Electronic Supplementary Information for

Ranges of B, Cd, Cr, Cu, Fe, Pb, Sr, Tl and Zn concentrations and isotope ratios in environmental matrices from an urban area

Nicola Pallavicini[a,b], Emma Engström[a,b], Douglas C. Baxter[b], Björn Öhlander[a], Johan Ingri[a], Scott Hawley[c], Catherine Hirst[d], Katarina Rodushkina[e] and Ilia Rodushkin[a,b].

[a]*Division of Geosciences and Environmental Engineering, Luleå University of Technology, S-971 87 Luleå, Sweden*

[b]*ALS Laboratory Group, ALS Scandinavia AB, Aurorum 10, S-977 75 Luleå, Sweden*

[c]*Department of Earth Sciences, Durham University, Durham, UK*

[d]*Department of Geosciences, Natural History Museum, Stockholm, Sweden*

[e]*Department of Chemistry, Uppsala University, Uppsala, Sweden*

1.1.1 Chemicals and reagents

All sample preparation was conducted utilizing high purity reagents: de-ionized Milli-Q water (Millipore, Bedford, MA, USA) further purified by sub-boiled distillation in Teflon stills (Savillex, Minnetonka, Minnesota, USA), Nitric acid (HNO3) and hydrochloric acid (HCl), both from Sigma-Aldrich Chemie GmbH (Munich, Germany), acetic acid (CH_3COOH, Merck KGaA, Darmstadt, Germany), hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$, 98.0%, Sigma-Aldrich Chemie GmbH, Munich, Germany), hydrogen peroxide (H_2O_2 ≥30%, Sigma-Aldrich Chemie GmbH, Munich, Germany), ammonium acetate (CH_3COONH_4, Merck KGaA, Darmstadt, Germany), aqua regia (HNO_3 + 3HCl, both acids from Sigma-Aldrich Chemie GmbH, Munich, Germany) and hydrofluoric acid (HF, 48%, Merck Merck KGaA, Darmstadt, Germany). All laboratory ware coming into contact with samples/sample digests was soaked in 0.7 M HNO3 (>24 h at room temperature) and rinsed with MQ water prior to use.

1.1.2 Biological samples

All biological samples i.e. common birch (*Betula pubescens*) leaves, Norway spruce (*Picea abies*) needles and fruit bodies of edible mushrooms (*Boletus edulis, Leccinum scabrum, Leccinum versipelle, Leccinum aurantiacum and Suillus variegatus*) were collected in 2013–2015 from approximately 50 individual locations. Each sample comprised of 0.5–1.5 g dry weight of leaves (10–50 depending on the growth stage) collected from different branches/trees, needles from the last year grown on parts of lower branches and mushrooms collected under sampled trees. Sampling height was limited to roughly 2.5 m from the ground for all leaf and needle samples. Sampling was performed either at the beginning of the growing season (May early June, birch leaves only) or just before senescence (early September). In the majority of locations samples were taken from different trees in spring and autumn. All samples were collected wearing powder-free laboratory gloves into zip lock plastic bags marked with geographic coordinates, sample type and collection date. Sampling locations were chosen utilizing a 1 km^2 sampling grid across a total area of ca. 200 km^2.

For method verification a certified reference materials (CRMs) were included and processed in parallel to "natural samples" (digestion, separation and analysis): ERM BB186 pig kidney (Institute for Reference Materials and Measurements), TORT-1 lobster hepatopancreas and NASS-4 open ocean water (National Research Council of Canada, Ottawa, Canada), NIST SRM 1547 peach leaves (National Institute of Standards and Technology) and NJV 94-5 wood fuel (Swedish University of Agricultural Sciences, Sweden). None of the materials mentioned above has a certified isotopic composition, but they do provide a representative natural range of analytes concentrations and isotope compositions [1].

Moore et al. (2017) have obtained an averaged value for different digests of ERM BB186 of -0.65±1.3 for $\delta^{66/64}Zn$ which is identical within uncertainty to the value found in our study (-0.608±0.105).

