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Improving sample representativeness in environmental studies: a major component for the uncertainty budget

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Abstract For environmental quality assessment, INAA has been applied for determining chemical elements in small (200 mg) and large (200 g) samples of leaves from 200 trees. By applying the Ingamells' constant, the expected percent standard deviation was estimated in 0.9–2.2% for 200 mg samples. Otherwise, for composite samples (200 g), expected standard deviation varied from 0.5 to 10% in spite of analytical uncertainties ranging from 2 to 30%. Results thereby suggested the expression of the degree of representativeness as a source of uncertainty, contributing for increasing of the reliability of environmental studies mainly in the case of composite samples.

Keywords LS-INAA · Sampling error · Ingamells' constant · Dense Ombrophilous Forest

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Introduction

The size of the test portion in chemical analysis is usually quite small, varying from sub-milligram amounts (e.g. in laser-ablation ICP) to a few grams (e.g. in XRF). As such, very high demands are set to assure representativeness of these test portions for the sample collected and/or the population studied, in which composite sampling is usually employed. Inhomogeneity is one of the basic causes of the sampling error. Macroscopic inhomogeneities can be simply observed, e.g. with samples composed of clearly different materials, or of different particle sizes. Microscopic inhomogeneities also exist, and are much more difficult to account for. This is e.g. the case with trace substances, like trace elements with mass fractions in the mg kg^{-1} to μg kg^{-1} ranges. In addition, inhomogeneities may occur due to physical phenomena with the material collected, like segregation, grouping and sometimes microbiological activities. The sampling error is seldom properly assessed, simply because it would imply costly homogenization studies involving at least 10 replicates of a test portion [1–3]. Consequently, the percent standard deviation of mass fraction of chemical elements is often underestimated in many environmental studies.

As a complete alternative, the direct analysis of large samples has been proposed, taking advantage of the analytical characteristics of INAA [4–6]. Large sample (LS) INAA also allows the direct assessment of the sampling error since the large sample result can be compared with the result of conventional analysis on a small test portion of the same population studied [7]. The large sample analysis technique even allows for determination and identification of local inhomogeneities [8, 9]. However, for almost all environmental studies, the sampling of a huge amount of material can be unreasonable mainly in natural protected

areas, seldom resulting in a composite sampling design. As such, the minimum sample mass to attain a predefined percent standard deviation cannot be easily verified.

For that reason, the degree of representativeness may be considered as a source of uncertainty. The basic problem, however, is the estimation of the minimum sample mass at which the coefficient of variation of a chemical element mass fraction is—with a certain degree of confidence—below a predefined level. This has been elaborated through the development of empirical relations, the most well known are the sampling constants by Ingamells [1, 2] and by Wallace and Kratochvil [3]. The sampling constant, K_s, is firstly estimated for each element on basis of the standard deviation of analysis of replicates of a given sample mass. Next, the square root of this constant is numerically equal to the expected percent standard deviation for the obtained results in sub-samples of 1 g for a method free from analytical errors [1]. This sampling constant can be also used to estimate the minimum sample mass for attaining a given acceptable (minimum) variation, e.g. 1% due to the subsampling.

In this work, the degree of representativeness of individual and composite samples of the leaf compartment from an Atlantic Forest ecosystem was studied applying the Ingamells' sampling constant [1, 2]. The test portions were analyzed by instrumental neutron activation analysis (INAA) aiming at the evaluation of the expected percent standard deviation as a source of uncertainty for leaf analysis of one tree (ten test portions), one species (ten trees) and composite samples. In the last case, samples of approximately 200 g were analyzed by LS INAA to facilitate the evaluation of the degree of representativeness for species population.

Experimental

Sampling for one tree/one species

Leaves (500 g) of a *Marlierea tomentosa* tree were used to assess the representativeness of 200 mg samples, which is the mass routinely used for normal INAA. Details of experimental design are described elsewhere [10]. The most abundant tree species from a long-term plot of 0.1 km^2 in the Parque Estadual Carlos Botelho (PECB), São Paulo State, Brail, are presented in Table 1. This conservation unit has about 380 km^2 of Atlantic Forest. Summarily, for each species, leaves (500 g) of ten trees were sampled from middle- and lower-crown in March 2003, January 2004 and July 2004. Samples were washed with tap water, oven-dried at 60 °C until constant weight and milled in titanium mill to reduce particle size.

