations of Pd (source of isobaric interferences on ^{108}Cd and ^{110}Cd), Zr (source of ZrO^+ interferences on ^{107}Ag, ^{108}Cd, ^{110}Cd and ^{112}Cd), Nb (source of NbO^+ interference on ^{109}Ag) and Th (source of Th^{2+} interference on ^{116}Cd) in the analyte fraction were below respective limits of detection ensuring that Cd to interfering element concentration ratios are >100000 and therefore contribute negligible level of spectral interferences. The Cd to Zn concentration ratio in the analyte fraction was >100 and given the low argide formation rate in the ICP[4], the interferences from $ZnAr^+$ on ^{107}Ag, ^{108}Cd and ^{110}Cd can be neglected. Both Mo (source of MoO^+ interferences on ^{108}Cd, ^{110}Cd, ^{112}Cd, ^{114}Cd and ^{116}Cd) and Sn (source of isobaric interferences on ^{112}Cd, ^{114}Cd and ^{116}Cd) were present in the Cd fraction at levels approaching 1% of the Cd concentration in some samples. Addition of an extra elution step (12 ml 0.06M HCl) between the Zn and Cd fractions, as recommended by Gao et al.[3], helps to decrease Sn concentration in the latter by approximately 30-50%, but as this also results in larger volumes required for complete Cd elution and prolongs the separation procedure by almost 20%, no overwhelming benefit was evident.

It should be noted that the mushroom and soil samples used in the aforementioned tests contain relatively high Cd concentrations (>10 mg kg^{-1}) and the severity of problems originating from concomitant elements in the analyte fraction may be considerably higher for matrices with sub mg kg^{-1} Cd concentrations. Moreover, there is a risk that additional contamination might be introduced during the next preparation step – namely evaporation of the Cd fraction to dryness in 25 ml Teflon vials at 95°C on a ceramic-top hot plate and dissolving the residue in 3 ml 0.14 M HNO_3. This was necessary in order to prevent effects from remaining chloride ions on the Ag internal standard, for close matching of the acid strength between all measurement solutions in the MC-ICP-MS analytical sequence and to increase the Cd concentration four-fold, which is advantageous for samples low in Cd. For all samples prepared in the course of this study, a 0.2 ml aliquot of this solution was diluted 25-fold in 0.7 M HNO_3 and analyzed by ICP-SFMS. In combination with concentrations obtained from the analysis of digests prior to separation and volumes of corresponding fractions this enabled assessment of Cd recovery and the quantitation of interfering elements.

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Table 3 Delta values (in ‰) for Cd isotope ratios normalized by respective mass difference for birch leaves, fish liver and NIST SRM 3108 spiked with Mo and Sn

	Cd/Mo>100 Cd/Sn>100 Birch leaves	Cd/Mo>100 Cd/Sn>100 Fish liver	Cd/Mo>100 Cd/Sn=10 NIST SRM 3108	Cd/Mo=4 Cd/Sn>100 NIST SRM 3108	Cd/Mo=4 Cd/Sn>100 NIST SRM 3108 Aridus	Cd/Mo=10 Cd/Sn=2 NIST SRM 3108 Aridus
$(\delta^{110}Cd/^{108}Cd)/2$	0.129	-0.177	0.012	-5.915	-0.770	-0.238
$(\delta^{111}Cd/^{108}Cd)/3$	0.130	-0.180	-0.003	-3.823	-0.560	-0.160
$(\delta^{112}Cd/^{108}Cd)/4$	0.135	-0.181	-0.005	-2.969	-0.462	-0.115
$(\delta^{114}Cd/^{108}Cd)/6$	0.136	-0,182	-0.008	-1.959	-0.344	-0.081
$(\delta^{116}Cd/^{108}Cd)/8$	0.133	-0.184	0.012	-1.425	-0.288	-0.009
$\delta^{111}Cd/^{110}Cd$	0.131	-0.185	-0.014	0.357	0.063	0.005
$(\delta^{112}Cd/^{110}Cd)/2$	0.140	-0.185	-0.019	-0.023	-0.016	0.009
$(\delta^{114}Cd/^{110}Cd)/4$	0.139	-0.185	-0.020	0.019	0.009	-0.008
$(\delta^{116}Cd/^{110}Cd)/6$	0.137	-0.187	0.011	0.169	0.066	0.051
$\delta^{112}Cd/^{111}Cd$	0.150	-0.185	-0.011	-0.403	-0.058	-0.022
$(\delta^{114}Cd/^{111}Cd)/3$	0.142	-0.185	-0.019	-0.094	-0.029	-0.009
$(\delta^{116}Cd/^{111}Cd)/5$	0.139	-0.187	0.021	0.012	0.003	0.086
$(\delta^{114}Cd/^{112}Cd)/2$	0.138	-0.185	-0.043	0.061	0.021	-0.025
$(\delta^{116}Cd/^{112}Cd)/4$	0.136	-0.188	0.028	0.150	0.039	0.102
$(\delta^{116}Cd/^{114}Cd)/2$	0.134	-0.190	0.099	0.170	0.034	0.229
Mean(SD)	0.137(0.005)	-0.184(0.003)				

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Isotope ratio measurements and data evaluation

For MC-ICP-MS measurements, the Cd concentration was adjusted to 200 µg l^{-1} with 0.14 M HNO_3 and spiked with Ag to 50 µg l^{-1} before analysis with the standard introduction system or to 20 µg l^{-1} and spiked with Ag at 5 µg l^{-1} for the high sensitivity introduction system. When the latter system was used, the Aridus was turned on approximately 30 min before starting the MC-ICP-MS device. The instrument was allowed to stabilize for at least 1 h with the plasma lit while aspirating 0.14 M HNO_3 blank solution prior to performing the optimization of the operational parameters (gas flows, torch position, and lens settings) and mass calibration in the 107-120 *amu* range. Typical ^{114}Cd intensity was 5 V for 200 µg l^{-1} using the standard introduction system, and 4 V for 20 µg l^{-1} using the Aridus and standard cones. Similar instrumental sensitivity was reported by Wombacher et al.[33]for a Nu Plasma MC-ICPMS instrument. Using the Aridus in combination with the 'Jet' sampler and 'X-type' skimmer cone, our NEPTUNE PLUS typically generated 12 V intensity for the ^{114}Cd isotope at a concentration of 20 µg l^{-1}.