1.1.3 Soil samples preparation and digestion

The topsoil samples were dried at 100°C until they attained a constant weight and then 0.5 g was weighed into 50 mL polypropylene vessels before being dissolved in 20 mL of aqua regia. Vessels were loosely capped and placed in a graphite-topped heating block with cavities matching the

vessels' diameter. Digestion was carried out for 1 h at 110ºC under reflux conditions. An aliquot of digest was preserved for element concentrations analysis by double-focusing sector field inductively coupled mass spectrometry (ICP-SFMS) as described in detail by Engström et al. [3]. The solid and liquid phases were then careful separated and the rest of the digest was evaporated to dryness. Two mL of H_2O_2 was then added and evaporated to oxidize remaining organic phases, and the residue was re-dissolved in 2 mL 10 M HCl for separation of individual elements prior to isotopic ratio measurements.

1.1.4 Biological samples preparation and digestion

Mushrooms were mechanically cleaned, to remove external exogenous material, and divided into approximately 1 cm^3 pieces using a ceramic knife on a Teflon plate. Samples were then dried at 50°C, until they attained a constant weight, and homogenized by crushing in plastic bags before being stored in air-tight packed at room temperature.

About 0.5 g of dried material from each sample bag was accurately weighed into a 12 mL Teflon vial before the addition of 5 mL 14 M HNO_3. After the initial oxidation of organic matter subsided, vials were gently agitated and any solid material adhering to the walls was washed down by an additional 1 mL of HNO_3. Vials (up to 40 per batch) were placed into a carousel with numbered slots, which was then loaded into the Teflon-coated UltraCLAVE reaction chamber containing a deionized water–H_2O_2 mixture (10:1 v/v). The chamber was pressurized with compressed argon and a pre-programmed digestion cycle (30 min ramp to 220°C followed by 20 min holding time at that temperature) was initiated. The total processing time, including cooling and subsequent transfer and dilution of sample digests to a final volume of 10 mL into storage polypropylene tubes, was approximately three hours per digestion batch.

In some samples, minor quantities of white precipitates of siliceous material were formed. Rapid dissolution of the precipitate was achieved after addition of 30 mL of 16 M HF and manual agitation for a few minutes. Sets of method blanks and CRMs were prepared with each batch of samples.

As for any of the wide variety of methods used for the preparation of biological matrices for elemental analysis e.g. ashing, hot-block and microwave digestions, and high pressure ashing [4–6], digestion using the UltraCLAVE has its merits and limitations. The former include the complete oxidation of carbonaceous material which ensures undigested organics have a negligible impact on

the subsequent separation procedure, the applicability of the method to all matrixes tested in this study, the ease of sample handling/loading (limited material manipulation and thus lowered risk of contamination), and a relatively high throughput. The major limiting factor is the amount of material that can be digested in a 12 mL vessel (approximately 0.5 g dried material). This can require processing parallel digestions for samples low in some analytes, though this approach was not required in the present work.

Aliquots of the digests were diluted 50-fold with 1.4 M HNO_3, providing a total digestion factor of approximately 1000 v/m, and analyzed by ICP-SFMS using a combination of internal standardization and external calibration [5]. Portions of diluted digests remaining after this analysis (approximately 6 mL) were used for B isotopic ratio measurements either directly or after additional dilution. The rest of the original sample digest was evaporated to dryness in a 25 mL Teflon beaker at 95°C on a ceramic-top hot-plate, followed by dissolution in 4 mL of 9.6 M HCl, in preparation for subsequent purification.

1.1.5 Sequential extraction procedure for soil samples

For the study of operationally defined fractions in soil we used a sequential extraction procedure (SEP). The SEP adopted for the present study is slightly modified version of a scheme developed in the Standards, Measurements and Testing program (SM & T–formerly BCR) of the European Union [7,8]. The original leaching scheme included four solutions (acetic acid, hydroxylamine hydrochloride, hydrogen peroxide and ammonium acetate). This study follows an enhanced scheme previously performed by Tokalioğlu et al. [9]. The modifications consists of: 1) the addition of a distilled water leach at the beginning of the process; 2) the addition of a HF leach at the end of the process ; and 3) the merging of two originally separate phases (hydrogen peroxide and ammonium acetate). Details of the extraction procedure can be found in Table 2 in the main article.