Sampling for composite samples

In earlier work [11] it has been demonstrated that, at the 95% confidence level, the seasons have no influence on the element mass fractions in the compartments studied in

Table 1 Most abundant tree species for leaf sampling in the Parque Estadual Carlos Botelho (PECB)

Family	Species	*N*	*n*
Cyatheaceae	*Alsophila sternbergii* (Pohl) Conant.	342	10
Rubiaceae	*Bathysa australis* K. Schum.	210	10
Myrtaceae	*Calycorectes australis* D. Legrand	108	7
Sapotaceae	*Chrysophyllum innornatum* Mart.	83	9
Sapotaceae	*Chrysophyllum viride* Mart. & Eichler ex Miq.	104	10
Myrtaceae	*Eugenia cuprea* (O. Berg) Nied.	143	8
Arecaceae	*Euterpe edulis* Mart.	1761	19
Myrtaceae	*Eugenia mosenii* (Kausel) Sobral	117	9
Myrtaceae	*Eugenia melanogyna* (D. Legrand) Sobral	124	10
Lauraceae	*Endlicheria paniculata* (Spreng.) J. F. Macbr.	103	11
Myrtaceae	*Gomidesia flagellaris* D. Legrand	122	10
Clusiaceae	*Garcinia gardneriana* (Planch. & Triana) D. Zappi	259	10
Nyctaginaceae	*Guapira opposita* (Vell.) Reitz	333	10
Phyllantaceae	*Hyeronima alchorneoides* Allemão	126	10
Myrtaceae	*Marlierea suaveolens* Cambess.	126	10
Myrtaceae	*Marlierea tomentosa* Cambess.	104	9
Myrtaceae	*Neomitranthes glomerata* (D. Legrand) D. Legrand	99	10
Rubiaceae	*Rudgea jasminoides* (Cham.) Müll. Arg.	137	10
Olacaceae	*Tetrastylidium grandifolium* (Baill.) Sleumer	216	10
Myristicaceae	*Virola bicuhyba* (Schott ex Spreng.) Warb.	156	9

N total number of trees in the long-term plot, *n* number of sampled trees

the PECB conservation unit. As such, sampling of the leaf material for the large sample analysis took place in November 2004. Leaves from 10 trees of each species were combined into composite samples for analysis by LS-INAA. All samples were dried at room temperature and humidity (24 °C; 60%). After packing into plastic bags, samples were gamma-ray-sterilized (dose: 30 kGray) by Companhia Brasileira de Esterilização, Jarinú, Brazil, to avoid microbiological degradation and proliferation. The Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA), Ministério do Meio Ambiente, Brazil, has authorized the transfer of the samples to The Netherlands for chemical analysis by LS INAA.

Normal instrumental neutron activation analysis

INAA performed at the Radioisotopes Laboratory (LRi) is based on the k_0 standardization [12] and utilizes the Quantu software [13] for determining chemical elements in diverse kind of material. The analysis steps are summarized below:

- Sample weighting in polyethylene vials specific for neutron irradiation. Test portions of approximately 200 mg. Independent portions of 1 g were separated for moisture determination. Typical moisture content was 5%.
- Irradiation of the small samples and neutron flux monitors for 8 h in the nuclear research reactor IEA-R1, Instituto de Pesquisas Energéticas e Nucleares (IPEN). For neutron flux monitoring, Ni–Cr alloy of known mass fractions of monitor chemical elements was employed [14]. Test portions of 10 mg were sandwiched between the sample vials. Typical thermal neutron flux was 10^{13} cm^{-2} s^{-1}.
- Measurement of the induced radioactivity was carried out at LRi with Ge detectors (rel. eff. 45 and 50%) after 4, 6, 10 and 20 days decay with respective counting times of 1, 2 and 10 h. The induced radioactivity of the neutron flux monitors was measured for 5 min after 10 and 15 days decay time.
- Convolution of the gamma-ray spectra and calculation of chemical element mass fractions. The uncertainty of measurement included contributions of weighting, neutron flux variation, counting statistics, gamma-ray self-attenuation correction and k_0 standardization [13].