Samples were analyzed using bracketing isotope standards (NIST SRM 3108) with matching Cd and Ag concentrations and acid strength. Three samples or QCS solutions were analyzed between each pair of standards. Two consecutive measurements were performed for each solution in the sequence. The software option of excluding pass, run and block outliers was deactivated as it was found that this improved correlation between instrumental mass bias for Cd and Ag isotope ratios. Even without using this option the typical in-run precision in isotope ratios was in the 0.02‰-0.04‰ range and 0.06‰-0.20‰ range for measurements performed with standard or high sensitivity introduction systems, respectively. Higher signal fluctuations observed using the latter system are the most likely root cause for the deterioration in measurement precision.

Intensity data for all monitored isotopes (**Table 1**) were transferred to spreadsheet software for calculations. Firstly, contributions from Sn isobaric interferences on ^{112}Cd, ^{114}Cd and ^{116}Cd were corrected mathematically using the ^{117}Sn signal, tabulated Sn isotope abundances[34] and computed instrumental mass bias for each analytical session. Corrected intensities were then used to calculate fifteen Cd isotope ratios and the ^{109}Ag/^{107}Ag ratio. Instrumental mass bias was corrected in two steps. Firstly, revised exponential correction[35] using the internal standard (Ag ratio) was applied to all Cd ratios. Secondly, δ-values for all Cd ratios were calculated against bracketing NIST SRM 3108 standards:

$$\delta^{x/y}Cd = \left[\frac{\left({}^{x}Cd/{}^{y}Cd\right)_{sample}}{\left({}^{x}Cd/{}^{y}Cd\right)_{NIST3108}} - 1\right] \times 1000$$

where ${}^{x}Cd$ and ${}^{y}Cd$ correspond to the two different Cd isotopes, the $\left({}^{x}Cd/{}^{y}Cd\right)_{sample}$ value refers to the measured ratio and $\left({}^{x}Cd/{}^{y}Cd\right)_{NIST3108}$ is the isotope ratio of the standard. The factor 1000 is used to convert the δ-values to per mil notation. When the δ-value refers to a ratio of a heavier to a lighter isotope, a positive δ-value corresponds to an enrichment in the heavier isotope compared to the standard.

Mean ratios from two consecutive measurements of the first and the third samples (or QC) in each analytical block (standard1-sample1-sample2-sample3-standard2) were calculated against ratios for standards 1 and 2 respectively. For the second sample, mean ratios from bracketing standard were used assuming linear changes in instrumental mass bias persisting after internal standard correction. Results from two consecutive measurements of each sample allow calculation of mean δ-values and respective standard deviations for all Cd isotope ratios that are less affected by variations caused by imperfect amplifier gain calibration. As an additional aid to check for internal consistency of isotope data, all the δ-values for each sample were normalized by mass difference (e.g. **Table 3**), a data presentation that resembles the frequently used three isotope plot[32].

Pb and Zn isotope ratio measurements in separated fractions (**Fig. 1**) were performed using internal standardization with Tl and Cu, respectively, and bracketing standards, NIST SRM 981 Common Lead and IRMM 3702, respectively, as described in detail elsewhere[36,37].

For Os isotope measurements a detailed method description available in previous studies[16,17]

Results and discussion

Performance of separation procedure

The average Cd method blank for the entire procedure, as assessed by applying all preparation and separation steps to a set of reagent blanks handled as samples, was 0.14±0.09 ng (n=17). This corresponds to <0.2% contribution for samples with the lowest Cd content tested and therefore has negligible impact on measured ratios. The Cd recovery from all samples separated during this study (n>100) was above 95%. Levels of common contaminant-prone elements (Na, Ca, K, Mg, S) in analyte fraction prepared for MC-ICP-MS were, as a rule, below 100 µg l-1. In some matrices, µg l-1 concentrations of Fe (Mn-Fe nodules), Zn, Tl, and Sb were also present. Such low levels of impurities are unlikely to cause significant matrix effects or spectral interferences. As far as elements that can spectrally interfere with Cd and Ag isotopes are concerned, the concentrations of Pd, Nb, Th and Zr were below 0.01 µg l-1 in all Cd fractions. For birch leaves, mushroom, liver and kidney

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Table 4 $\delta^{114}Cd/^{110}Cd$ for reference and control samples