Adding the distilled water extraction (step F1) before the acetic acid dissolution aims to mimic the percolated/saturated of rain or melted snow through soils. This should mobilize elements which are most liable to plant root uptake without major chemical or physical modification, e.g. by phytosiderophores aiding nutrient availability. Sample tubes containing 3 g - 5 g of soil were weighted against empty tubes and 40 mL of distilled (high purity) Milli-Q water was added. Tubes were then placed on an end-over turntable and left for 16 h. After centrifugation (2000 rev min^{-1}

for 4 min), the water supernatant was carefully transferred to empty containers paying special attention not to transfer any solid material.

The second step (F2) preferentially targets elements bound to carbonates. Forty mL of 0.11 M acetic acid was added to the solid residue in the original sample tubes. The tubes were then placed on the end-over turntable for 16h before centrifugation and decanting of the leachate to a new tube.

The next stage (F3) was performed as the previous one, except with the reducing agent hydroxylamine hydrochloride. At this stage, elements bound to Fe-Mn oxides are leached. Such elements are affected by changes in oxic/anoxic conditions [10] and need a reagent which is adequate for the reduction to the ferrous and manganous forms of the respective oxides [11]. Hence the use of hydroxylamine hydrochloride as reducing medium is a suitable choice.

The next phase of the extraction targeted elements bound to organic matter. This has traditionally involved the use of hydrogen peroxide (H_2O_2) as oxidation medium. Tessier et al. [11] claimed that even though other more effective methods for the oxidation of organic matter could be employed, H_2O_2 is the most suitable in terms of specificity as the use of other oxidizers such as combinations of nitric, hydrochloric and perchloric acids could result in the liberation of some of the silicate-bound metals. The oxidation stage (F4) in the present study consisted of the following procedure: 10 mL of H_2O_2 was carefully added in small aliquots to the soil residue remaining after the F3 step to avoid violent oxidation reactions. The digest was left at room temperature for about 1 h with occasional manual shaking. The solution was subsequently heated at 85°C for 1 h. At this stage, the volume of the liquid phase was reduced to a few millilitres so a second aliquot of 10 mL of hydrogen peroxide was added to the residue and the extraction procedure was repeated. The solution was then heated to near dryness on a hot block and 50 mL of 1 M ammonium acetate solution was added to the residue, followed by 16 h extraction on the end-over turntable, centrifugation and separation of leachate.

The final stages (F5 and F6) release elements from the most refractory mineral phases. In brief, 20 mL of aqua regia (15 mL HCl + 5 mL HNO_3) was added to the residue after F4 step and left to react overnight at room temperature followed by treatment on a hot block at 110ºC for 1 h under reflux conditions. The acid digest was decanted to a new tube, 20 mL of Milli-Q water were added to the residue, the content was thoroughly mixed and the tubes were centrifuged for 15 minutes at

1000 rpm. This Milli-Q solution was combined with the aqua regia digest. Traditionally the SEP schemes would have stopped at this stage. However, a substantial amount of undigested residue remains after the latter treatment due to the insolubility of many primary silicate minerals in any of aforementioned treatments. This is likely to cause incomplete analyte recoveries in silicate rocks. Therefore, 10 mL of HF was added to the washed residue after F5 step and left on the end-over rotator/shaker overnight. The contents were diluted to 40 mL with Milli-Q water, centrifuged and the supernatant decanted.

A set of reference materials and two procedural blanks were prepared in parallel with the soil samples for quality control purposes.

1.1.6 Separation

Element concentrations in all fractions were determined by ICP-SFMS using small aliquots of extracts while the rest of each leachate was evaporated to dryness for subsequent matrix separation. Owing to the significantly lower concentrations of many analytes in soil samples from the suburbs the first (F1+F2+F3) and the last (F4+F5+F6) three SEP fractions from each layer were pooled before evaporation in a number of cases. Prior to isotopic ratio measurements, the analytes of interest and matrix interfering elements have to be chromatographically separated by ion-exchange purification procedures. The chromatographically procedure used in the present study for the isolation of all elements of interest, except for Cr, has been developed, optimized and tested on real life samples in our previous work [12] and can be summarized as follows.