Large sample instrumental neutron activation analysis

At the Reactor Institute Delft (RID) of the Delft University of Technology, the dried material was transferred into polyethylene bottles for irradiation in the LS INAA facilities. Details of this procedure can be found elsewhere [4, 5]. The average mass of the leaf samples was 200 g, i.e. about 1,000 times higher than the usual test portion (200 mg) in normal INAA. Separate test portions of 50 g were used to determine the moisture content through freeze-drying until constant weight (48 h). Typically, the moisture content was 10%. The LS-INAA procedure consists of the following steps:

- Measurement of the natural radioactivity of each large sample (1 h).
- Measurement of the transmission, through each sample, of the gamma-rays emitted by an external ^{152}Eu + ^{154}Eu source (5 min).
- Calculation of the effective gamma-ray mass attenuation coefficients of each large sample [5].
- Irradiation of the samples and neutron flux monitors in the HOR research nuclear reactor (40 h). Neutron flux monitors were made from 99.99% high pure Zn foil to be inserted in the carbon-carbon composite flux-monitor holder of the LS-INAA irradiation container. Typical thermal neutron flux was 3×10^8 m^{-2} s^{-1}.
- Measurement of the induced radioactivity in the sample and flux monitors. The LS-INAA counting facility consists of a gamma-ray spectrometer with horizontal Ge detector (rel. eff. 96%). Samples are located at 20 cm distance from the detector end cap and rotated during counting. Measurements of the induced radioactivity in the large sample took place after 0, 7 and 20 days decay with respective counting times of 1, 2 and 10 h. The induced radioactivity of neutron flux monitors was measured by 15 min using a gamma-ray spectrometer with a well-type Ge detector.
- Calculation of the neutron diffusion length and neutron diffusion coefficient [5].
- Calculation of the correction factors for gamma-ray and neutron self-attenuation. In addition, an empirical correction was applied to account for the neutron attenuation in the void fraction in the bottles.
- Analysis of the gamma-ray spectrum of the induced radioactivity; application of all correction factors and interpretation of the peak areas towards element mass fractions using the calibration constants from the k_0 method [15].

Ingamells' sampling constant

The sampling constant K_s is estimated on basis of the reproducibility standard deviation of analyzed subsamples of a given mass [1, 2]. If the analytical uncertainty is small (less than 1/3 of the assumed subsampling error) and x_1, x_2,…, x_i,…, x_m are the results of M measurements in the subsamples of weight w (g), K_s is given by Ingamells [1, 2]:

$$\hat{K}_s = \hat{R}^2 w = \frac{10^4 w \sum_{i=1}^{M} (x_i - \bar{x})^2}{(M-1)\bar{x}^2} \quad (1)$$

in which $\bar{x}$ is the mean element concentration of M determinations and $\hat{R}$, the expected percent standard deviation (%). The percent standard deviation for the same element of sample in a further subsample of mass w_F is estimated by [1, 2]

$$\hat{R}_F = \sqrt{\frac{\hat{K}_s}{w_F}} \quad (2)$$

Calculating $\hat{R}_F$ allows combining this estimate of "uncertainty of sampling" with the uncertainty of measurement. This is permitted for K_s derived from a sufficiently large number of previous determinations, in which data are normally distributed (referring to subsample w_F). Generally, this is already possible if K_s is based on the ten or more measurements, except in the case of fractioning and heterogeneity due to the presence of diverse materials of grossly different composition [1, 2].

The sampling constants were estimated for assessing the representativeness of ten small individual leaf subsamples analyzed by normal INAA. For the evaluation of the degree of representativeness of the composite samples analyzed by LS INAA, results from 200 trees collected in three periods were used to estimate the expected percent standard deviation and compared to the analytical uncertainties aimed at assuring a minimum SE.