Test sample	Preparation	Cd, mg kg^{-1} **Found±σ**(Certified±σ)	$\delta^{114}Cd/^{110}Cd$ (2σ), ‰
QCS A	Dilution, n=34	(1000)	-0.020 (0.024)
QCS B	Dilution, n=17	(1000)	-0.008 (0.020)
QCS C	Dilution, n=17	(1000)	-0.584 (0.023)
UM-Münster-Cd	Dilution, n=8	(20.6)	4.437 (0.042)
CdO	Dissolution in HNO_3, n=8	(875000)	0.007 (0.016)
$Cd(CH_3COO)_2*2H_2O$	Dissolution in HNO_3, n=8	(422000)	0.018 (0.022)
Moose Kidney	Procedure E, n=8	**12.3±0.4**	0.635 (0.034)
	Procedure D(HPA), n=4	**12.5±0.3**	0.597 (0.049)
Fish liver	Procedure D(UltraWave), n=6	**1.82±0.10**	-0.789 (0.048)
Mushroom	Procedure E, n=10	**10.1±0.6**	0.365 (0.037)
	Procedure D(HPA), n=6	**10.4±0.3**	0.347 (0.089)
Leaves A	Procedure E, n=4	**1.75±0.09**	0.525 (0.068)
	Procedure D(UltraWave), n=4	**1.73±0.12**	0.500 (0.031)
Leaves B	Procedure E, n=4	**1.14±0.07**	0.733 (0.099)
	Procedure D(UltraWave), n=4	**1.19±0.07**	0.772 (0.102)
ERM BB186 Pig Kidney	Procedure E, n=4	**1.04±0.06** (1.09±0.05)	0.465 (0.062)
TORT-1 Lobster Hepatopancreas	Procedure E, n=4	**25.7±1.3** (26.3±2.1)	-0.123 (0.025)
NIST SRM 2711 Montana soil	Procedure C, n=8	**42.5±1.9** (41.70±0.25)	0.803 (0.071)
	Procedure D(HPA), n=8	**40.9±1.6** (41.70±0.25)	0.711 (0.106)
NIST SRM 2709 soil	Procedure C, n=4	**0.37±0.02** (0.38±0.01)	0.007 (0.089)
GBW 07311 stream sediment	Procedure C, n=4	**2.19±0.10** (2.3±0.1)	-0.305 (0.054)
VKI-QC municipal sludge	Procedure C, n=4	**1.19±0.09** (1,34±0.17)	-0.067 (0.036)
LGC6187 river sediment	Procedure C, n=4	**2.77±0.14** (2.7±0.3)	0.313 (0.048)
PACS-2 marine sediment	Procedure C, n=4	**2.04±0.08** (2.11±0.15)	-0.204 (0.040)
NOD-A-1 manganese-nodule	Procedure B, n=4	**7.28±0.31** (7.5±0.16[a])	0.086 (0.031)
NOD-P-1 manganese-nodule	Procedure B, n=4	**21.5±1.1** (22.6±0.3[a])	0.120 (0.038)

[a]Non certified values from Axelsson et al. [47]

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samples, Mo and Sn levels in analyte solution were also below 1% of respective Cd concentrations. However, in some environmental CRMs, significantly higher concentrations of these interfering elements were found, sometimes at levels exceeding 20% of the Cd content. In contrast to the majority of elements, the elution behaviour of Sn and Mo varies significantly depending on the sample matrix, the preparation procedure, and even on the individual column used. For example, during the separation of digests prepared without an ashing step, most (>95%) Mo elutes in the last HNO3 fraction, while when the same matrix is prepared by ashing, up to 50-60% of Mo elutes in the Pb and Zn fraction with partial tailing into the Cd cut, and a secondary elution peak in the last fraction. Hence, the share of the total loaded amounts of these elements co-eluting in the Cd fraction may differ by a factor of 2-3 even for replicate preparations and separations.

In the case of Sn, the efficiency of mathematical corrections might be affected by the uncertainty in the actual instrumental mass bias deduced from measured and tabulated[34] Cd or Ag ratios and also by Sn fractionation, either in the original sample or introduced during column separation. The severity of both factors will increase with decreasing Cd to interferent ratio in the measurement solution. Instrumental mass bias per mass unit calculated from measured (in bracketing standards) and calculated (using tabulated isotope abundances) Ag or Cd isotope ratios varies by up to 0.3% depending on the ratio used, possibly reflecting uncertainties in tabulated abundances or limitations of the correction model. A set of SRM 3108 solutions spiked with increasing Mo concentrations show strong positive correlation (R^2>0.94) with the factor ($^{(x-16)}$Mo×^{y}Cd)/(^{x}Cd×$^{(y-16)}$Mo), confirming that the contributions from $Mo^{17}O^+$, $Mo^{18}O^+$ or MoN^+ are negligible to a first approximation. These tests also demonstrate that for solutions with Cd/Mo concentration ratios of 4 or above, there are a few ratios (^{112}Cd/^{110}Cd, ^{114}Cd/^{110}Cd and ^{116}Cd/^{111}Cd) that are not significantly affected (**Table 3**). The formation of MoO^+ decreases almost 10-fold in a sample introduction system incorporating a desolvating nebulizer compared to one using a standard configuration due to the lower solvent vapour loading to the ICP. Therefore the former sample introduction system offers important advantages for Cd isotope ratio measurements in samples containing Mo.