Sampled are loaded in in 4 mL of 9.6 M HCl onto AG MP-1M (macroporous, 100–200 dry mesh size, 75–150 mm wet bead size, Bio-Rad Laboratories AB, Solna, Sweden) resin-containing columns. Matrix elements are then washed through the column utilizing the same acid. Copper, Fe, Zn, Cd + Tl and Hg are quantitatively eluted from the resin using HCl loads of decreasing molarities followed by a mixture of 6 M HNO_3 containing traces of HF. In contrast to matrix separation in geological/industrial materials, there is no risk of overloading the resin capacity with any of the analytes and therefore the entire digest volume can be used. Sample loading in more concentrated HCl allows separation of Cu, Fe and Zn using the same column, while neither Ag nor Pb is efficiently retained by the resin. The sample load and matrix wash fractions (collected into a 25 mL Teflon beaker) contained >99.5% of initial Sr and >85% of initial Pb. After evaporation and

redissolution in 4 mL of 7 M HNO_3, Sr and Pb were separated using Sr-specific columns (Eichrom Technologies, IL, USA), by selective elution with 0.05 M HNO_3 and 0.1 M ethylenediaminetetraacetic acid (EDTA), respectively. The sample load and matrix wash fractions from this column contain >95% of the original Ag which can be purified by loading the eluent in 4 mL of 2 M HCl onto AG MP-1M resin-containing columns and eluting with 14 M HNO_3 [13].
All columns can be re-used several times, although the efficiency of matrix separation gradually deteriorates after 5–6 cycles with the matrix/Cu and Zn/Cd cut-offs affected the most. It should be noted that approximately 0.1% of the initial Sr and 0.2–0.4% of the initial Pb remain on Sr-specific columns which may affect subsequent separations for samples with much lower analyte concentrations or grossly different isotopic compositions.
All separated analyte fractions, except those for Sr and Hg, were evaporated to dryness and dissolved in 2–10 mL of 0.3 M HNO_3. (An aliquot of 14 M HNO_3 was pipetted directly onto the solid residue as a first step, allowed to react for 15–25 min, and then diluted appropriately by addition of MQ water.) 0.1 mL aliquots of the separates were diluted 50-fold with 1.4 M HNO_3 and analyzed by ICP-SFMS (same approach as for sample digests). This provides: (I) information on analyte contents needed to prepare concentration e.g. acid strength-matched solutions for isotopic ratio measurements; (II) direct assessment of analyte recovery; (III) control over separation efficiency from matrix elements; and (IV) a test for potentially spectrally interfering elements either from the sample matrix or from handling contamination. Cr purification was performed using Dowex AG 1-X8 Resin (100–200 dry mesh size, 106–180 μm wet bead size, Bio-Rad Laboratories AB). For the details of the Cr separation have are reported in detail by Pontér et al. [14]. Analyte recovery and the efficiency of matrix separation were tested by analysis of load solutions and purified fractions by ICP-SFMS.

The potential of artificially introduced fractionation during separation/evaporation and analysis stages was tested by processing a mixture of ‘δ-zero’ standards with concentrations typical for birch leaves. Acceptance criteria were recoveries above 90%, except for Pb where lower recoveries can be tolerated [12], and concentration ratio(s) of interfering elements to analyte below pre-defined, experimentally determined values ensuring manageable levels of spectral interferences and matrix effects. On the rare occasions when separation failed to meet these criteria, solutions were rejected and no further processing took place. Successfully purified fractions were evaporated to dryness in 40 mL Teflon beakers on a hot plate at 100°C. An aliquot of 14 M HNO_3 was pipetted directly onto

the residue, left to react for 10-15 min and then diluted to the desired concentration for isotopic ratio measurements.
AG MP-1M ion-exchange resin was used as supplied.

1.1.7 Element concentration and isotopic analyses

Element concentrations were determined by double-focusing sector field inductively coupled mass spectrometry (ICP-SFMS) on an ELEMENT XR, (Thermo Scientific, Bremen, Germany) with instrumental set-up, isotope selection and other relevant information as previously reported [5].
Isotopic ratios in purified fractions were measured on a NEPTUNE PLUS (Thermo Scientific) MC-ICP-MS or ICP-SFMS (the latter being used for B and Pb in samples with low analyte concentrations) using a combination of internal standardization and bracketing standards for instrumental mass bias correction (Table 1 in the main article). All isotopic compositions are reported in standard delta (δ) notation, except for Pb and Sr that are reported as ratios. The following reference materials were used as 'δ-zero' reference standards: NIST SRM 3108 Cd solution Lot 130116, NBS SRM 979 Cr standard, NIST SRM 976 Cu standard solution, NIST SRM 951a boric acid, NIST SRM 981 common Pb, and NIST SRM 987 (NBS-987) Sr carbonate (all from the National Institute of Standards and Technology, Gaithersburg, MD, USA); IRMM 3702 Zn solution and IRMM-014 Fe metal (both from the Institute for Reference Materials and Measurements, Geel, Belgium). For Tl isotopic analyses, commercial standards (1000 mg L^{-1} mono-elemental solutions supplied by Ultra Scientific, North Kingstown, RI, USA; Lot L00709) were used as 'δ-zero' standards. All sample manipulations were performed in clean laboratory areas (Class 10000) by personnel wearing clean room gear and following all general precautions to reduce contaminations [15].