Results and discussion

For INAA, the contribution of contamination during the particle size reduction was not taken into account since titanium grinding instruments have been used, and a possible titanium contamination would not interfere on the determinations of chemical elements in biological material. The quality of analytical procedure was evaluated by analysis of the biological certified reference materials IAEA 336 Lichen, IAEA V-10 Hay Powder and INCT-TL-1 Tea Leaves, produced by International Atomic Energy Agency and Institute of Nuclear Chemistry and Technology, respectively [11].

One tree representativeness

Table 2 shows the estimated values of K_s for guaranteeing representativeness of one leaf sample analyzed by normal INAA. The mass needed to assure a sampling error of 1% for Co was estimated at 170 mg, while the averaged mass was 195 mg. There was considerable contribution of sampling error for the other elements observed in the samples, considering that the uncertainty of measurement is smaller than the sampling error. If compared to earlier published values for K_s^2, a discrepancy is observed for Fe and K. This might be attributed to the relatively high concentration of these elements since the sample matrix was of geological origin, in which mass fractions can be much higher than in leaves. Moreover, particularly for Fe, the mass fractions can be affected by leaf surface contamination with soil particles [16]. The $K_s^{0.5}$ values obtained for Na and Rb were considered in very good agreement with those from the literature. It has been demonstrated by the expected standard deviation ($\hat{R}_F$) that the degree of representativeness reached contributions of up to 2% for Co. It would be expected that the $\hat{R}_F$ values for analyzing of 200 mg test portions from Table 2 should be combined to combined standard uncertainty of measurement to reflect a realistic indication of the range of variation of the results, obtained by normal INAA.

One species representativeness

As the main problem in biomonitoring studies, the analysis of only one tree/species could not be related to the uptake of all chemical elements in the ecosystem to be evaluated in terms of environmental quality [17]. For that reason, it is common to analyze so many species/trees is possible. However, the results of chemical element mass fractions obtained by normal INAA given in Table 3 are very undesirable since local variances have been determined as very high throughout the analysis of ten trees belonging to the same species. The coefficient of variation ranged from 12 to 137% depending on the chemical element and the treespecies. As it can be expected, the K_s values (Table 4), that is, the minimum sample mass to be analyzed for assuring sampling error equal to 1%, for the analysis of ten trees of one species were much higher (minimum: 43 g for determining Fe in *Eugenia cuprea* population; maximum: 16.6 kg for Co determination in *Neomitranthes glomerata* population) compare to the values obtained for 200 mg samples (Table 2). This may be attributed to the larger size of the group of individual samples per species compared to all trees from the same anterior species. As a consequence, $K_s^{0.5}$ values were quite higher due to diverse sources of error involving in the analysis of composite samples by normal INAA. Reducing particle size might solve this problem, although the mass of the samples would be quite high for normal sample preparation procedures [18].

Composite samples

The immediate answer for evaluating plant population in terms of chemical composition would be the analysis of a huge amount of material as possible. It was suggested by the Ingamell' constant, in which some grams of material could

Table 2 Chemical element mass fraction (mg kg^{-1}) in *Marlierea tomentosa* leaves

Subsample	Br	Co	Cs	Fe	K	Na	Rb	Sr
1	8.40	0.497	0.163	74.7	8663	336	28.4	62.4
2	8.38	0.506	0.156	74.8	8385	332	28.1	62.9
3	8.66	0.505	0.166	73.9	8535	343	28.4	63.7
4	8.52	0.495	0.161	73.0	8513	336	28.1	61.0
5	8.57	0.502	0.161	74.9	8673	338	28.4	59.9
6	8.57	0.505	0.167	74.2	8737	342	29.5	64.0
7	8.42	0.496	0.159	76.3	8545	340	28.7	62.1
8	8.69	0.507	0.163	74.0	8876	347	28.7	62.7
9	8.48	0.500	0.163	73.7	8588	341	28.5	62.8
10	8.46	0.504	0.162	78.7	8588	335	28.2	61.8
Mean	8.52	0.502	0.162	74.8	8610	339	28.5	62.3
Uncertainty%	2.9	1.8	3.7	2.2	1.7	2.2	2.6	3.1
K_s	0.31	0.17	0.70	0.92	0.48	0.31	0.40	0.73
$K_s^{0.5}$ (this work)	0.56	0.42	0.84	0.96	0.69	0.56	0.64	0.85
$K_s^{0.5}$ (Ingamells & Switzer) [2]	–	–	–	0.07	0.3	1	1.7	–
$\hat{R}_F$(200 mg)	1.25	0.93	1.88	2.15	1.55	1.25	1.42	1.91