The manner of presenting normalized δ-values exemplified in **Table 3** makes identification of results affected by spectral interferences from MoO^+ or from Sn very straightforward, being manifested by large uncertainties in mean normalized δ-values calculated using all 15 isotope ratios, and by distinct reproducible deviation patterns characteristic of each interfering species. Deviating δ-values for ratios involving the ^{116}Cd isotope are indicative of inadequate correction for Sn interference and imply that caution should be exercised if using results for other ratios that can be affected, *viz.*, those involving ^{114}Cd and ^{112}Cd isotopes. Negative δ-values for ratios with the ^{108}Cd isotope as denominator, monotonically increasing from ^{110}Cd/ ^{108}Cd to ^{116}Cd/^{108}Cd are caused by MoO^+ interference and only the least affected Cd ratios (δ^{114}Cd/^{110}Cd and δ^{116}Cd/^{111}Cd) should be used. It should be noted that, for almost all birch leaves analyzed in this study, Mo and Sn contamination of the Cd fraction was negligible, providing typical spread for mean normalized δ-values below 0.01‰. This also suggests the absence of measurable mass independent fractionation[6].

tests, using the measurement protocol described above and applied to NIST SRM 3108 solutions spiked with increasing Sn concentrations, shows that the best agreement between corrected ratios and those for unspiked standards was obtained when employing mass bias deduced from the tabulated ^{112}Cd/^{111}Cd ratio. This yielded efficient correction, evident from δ-values for affected ratios after correction in the -0.02‰<δ<0.02‰ range, for the majority of ratios except those involving the ^{116}Cd isotope in solutions with Cd/Sn ≤ 10 (**Table 3**). Assessment of potential natural or artificially-introduced isotopic fractionation of the Sn eluted in the Cd cut would require either a separate measurement session for Sn isotope ratios or obtaining such information by performing MC-ICP-MS measurements in the dynamic mode. Neither of these approaches was tested in the present study, but it should be considered for matrices where the Cd/Sn concentration ratio in separated fractions is below 10.

Spectral interferences caused by MoO^+ affect all Cd isotopes monitored, but to significantly different degrees. For a given Cd/Mo concentration ratio and assuming stable oxide formation in the ICP or MC-ICP-MS interface, the severity of these interferences will depend on the relative abundances of the Mo isotopes causing $Mo^{16}O^+$ interferences and those of the Cd isotopes affected. For example, interference will be significantly more pronounced on the ^{108}Cd (0.89% natural abundance) affected by ^{92}Mo (14.8 %) than for ^{110}Cd (12.6 %) affected by ^{94}Mo (9.2 %), and this in turn will affect the Cd isotope ratios very differently. Apparent deviations in δ-values for different Cd ratios calculated for NIST

Reproducibility

In theory, sample matrix, analyte content and all stages of the measurement procedure might affect method reproducibility. Thus accurate assessment of the overall reproducibility of the method would require replicate preparation, separation and analyses of all samples that might be impractical or even unfeasible for large studies. As a more cost and time efficient approach, typical method reproducibility can be estimated using a set of test samples prepared and analyzed in different analytical sessions. Therefore either just

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the measurement and data evaluation steps for synthetic Cd solutions and Cd chemicals prepared by dissolution, or the entire analytical procedure for solid environmental and geological matrices, was applied to a set of reference and control samples with variable matrix composition and Cd concentrations[38] (**Table 4**). A minimum of two aliquots of each material was prepared and each Cd solution analyzed in duplicate during at least two measurement sessions. For 'zero-matrix' samples (QCS A-C and solid Cd chemicals) with neither extensive sample preparation nor column separation necessary, long-term reproducibility (2σ) is approximately 0.02‰ (**Table 3**) that is slightly better than the range of (0.02-0.1)‰ per *amu* quoted by Wombacher et al. using either external standardization or bracketing standards approaches[6]. This defines the highest level of precision that can be achieved for Cd isotope ratios measured by MC-ICP-MS in solutions given perfect concentration and matrix matching with isotope standards in the absence of spectral interferences.

Reproducibility depreciates to approximately 0.05‰ for the majority of solid environmental matrices, though in some cases it may deteriorate to 0.1‰ (**Table 4**). As a rule, poorer reproducibility was noted for samples with either initially low Cd content or when there are restrictions on the sample intake per preparation (Procedures B-D). Therefore MC-ICP-MS measurements had to be made on undiluted Cd fractions (higher risk for minor matrix effects) with high sensitivity set-up. Apart from higher in-run precision (see Experimental), the stability of instrumental mass bias using the Aridus is also notably inferior to that of the standard introduction system, and memory effects for Cd, Mo and Sn are more pronounced requiring much longer wash-out times. All these factors most probably contribute to poorer reproducibility and the use of Aridus should be avoided when ultimate precision is a major goal, e.g. in certification or inter-laboratory comparison campaigns. Differences in reproducibility between pure standard solutions and samples of biological origin have previously been noted in other stable isotope studies[39] as well.

Accuracy

As to the best of our knowledge no reference materials with certified Cd isotope composition are available, the only means to evaluate method accuracy is to compare δ-values obtained in our study (**Table 3**) with previously published data where such exist. This was possible for one Cd solution (UM-Münster-Cd) and three reference materials (NOD-P-1, NOD-A-1 and NIST 2711) as shown in **Table 4**. The degree of agreement between data found here and previously reported for these materials was rather mixed:

- UM-Münster-Cd δ-values obtained in this study (4.44 ± 0.04) ‰ proved to be in good agreement with those proposed by Cloquet et al.[8] (4.48 ± 0.04) ‰. This is despite differences in the "δ-zero" reference materials used (NIST SRM 3108 and Cd Spex). Our value is also within the range obtained for this standard in an inter-laboratory exercise[20]. It must be noted though that this solution consists of artificially fractionated Cd and that no sample pre-treatment other than dilution was needed;
- Acceptable correspondence was also found for NOD-P-1 δ-values between this study (0.12 ± 0.04) ‰ and literature data (0.13 ± 0.12) ‰[8], which should be considered more than satisfactory given measurement uncertainties;
- Agreement was less impressive between the results for the second Fe-Mn nodule (NOD-A-1), i.e. (0.09 ± 0.03) ‰ *versus* (-0.07 ± 0.12) ‰[8];
- The poorest agreement was between δ-values for NIST 2711 (Montana soil), with found values of (0.80 ± 0.07) ‰ and (0.71 ± 0.11) ‰ deviating from published data, (0.51 ± 0.02) ‰[8].