All solutions were analyzed in duplicate. Signal intensities were transferred to commercially-available spreadsheet software for further off-line calculations, including blank, isobaric interference(s) as well as instrumental mass bias corrections. The latter was corrected by the revised exponential correction model by Baxter et al. [16] using the internal standard (for all isotopic systems but B) and the corrected ratios or δ-values were calculated against bracketing standard solutions. Three samples were analyzed between two standards, together forming a block (block: standard 1 – sample 1 – sample 2 – sample 3 – standard 2). The mean value of the two consequent

measurements of the sample ratio was calculated against ratios for standards in each block. Assuming a linear change in mass bias, ratios for samples 1 and 3 were calculated relative to those for standards 1 and 3, respectively, while sample 2 was calculated against the mean ratio for both standards. Results from the two measurements were used to calculate mean ratios or δ-values and in-run repeatability for each sample.

References

1 I. Rodushkin, N. Pallavicini, E. Engström, D. Sörlin, B. Öhlander, J. Ingri and D. C. Baxter, *J. Anal. At. Spectrom.*, 2016, **31**.

2 R. E. T. Moore, F. Larner, B. J. Coles and M. Rehkämper, *Anal. Bioanal. Chem.*, 2017, **409**, 2941–2950.

3 E. Engström, A. Stenberg, D. C. Baxter, D. Malinovsky, I. Makinen, S. Ponni and I. Rodushkin, *J. Anal. At. Spectrom.*, 2004, **19**, 858–866.

4 I. Rodushkin, T. Bergman, G. Douglas, E. Engström, D. Sörlin and D. C. Baxter, *Anal. Chim. Acta*, 2007, **583**, 310–318.

5 E. Engström, A. Stenberg, S. Senioukh, R. Edelbro, D. C. Baxter and I. Rodushkin, *Anal. Chim. Acta*, 2004, **521**, 123–135.

6 I. Rodushkin, T. Ruth and Å. Huhtasaari, *Anal. Chim. Acta*, 1999, **378**, 191–200.

7 P. Quevauviller, A. Ure, H. Muntau and B. Griepink, *Int. J. Environ. Anal. Chem.*, 1993, **51**, 135–151.

8 P. Quevauviller, G. Rauret, J. F. López-Sánchez, R. Rubio, A. Ure and H. Muntau, *Sci. Total Environ.*, 1997, **205**, 223–234.

9 Ş. ̧Tokalioğlu, Ş. ̧Kartal and G. Birol, *Turkish J. Chem.*, 2003, **27**, 333–346.

10 A. J. Zimmerman and D. C. Weindorf, *Int. J. Anal. Chem.*, 2010, **2010**, 1–7.

11 A. Tessier, P. G. C. Campbell and M. Bisson, *Anal. Chem.*, 1979, **51**, 844–851.

12 I. Rodushkin, N. Pallavicini, E. Engström, D. Sörlin, B. Öhlander, J. Ingri and D. C. Baxter, *J. Anal. At. Spectrom.*, 2016, **31**, 220–233.

13 N. Pallavicini, E. Engström, D. C. Baxter, B. Öhlander, J. Ingri and I. Rodushkin, *J. Anal. At. Spectrom.*, 2014, **29**, 1570–1584.

14 S. Pontér, N. Pallavicini, E. Engström, D. C. Baxter and I. Rodushkin, *J. Anal. At.*

Spectrom., 2016.

15 I. Rodushkin, E. Engström and D. C. Baxter, *Anal. Bioanal. Chem.*, 2010, **396**, 365–377.

16 D. C. Baxter, I. Rodushkin, E. Engström and D. Malinovsky, *J. Anal. At. Spectrom.*, 2006, **21**, 427–430.