$K_s^{0.5}$ is the expected standard deviation (1 g test portions). $\hat{R}_F$ is the expected standard deviation for the analysis of 200 mg test portions

Table 3 Mass fractions of chemical elements (mg kg^{-1}) and the respective local variances in percentage (CV%) determined by conventional INAA

INAA	Br		Ca[a]		Co		Fe		K[a]		Na[a]		Rb		Sc	
Sp	Mea	CV%	Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%
Aste	24.8	31	5.53	28	0.32	38	279	27	6.07	29	3.22	54	32.1	29	0.445	63
Baus	6.40	35	6.74	31	1.27	116	159	24	22.5	14	1.93	16	38.6	40	0.040	17
Caus	3.96	137	12.2	47	0.14	47	67	19	18.1	23	1.07	47	55.8	27	0.012	29
Cvir	12.7	23	7.67	14	0.10	53	78	34	13.6	30	0.26	36	37.2	30	0.014	64
Ecup	17.8	23	10.5	16	0.11	30	126	26	9.55	18	0.54	44	28.6	67	0.030	30
Eedu	5.61	15	4.52	16	0.04	60	98	9	11.4	17	1.56	23	25.6	23	0.018	22
Emii	16.8	36	6.79	12	0.05	46	74	14	17.4	42	3.13	57	47.6	57	0.037	70
Emna	46.1	49	13.0	32	0.06	47	99	55	14.4	31	3.68	60	43.8	36	0.181	76
Gfla	14.8	23	7.08	22	0.13	32	97	21	10.5	21	0.95	23	28.9	26	0.020	26
Ggar	5.66	39	8.25	27	2.82	66	59	21	8.59	35	0.87	29	28.8	58	0.012	30
Gopp	30.8	51	10.6	30	0.07	45	101	27	25.7	15	6.58	26	76.8	21	0.020	48
Halc	8.07	29	7.32	23	0.44	55	95	20	11.7	19	0.78	43	27.1	42	0.015	27
Msua	5.80	14	6.61	24	0.17	30	77	17	6.70	27	1.62	39	13.9	35	0.014	26
Mtom	7.12	28	5.92	29	0.18	10	83	33	9.36	28	1.83	46	33.3	62	0.016	38
Nglo	3.11	44	12.7	24	0.38	206	61	25	11.8	39	0.35	27	40.3	32	0.010	31
Rjas	36.7	30	10.4	29	0.05	26	86	15	13.8	21	2.01	31	42.7	39	0.067	47
Tgra	16.5	70	3.83	34	0.03	49	106	43	10.3	51	1.54	80	36.7	35	0.021	75
Vbic	3.12	39	6.17	33	0.10	67	84	20	9.19	12	0.43	52	25.8	24	0.013	22

Aste *Alsophilla sternbergii*, Baus *Bathysa australis*, Caus *Calycorectes australis*, Cvir *Chrysophyllum viride*, Ecup *Eugenia cuprea*, Eedu *Euterpe edulis*, Emii *Eugenia mosenii*, Emna *Eugenia melanogyna*, Epan *Endlicheria paniculata*, Gfla *Gomidesia flagellaris*, Ggar *Garcinia gardneriana*, Gopp *Guapira opposita*, Halc *Hyeronima alchorneoides*, Msua *Marlierea suaveolens*, Mtom *Marlierea tomentosa*, Nglo *Neomitranthes glomerata*, Rjas *Rudgea jasminoides*, Tgla *Tetrastylidium grandifolium*, Vbic *Virola bicuhyba*

[a] Values in g kg^{-1}

guarantee SE lower than 1% (Table 3). By applying LS-INAA to composite samples of about 200 g, chemical element mass fractions of plant species were determined (Table 5), thereby becoming possible to estimate chemical element composition for plant population. According to the K_s results from Table 4, it is clear that LS-INAA has