All the mentioned Cd isotopic data from the literature were obtained using a MC-ICP-MS Micromass® Isoprobe™ and instrumental details can be found elsewhere[8].

The exact reasons for such discrepancies between results obtained in different laboratories is unknown and current difficulties in the assessment of method accuracy call for the preparation of reference materials that have certified Cd isotope composition.

No significant differences were found for $\delta^{114}Cd/^{110}Cd$ in kidney, mushroom, and leaves prepared by procedures D and E (**Table 4**, mean difference <0.03‰) thus assuring that the ashing step does not notably affect the Cd isotope composition for carbon-rich matrices. Agreement is somewhat poorer (0.09‰ difference) for NIST SRM 2711 prepared by procedures C and D, but is still acceptable if allowing for a 0.1‰ estimation of long-term reproducibility. The fact that similar Cd isotope fractionations in favour of heavy isotopes were obtained for in-house moose kidney control sample and for ERM BB186 Pig kidney is also reassuring. As moose diet mainly consists of terrestrial vegetation, the observed similarities between mean $\delta^{114}Cd/^{110}Cd$ values in moose kidney and in birch leaves (**Table 5**) are to be expected.

The isotopically lighter Cd in Lobster Hepatopancreas and fish liver (**Table 4**) falls within the published range for bivalves collected from western Canada, Hawaii, and the US East Coast[9].

Four out of five chemicals and commercial standard solutions tested in this study have Cd isotope compositions indistinguishable from those for NIST SRM 3108 (**Table 3**) with $\delta^{114}Cd/^{110}Cd$ in the range from -0.02‰ to 0.02‰, while Cd in QCS C is enriched in light isotopes. Interestingly, both QCS B and QCS C have a common supplier, the same catalogue number and the only difference is the lot of starting material, Cd nitrate hydrate. An almost 0.6‰ difference in $\delta^{114}Cd/^{110}Cd$ between two standard solutions from the same supplier highlights the importance of having a common 'δ-zero' Cd isotope standard[20] for all research groups studying Cd isotope fractionation.

Cd isotope composition in birch leaves

A summary, of Cd data birch leaves and lichens collected in Sweden and analyzed in the course of this study, is presented in **Table 5**. The first general observation that can be made from the entire set of data is that the Cd in birch leaves has a composition enriched in heavy isotopes (mean $\delta^{114}Cd/^{110}Cd$ value of 0.7‰, range 0.3‰-1.3‰). This is slightly surprising as the preferential accumulation of lighter isotopes in leaves is far more common (Fe[38], Zn[40],Cu[41–43]).

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Table 5 Cd concentrations and $\delta^{114}Cd/^{111}Cd$ in birch leaves and lichens from Sweden

All locations, 2005-2013	Cd, mg kg^{-1}		$\delta^{114}Cd/^{110}Cd$	
	Mean±SD	Min÷Max	Mean±SD	Min÷Max
All samples (n=83)	0.40±0.23	0.07÷1.70	0.70±0.20	0.30÷1.28
Luleå (n=65)	0.36±0.15	0.07÷0.71	0.73±0.19	0.34÷1.28
Rest of Sweden (n=18)	0.59±0.41	0.23÷1.70	0.59±0.17	0.30÷0.99
Luleå, 2013-08-02				
Low branches (H=4 m) (n=2)	0.68±0.07		0.622±0.070	
Medium branches (H=16 m) (n=2)	0.65±0.06		0.454±0.055	
High branches (H=24 m) (n=2)	0.63±0.06		0.430±0.049	
Luleå, 2013-05-19				
Old trees, pooled sample A (n=2)	0.31±0.03		0.810±0.088	
Young trees, pooled sample A (n=2)	0.26±0.02		0.689±0.021	
Luleå, 2013-05-25				
Old trees, pooled sample B (n=2)	0.30±0.03		0.837±0.079	
Young trees, pooled sample B (n=2)	0.35±0.03		0.662±0.043	
Luleå, 2012-2013				
2013-05-19 (n=5)	0.46±0.17		0.61±0.05	
2013-05-25 (n=5)	0.44±0.16		0.66±0.10	
2012-05-27 (n=5)	0.40±0.18		0.59±0.11	
2013-07-17 (n=5)	0.27±0.11		0.81±0.18	
2012-08-05 (n=5)	0.33±0.13		0.73±0.14	
2013-08-31 (n=5)	0.28±0.11		0.81±0.19	
2013-09-15 (n=5)	0.36±0.13		0.84±0.15	
2013-10-05 (n=5)	0.37±0.19		0.87±0.13	
Pooled litter samples (n=3)	0.74±0.29		0.46±0.19	
Pooled lichen samples (n=4)	0.07±0.02		0.09±0.14	

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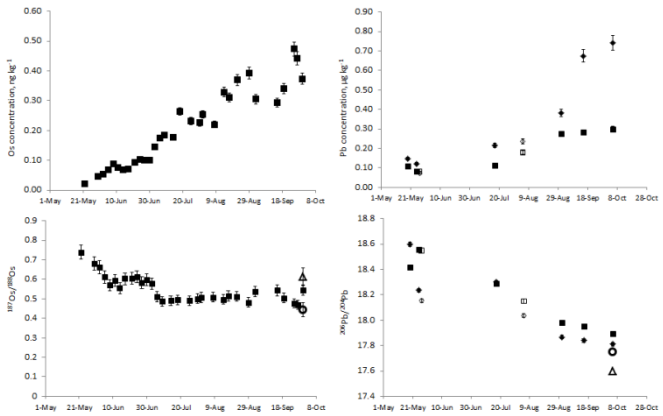

Figure 2 Changes in Os and Pb concentrations and $^{187}Os/^{188}Os$ and $^{206}Pb/^{204}Pb$ isotope ratios in birch leaves with squares and diamonds representing leaves from two different trees, lichens (open circle) and litter (open triangle). Samples for Os isotope ratio measurements were collected during the 2006 growing season. Samples for Pb isotope ratio measurements were collected during the 2012 (open squares and diamonds) and 2013 (filled squares and diamonds) growing seasons.

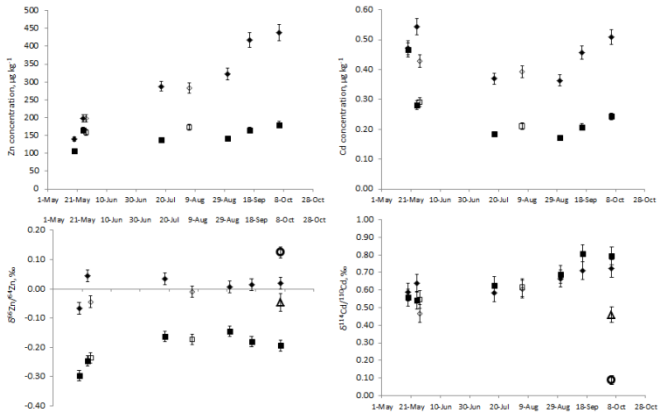

Figure 3 Changes in Zn and Cd concentrations and $\delta^{66}Zn/^{64}Zn$ and $\delta^{114}Cd/^{110}Cd$ in birch leaves with squares and diamonds representing leaves from two different trees, lichens (open circle) and litter (open triangle). Samples were collected during the 2012 (open sq squares and diamonds) and 2013 (filled squares and diamonds) growing seasons.

With reservation for the relatively limited number of sampling locations tested, there are no obvious site-specific differences between mean concentrations and the ranges of Cd isotope compositions observed for 18 sampling sites. The latter span over almost 1250 km along the Swedish coast line, from in the vicinity of the town of Höganäs in the south-west to the neighbourhood of Haparanda in the north-east, as well as one very confined sampling area in the vicinity of Luleå. For the entire data set, there is no significant correlation between Cd concentrations and δ-values (R^2 = 0.08). Birch leaves with the highest Cd concentrations (>1µg kg^{-1}) found in some locations do not exhibit isotope compositions deviating significantly from the mean.

There are no differences in Cd concentrations in leaves growing at different heights (**Table 5**), although the Cd isotope composition tends to become slightly lighter at the top of the crown, with fractionation during diffusion[44] in sap solution being a plausible explanation. As only based on data from a single birch, no far-reaching conclusions can be drawn from these findings.

On two sampling occasions, statistically significant differences in $\delta^{114}Cd/^{110}Cd$ were observed between pooled samples representing lower branches of old trees or all branches of young trees (**Table 5**). As these leaves were sampled at the very beginning of the growing season, it might be that Cd in samples from large trees originates from a pool accumulated in the stem or shoots, while in young trees a higher proportion is derived from the soil solution, and that there are isotopic differences between these pools.

During the first part of the growing season, changes in foliar Cd concentrations strongly resemble the seasonal dynamics of such nutritional elements as P and Cu (R^2>0.9). Namely, the highest concentrations occur at the onset of leaf growth, followed by a decline towards June-July probably due to 'dilution' by organic matter. From late August until autumn, Cd concentrations in leaves increase somewhat (**Table 5**), exhibiting positive correlations with the levels of Ca, Pb, Sb and many other elements during this period. This might be caused either by accumulation through the concurrent supply of element with the flow of nutrients via the root system no longer being counterbalanced by growth dilution, or by surface absorption from aerial sources, or indeed a combination of both. In all five trees sampled to study seasonal effects, there is a clear trend towards heavier isotope composition from May to October. Indeed, $\delta^{114}Cd/^{110}Cd$ correlates with sampling date having R^2>0.8 in all trees. Both Cd concentration and isotope composition reproduce well in leaves sampled in 2012 and 2013 (**Table 5**).

Multi-tracer information

In order to gain a better understanding of observed trends and to find eventual similarities or differences in the seasonal dynamics of isotope composition of other elements, results obtained during this study were complimented by concentration and isotope data for Os, Pb and Zn in leaves, lichens and litter. Obtained as a part of a study on the Os baseline status in the environment[16,17], the Os concentration and isotope ($^{187}Os/^{188}Os$ ratio) data represent a very detailed temporal record of the 2006 growing season using one of the birch trees sampled later on for the current work. Pb and Zn concentrations were available from analyses done to assess Cd concentrations (using preparation procedure A) while corresponding isotope data were obtained using MC-ICP-MS and relevant fractions from the column separations (**Fig. 1**). Seasonal changes in element concentrations and isotope ratios of these four elements in birch leaves are shown in **Fig. 2-3**.