Table 4 Estimated sampling constants K_s (g) for chemical element determination in leaves considering a sampling error equal to 1%

		Br	Ca	Co	Fe	K	Na	Rb	Sc
Aste	K_s	3.39E02	3.71E02	6.22E02	4.13E02	7.56E01	7.35E02	5.56E02	1.51E03
	$R_{LS\text{-}INAA}$	1.50E00	1.57E00	2.04E00	1.66E00	7.10E−01	2.21E00	1.92E00	3.17E00
Baus	K_s	6.93E02	1.14E02	1.26E03	6.54E02	2.83E02	2.64E02	4.40E02	1.05E03
	$R_{LS\text{-}INAA}$	2.15E00	8.71E−01	2.90E00	2.09E00	1.37E00	1.33E00	1.71E00	2.64E00
Caus	K_s	1.56E03	3.94E02	3.77E02	2.44E02	1.03E02	4.86E02	1.32E02	6.60E02
	$R_{LS\text{-}INAA}$	3.22E00	1.62E00	1.59E00	1.28E00	8.29E−01	1.80E00	9.37E−01	2.10E00
Cvir	K_s	2.36E02	1.23E02	6.73E02	1.73E03	1.37E02	2.79E02	2.08E02	3.41E02
	$R_{LS\text{-}INAA}$	1.25E00	9.05E−01	2.12E00	3.40E00	9.57E−01	1.36E00	1.18E00	1.51E00
Ecup	K_s	3.33E02	8.01E01	6.80E02	4.27E01	2.32E02	1.22E03	4.42E02	1.23E02
	$R_{LS\text{-}INAA}$	1.49E00	7.31E−01	2.13E00	5.33E−01	1.24E00	2.85E00	1.72E00	9.07E−01
Eedu	K_s	1.99E02	2.53E02	1.16E04	9.16E02	1.73E02	8.57E02	1.52E02	1.27E03
	$R_{LS\text{-}INAA}$	1.15E00	1.30E00	8.78E00	2.47E00	1.07E00	2.39E00	1.01E00	2.91E00
Emii	K_s	4.66E02	6.34E01	1.31E03	5.62E01	2.15E02	4.85E02	2.79E02	9.72E02
	$R_{LS\text{-}INAA}$	1.76E00	6.50E−01	2.96E00	6.12E−01	1.20E00	1.80E00	1.36E00	2.55E00
Emna	K_s	3.83E02	1.08E02	3.80E02	2.24E02	1.30E02	8.49E02	2.19E02	9.82E02
	$R_{LS\text{-}INAA}$	1.60E00	8.49E−01	1.59E00	1.22E00	9.31E−01	2.38E00	1.21E00	2.56E00
Epan	K_s	9.96E01	3.34E02	8.02E02	1.90E02	2.03E02	5.30E02	3.14E02	4.10E02
	$R_{LS\text{-}INAA}$	8.15E−01	1.49E00	2.31E00	1.12E00	1.16E00	1.88E00	1.45E00	1.65E00
Gfla	K_s	1.71E02	9.20E01	2.51E02	7.87E01	9.59E01	4.73E02	1.24E02	1.31E02
	$R_{LS\text{-}INAA}$	1.07E00	7.83E−01	1.29E00	7.24E−01	7.99E−01	1.78E00	9.09E−01	9.34E−01
Ggar	K_s	1.79E02	1.21E02	1.19E03	1.98E02	1.12E02	1.98E02	3.51E02	1.82E02
	$R_{LS\text{-}INAA}$	1.09E00	8.98E−01	2.82E00	1.15E00	8.63E−01	1.15E00	1.53E00	1.10E00
Gopp	K_s	2.30E02	1.93E02	4.60E02	1.02E02	1.21E02	7.75E01	1.20E02	3.78E02
	$R_{LS\text{-}INAA}$	1.24E00	1.13E00	1.75E00	8.26E−01	8.97E−01	7.19E−01	8.94E−01	1.59E00
Halc	K_s	1.49E02	2.14E02	7.27E02	1.72E03	1.53E02	2.33E02	2.94E02	5.34E02
	$R_{LS\text{-}INAA}$	9.97E−01	1.19E00	2.20E00	3.38E00	1.01E00	1.25E00	1.40E00	1.89E00
Msua	K_s	1.34E02	1.08E02	1.74E02	7.65E01	1.93E02	2.56E02	2.65E02	1.41E02
	$R_{LS\text{-}INAA}$	9.44E−01	8.48E−01	1.08E00	7.14E−01	1.13E00	1.31E00	1.33E00	9.71E−01
Mtom	K_s	3.18E02	5.89E02	3.17E03	1.80E02	2.48E02	4.34E02	1.07E03	1.50E03
	$R_{LS\text{-}INAA}$	1.46E00	1.98E00	4.60E00	1.10E00	1.29E00	1.70E00	2.67E00	3.16E00
Nglo	K_s	4.43E02	7.19E01	1.66E04	1.74E03	8.92E01	2.21E02	1.49E02	2.57E02
	$R_{LS\text{-}INAA}$	1.72E00	6.92E−01	1.05E01	3.40E00	7.71E−01	1.21E00	9.97E−01	1.31E00
Rjas	K_s	8.50E02	5.60E01	1.20E03	2.23E03	2.12E02	2.60E02	3.53E02	8.74E02
	$R_{LS\text{-}INAA}$	2.38E00	6.11E−01	2.83E00	3.86E00	1.19E00	1.32E00	1.53E00	2.41E00
Tgra	K_s	2.38E02	2.01E02	3.90E02	1.26E02	5.95E01	2.82E02	1.94E02	4.45E02
	$R_{LS\text{-}INAA}$	1.26E00	1.16E00	1.61E00	9.18E−01	6.30E−01	1.37E00	1.14E00	1.72E00
Vbic	K_s	5.98E02	2.47E02	6.21E02	1.98E02	1.25E02	8.01E02	2.62E02	8.94E02
	$R_{LS\text{-}INAA}$	2.00E00	1.28E00	2.04E00	1.15E00	9.13E−01	2.31E00	1.32E00	2.44E00

$R_{LS\text{-}INAA}$ refer to the expected standard deviation for samples analyzed by LS INAA (200 g sample size)

Aste *Alsophilla sternbergii*, Baus *Bathysa australis*, Caus *Calycorectes australis*, Cvir *Chrysophyllum viride*, Ecup *Eugenia cuprea*, Eedu *Euterpe edulis*, Emii *Eugenia mosenii*, Emna *Eugenia melanogyna*, Epan *Endlicheria paniculata*, Gfla *Gomidesia flagellaris*, Ggar *Garcinia gardneriana*, Gopp *Guapira opposita*, Halc *Hyeronima alchorneoides*, Msua *Marlierea suaveolens*, Mtom *Marlierea tomentosa*, Nglo *Neomitranthes glomerata*, Rjas *Rudgea jasminoides*, Tgla *Tetrastylidium grandifolium*, Vbic *Virola bicuhyba*

improved the sample representativeness since, for 70% of cases, the composite sample mass utilized in LS-INAA were compatible to those estimated for assuring a small sample errors (from 1 to 10%) bar the cases of Co, Na and Sc determination in some tree species. The analytical uncertainties obtained by LS-INAA (Table 5) were considerably smaller than the expected percent standard deviations (i.e. reproducibility) from conventional INAA results (Table 4). For example, the concentration of Co estimated for *Bathysa australis* showed a percent standard deviation of

Table 5 Mass fractions of chemical elements (mg kg^{-1}) and analytical uncertainties (u%) determined by LS-INAA