Common features of the seasonal dynamics of Os and Pb (**Fig. 2**) in birch leaves include the following:

- Increases in concentrations from spring to autumn, being particularly pronounced for Os;
- Isotope composition shifts from more to less radiogenic during the growing season;

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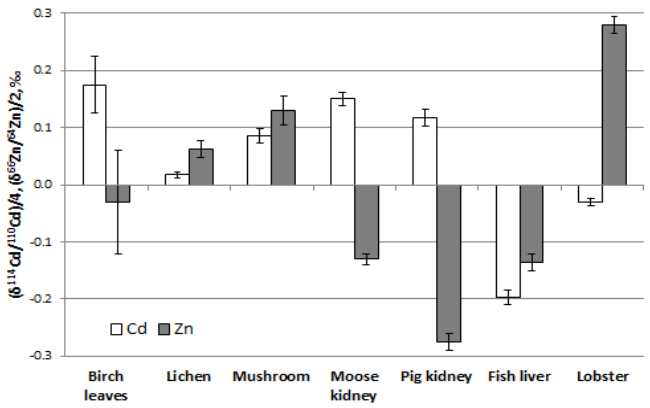

Figure 4 Normalized Cd and Zn isotope fractionation in carbon-rich matrices.

- Similarities in the isotopic composition of foliage and pendulous lichens at the end of growing season;
- The isotopic composition of mushrooms, used here to estimate isotope composition of soil solution available to plants, is significantly more radiogenic than in leaves with mean $^{187}Os/^{188}Os$ ratio of 1.10 ± 0.08 and mean $^{206}Pb/^{204}Pb$ ratio of 19.6 ± 0.2 (not shown in **Fig. 1**);
- The isotopic composition of litter is more radiogenic than of leaves at the end of the growing season.

A very simplistic model explaining these observations can be proposed. Young leaves have isotopic compositions that are a product of element mixing from soil solution (most radiogenic in a system) and accumulated reserves in the tree from the preceding season. Any contribution from a third, aerial, less radiogenic component is low because of the brief exposure period and low surface area of young leaves. As exposure of the growing leaf surface to airborne contaminants continues through the entire season, the contribution of this component to elemental isotopic compositions in foliage increases and finally becomes dominant in the autumn. This is confirmed by the very similar isotope compositions of leaves and lichens – organisms with predominantly aerial supplies of both nutrients and contaminants (**Fig. 2**). Concentrations of both elements in lichens are, by several orders of magnitude, higher than in leaves due to much longer exposure time and much higher surface area of the former.

For litter, a combination of soil-born contamination and losses of the surface layers most affected by aerial input through organic material decay will result in shifts back towards the 'spring' isotopic signatures. Due to significant (more than 10%) differences in isotope ratios for end-members, the 'mixing process' occurring in foliage can be followed through either Os or Pb isotopic data that nowadays can be obtained without any major analytical challenge. However, potential mass dependent fractionation effects occurring during either uptake through the root system or element translocation between different tree compartments will not be seen against this changing radiogenic 'background'.

Birch leaves have mostly negative $\delta^{66}Zn/^{64}Zn$ values (**Fig. 3**) which is in agreement with most previously published observations[40,45,46]. The lightest Zn was observed during the very first few weeks of leaf growth, and accompanied by the lowest measured Zn concentrations. During the rest of season, $\delta^{66}Zn/^{64}Zn$ remains relatively constant. There are reproducible differences in Zn isotope composition between trees from the same area with birch leaves having higher Zn contents exhibiting lower degrees of fractionation. Zn in litter is also light (-0.06‰), falling into the range of isotopic compositions found for birch leaves from the same location, while lichens have heavier Zn than tree leaves (0.12‰). A very similar Zn isotope composition was reported in BCR-CRM 482 Lichen by Viers et al.[40]. Mushrooms have even heavier Zn isotope composition (0.26‰).

The isotopic compositions of Cd in leaves are heavier than those for either mushrooms (0.36‰) or lichens (0.07‰) at all times (**Fig. 3**). Unlike the situation for the uptake of Os and Pb radiogenic isotopes, potential Cd isotope fractionation during incorporation in mushrooms cannot be neglected. Since vascular plants and fungi may have mycorrhizal association in their shared root system, the direction, but not necessarily the extent, of such fractionation should not be different for plants and mushrooms. Hence, neither soil solution nor aerial Cd isotope signatures are 'visible' in foliage, suggesting that significant isotope fractionation occurs during element incorporation in birch leaves. It is highly unlikely that the process of airborne Cd uptake through adsorption of particulate matter is accompanied by changes in isotopic composition. Indeed, if this source plays any notable role in increasing the element concentration during the autumn (**Fig. 3**) this should be manifested by an isotopic shift favouring lighter isotopes. As this is clearly not the case (**Fig. 3**), an aerial source of Cd (and Zn) can be neglected in birch leaves from this location. The lower Cd concentrations in lichens compared to leaves presents additional confirmation of this conclusion (**Table 5**). Therefore, soil solution is likely to be the only major Cd source in foliage and the observed fractionation in favour of heavier isotopes must occur in the organism itself, either during root uptake or element translocation through different tree compartments. Similarly, the amelioration of heavy isotope enrichment observed in litter (**Table 5, Fig. 3**) cannot be solely explained by soil-borne adulteration, as this would require an unrealistically high contamination load. Therefore it seems that heavier isotopes are preferentially leached during the initial stages of litter decomposition. The available experimental data are insufficient for clarification of the mechanisms responsible for an increasing proportion of heavier isotopes being incorporated during growing season (**Fig. 3**). It is possible that this phenomenon has common underlying mechanisms with those causing differences in isotope composition between leaves growing at various heights and between young and old trees (**Table 5**).

Notable differences in Cd (and Zn) isotope composition for leaves collected on the same day from different trees within a very constrained area (**Table 5**) exceed the overall reproducibility of the analytical method and suggests that the extent of fractionation varies between individual birch trees.