LS INAA	Br		Ca[a]		Co		Fe		K[a]		Na[a]		Rb		Sc	
Sp	Mean	u%	Mean	u%	Mean	u%	Mean	u%	Mean	u%	Mean	u%	Mean	u%	Mean	u%
Aste	15.4	3	<2.70		0.42	30	183	30	6.48	3	1.39	2	34.5	9	0.25	10
Baús	6.83	9	6.34	30	0.34	20	366	10	12.7	2	1.03	2	21.8	10	0.12	7
Caus	4.93	4	10.6	50	<0.80		<500		19.9	2	1.52	2	52.1	6	0.04	30
Cvir	15.6	2	9.86	20	0.25	30	169	30	16.2	2	0.38	2	35.9	8	0.02	40
Ecup	17.9	2	16.9	20	0.36	30	176	30	7.39	2	0.62	2	15.5	30	0.06	13
Eedu	4.22	3	4.29	20	0.32	9	98.6	13	4.20	2	0.12	2	12.9	7	0.03	12
Emii	12.7	2	6.34	40	0.25	9	91.5	14	12.4	2	3.36	2	26.1	4	0.06	12
Emna	56.3	2	17.6	20	0.33	20	134	40	14.0	2	2.43	2	33.1	5	0.30	4
Gfla	14.6	2	7.04	20	0.59	13	<500		9.72	2	1.20	2	11.3	30	0.05	9
Ggar	3.87	3	5.91	11	2.71	3	<500		5.21	2	0.63	2	11.3	14	0.02	10
Gopp	25.1	2	12.0	13	0.18	40	141	20	28.4	2	6.83	2	76.0	4	0.03	30
Halc	11.0	2	9.15	11	0.61	10	176	9	14.9	2	1.09	2	23.9	5	0.05	7
Msua	7.82	2	6.34	20	0.27	11	84.5	20	10.1	2	1.88	2	32.4	5	0.03	11
Mtom	6.34	2	7.04	20	0.30	14	63.4	50	7.25	2	1.62	2	17.6	9	0.02	20
Nglo	2.07	3	7.67	10	<0.80		56.3	20	5.28	2	0.25	2	16.9	7	0.02	20
Rjas	37.0	2	9.15	12	<0.80		127	20	12.9	2	1.94	2	33.8	7	0.08	6
Tgra	25.3	2	4.93	40	0.18	20	162	20	14.2	3	3.01	2	30.3	5	0.05	11
Vbic	5.14	2	6.83	13	0.24	12	183	13	10.2	2	0.75	2	23.9	6	0.02	30

Aste *Alsophilla sternbergii*, Baus *Bathysa australis*, Caus *Calycorectes australis*, Cvir *Chrysophyllum viride*, Ecup *Eugenia cuprea*, Eedu *Euterpe edulis*, Emii *Eugenia mosenii*, Emna *Eugenia melanogyna*, Epan *Endlicheria paniculata*, Gfla *Gomidesia flagellaris*, Ggar *Garcinia gardneriana*, Gopp *Guapira opposita*, Halc *Hyeronima alchorneoides*, Msua *Marlierea suaveolens*, Mtom *Marlierea tomentosa*, Nglo *Neomitranthes glomerata*, Rjas *Rudgea jasminoides*, Tgla *Tetrastylidium grandifolium*, Vbic *Virola bicuhyba*

[a] Values in g kg^{-1}

116% (Table 3), which is quite higher than the uncertainty of 20% obtained by LS-INAA (Table 5). However, it can be seen from Table 4 that, by the sampling constant calculation, at least 0.5–10% of expected variation for 200 g samples analyzed by LS INAA can be expected from sample representativeness variance. Although it might be argued that these values could be overestimated due to the rigorous sampling error of 1% required in the calculation, being fairly impracticable. Results thereby suggested a possible combination of the combined standard uncertainty of measurement with the expected percent deviation for a more realistic indication of the range of variation of the results obtained by LS INAA.

Conclusions

The sample representativeness study was carried out in analyzing various dimensions of test portion mass. In the case of small samples of leaf compartment, the analyzed portion was considered satisfactory. There was a significant contribution of the total sampling error estimated by the expected percent standard deviation for the standard uncertainty of measurement estimated for INAA. By increasing the sample dimension from leaves to tree species, LS INAA provided an increasing in sample representativeness due to the reduction of the sampling error by the analysis of large samples (approximately 200 g). To improve reliability of these results, it was suggested adding the expected percent standard deviation resulted from sample variance to the analytical uncertainty of measurement in INAA and LS INAA.

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