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Figure 4 summarizes Cd and Zn isotope fractionation (normalized to one *amu*) in the various carbon-rich matrices analyzed during this study. A detailed elaboration of the observed similarities (e.g. for lichens, mushrooms and fish liver) or differences (e.g. for birch leaves, kidney or lobster) in isotope shifts is outside the scope of this paper. However, in spite of chemical similarities between these two metals, there are clear indications of the existence of element-specific biotic fractionation mechanisms or isotopic differences in element pools available for living organisms. For example, the difference between normalized isotope fractionation in Fe-Mn nodules for Cd (+0.03‰ per *amu*) and Zn (+3.2‰ per *amu*) is almost identical to that in lobster, which is certainly deserves further studies.

Conclusions

The optimized analytical protocol tested in the course of this study allows precise, reproducible and high-throughput measurements of Cd isotope ratio measurements in a wide range of environmental matrices. Different sample preparation approaches were tested and all but those based on conventional MW-assisted digestion were found compatible with the Cd separation procedure. When available sample size is not a limiting factor, an ashing step, as an integral component of sample preparation for carbon-rich matrices, improves material representativeness and allows measurements to be performed on samples with Cd concentrations below 0.1 mg kg^{-1}.

Column separation using the AG-MP-1M ion-exchange resin allows efficient de-contamination from matrix elements and shows consistently high Cd (as well as Pb and Zn) recoveries. However, Sn and Mo – elements causing spectral interferences affecting Cd isotopes – partly co-elute in the analyte fraction. Though usually negligible in carbon-rich matrices, such interferences may severely limit the accuracy of Cd isotope ratio measurements in soils, sediments and sludge. Presentation of isotope data in the form of normalized δ-values (**Table 3**) helps to identify and discard potentially affected ratios. For samples analyzed in the present study the use of an additional column to separate Cd from Sn and Mo[6] was found to be unnecessary.

The use of a desolvating nebulizer extends the concentration range in measurement solution at which reproducible and accurate Cd isotope ratios can still be guaranteed down to 10 µg l^{-1} whilst reducing oxide formation that is useful for the analysis of sample solutions containing Mo. As in-run precision is degraded approximately two-fold while signal stabilization and wash-out times are significantly longer than with the standard configuration of the MC-ICP-MS sample introduction system, the latter is to be recommended for samples containing relatively high analyte concentrations. Though long term reproducibility (2σ for $\delta^{114}Cd/^{110}Cd$) can be as good as 0.02‰ for synthetic Cd solutions or chemicals (Table 4), a (0.05-0.1) ‰ range provides a more realistic assessment of overall reproducibility for the entire analytical procedure as applied to environmental samples. The agreement between $\delta^{114}Cd/^{110}Cd$ found during this study with previously published results varies from very good (for 'UM-Münster-Cd' and NOD-P-1) to less satisfactory (NOD-A-1 and NIST 2711). Accuracy assessment for Cd isotope ratio measurements is hampered by the current absence of matrix-matched materials with certified isotopic information, preferably presented as δ-values against a widely accepted isotope standard, and there is an urgent need for such CRMs to be developed and validated.

Cd in birch leaves fractionated in favour of heavier isotopes, the mean $\delta^{114}Cd/^{110}Cd$ for >80 single-tree and pooled samples being 0.7‰ with a range of (0.3-1.3) ‰. This fractionation most probably occurs during Cd uptake through the root system and element translocation in the birch. There is a shift towards heavier isotope composition in leaves from spring to autumn (**Fig. 3**), an observation confirmed by regular sampling of 5 birch trees from the same location. Even when collected on the same sampling date, foliage displays relatively broad variations in Cd isotope composition between trees growing in close proximity to one another. Moreover, there are indications that Cd fractionation in leaves depends upon growing height and tree age, though these findings are based on very limited experimental data and should be treated with caution. The magnitude of the observed natural variability relative to source signatures should be carefully considered before using Cd isotopic information in birch leaves, or other bio-indicators, for environmental exposure assessment, a task that in many situations can be better accomplished using traditional radiogenic isotope systems.

Quoting Bullen[38], there clearly remains much to be done to understand the causes of transition and post-transition metal, stable-isotope fractionation in living systems, and certainly Cd deserves at least a thorough reconnaissance for a variety of species and field situations. With greater understanding of the observed variations in isotope composition in terms of known processes, future work will gradually shift toward using isotopic signatures to identify as yet unknown or unconstrained processes in plants and other biological systems.

Acknowledgements

ALS Scandinavia AB is gratefully acknowledged for technical support. MetTrans Initial Training Network (funded by the European Union under the Seventh Framework Programme) is acknowledged for financial support. We wish to thank Katerina Rodiouchkina for field assistance and Dieke Sörlin for help with sample preparation.

The authors also wish to express their gratitude to Dr. Frank Wombacher (Institute for Geology and Mineralogy of the University of Cologne, Cologne, Germany) and Prof. Cristophe Cloquet (CRPG – Centre de Recherches Pétrographiques et Géochimiques, Vandœuvre les Nancy, France) for providing us respectively with UM-Münster-Cd standard and NIST SRM 2711 reference material.

The research leading to these results has received funding from the People Programme (Marie Curie Actions) of the European

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Union's Seventh Framework Programme FP7 2007-2013, under the REA grant agreement no 290336.

The views expressed in this *article* are those of the authors and may not necessarily reflect those of the European Union.

Notes

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Table of Contents

Various stages of an analytical method for high-precision cadmium isotope ratio measurements by MC-ICP-MS were critically evaluated and optimized for the processing of carbon-rich environmental samples. The method was used in a pilot study focusing on the assessment of factors affecting Cd isotope composition in birch leaves.

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