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The Quality of Element Determinations in Plant Materials by Instrumental Methods

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Abstract

This report presents an assessment of uncertainty related to sampling, sample preparation and analysis of plants. This specific issue is illustrated by examples derived from biogeochemical studies performed primarily in the Holy Cross Mountains (Góry Świętokrzyskie), as well as from interlaboratory comparative analyses made in the Central Chemical Laboratory of the Polish Geological Institute in Warsaw and other European laboratories. The chemistry of plants is affected by many environmental and biological variables that must be considered when sampling a given species or its part/organ. These variables considerably modify the uptake of elements from soils, rocks, water and air. The better we understand all these interrelationships that influence the chemical variability in plants, the more clear picture of uncertainty we obtain. Another problem that cannot be overlooked is sample preparation, as well as analytical method and technique used. These three principal stages influence the quality of results obtained, and consequently, the adequate assessment of environment quality.

Keywords: plants, sampling, sample preparation, elements, instrumental methods, uncertainty

Introduction

A major problem of biogeochemical studies is in understanding various interrelationships between organisms and environmental or biotic variables, as well as in interpretation of complex results. There are numerous, sometimes unpredictable, physicochemical and biological factors that influence concentrations of elements and organic compounds in vegetation. All these factors must be considered prior to sampling. In addition, to better interpret the results of chemical analyses, we must become more familiar with some uncertainties that are ingrained in a sequential treatment of a given plant sample. One of the most crucial issues in assessing the state of the environment is the quality of results derived from chemical analyses and uncertainty of analytical measurements. The quality of plant analysis depends mainly on the following principal stages: (1) sampling, (2) mechanical (washing, grinding) and chemical (ashing, acid digestion) sample preparation, (3) chemical analyses with different instrumental methods and techniques. Each of these stages can be a source of partial uncertainty that influences the results obtained, and consequently, the assessment of the environmental quality. This issue was discussed on the basis of the results derived from the plant biogeochemical studies carried out primarily in the Holy Cross Mountains (Góry Świętokrzyskie) [1–10], comparative interlaboratory analyses made in the Central Chemical Laboratory of the Polish Geological Institute and other European laboratories, and from different data presented in the selected references cited [11–14].

Plant Sampling – a Dose of Uncertainty

There are four basic precautions that must be followed during sampling. The sample collected should be:

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(i) representative for a given medium (geochemical environment), (ii) free from contaminants, (iii) homogenous, and (iv) natural, *i.e.* unaltered during storage, transportation and preparation [6, 11]. Each stage may bring about a random or systematic error (see table in [15]). However, this problem is far more complex when it comes to collecting plant samples. Diverse concentrations of elements in individual plant species are affected by a variety of environmental variables, *i.e.*, topographic (elevation, aspect), climatic (insolation, wind, pressure systems, moisture), hydrologic, and edaphic factors (soil moisture, texture, pH *etc.*). It should be stressed that most of them generally correspond to the geologic setting of study area [1–4, 7–10]. The angle of the slope and its aspect control the evapo-transpiration processes which in the northern hemisphere is more intense on south facing slopes. In the moderate climatic zone, northern slopes keep more moisture (snow lies longer there), but on the other hand, they are shorter exposed to the sun [16[. Another example is the production of metal chelating acids (especially usnic acid and atranorin) in larger amounts by lichens as elevation increases – causing metal concentrations in lichens at higher elevations to be higher [17].

However, the true "roulette" is related to physiological and genetic factors because pure chance can have a major influence. In general, the most effective uptake of many nutrients including trace elements occurs at the peak of growing season, *i.e.* in May – June in temperate climate. That is the main reason why the specific plant species or individual plant organs collected in May or June reveal distinctly higher concentrations of elements than their equivalents collected in October. An exception to this rule is the lichen species *Xanthoria parietina* that reveals an increase in thalli by about 25% in the winter compared to the summer [18]. In addition, some of the specific plant species (plant "hyperaccumulators") show the enormous metal-binding capacity [5, 6, 11]. Of the environmental variables that affect physiological processes, phenology is highly unpredictable. Daily, seasonal and annual variations in insolation, temperature and precipitation may greatly alter the spatial and temporal distribution of elements in various plant species or their tissues. Our studies also suggest that plant samples should not be collected after long rainfalls due to the substantial removal of some of chemical species. Our estimates are that the unpredictable environmental and biological variables mentioned above may be a source of considerable uncertainty, in some cases roughly 70-80%.

All these facts put some constraints on the selection of a specific plant species or its organ for biogeochemical studies. Different plants species, and even their tissues, show distinct variations in the concentrations of elements. This is induced primarily by diverse assimilation abilities (Table 1).

Considering this, the obtained results provide evidence that each plant species and its natural environment must be considered separately. The sampling uncertainty should reach 20% at the maximum provided that all the precautions mentioned above are followed during collecting plant samples. The best solution to this problem would be collecting plant samples by three separate investigation groups and then preparing a mixed sample for further analysis.

Sample Preparation

This stage encompasses mechanical (washing, grinding) and chemical (ashing, acid digestion) sample preparation [14, 19, 20]. However, there are different approaches to plant washing. Most researchers are strongly opposed to plant washing, due to the potential danger of removing waxes, airborne particulates and some of the elements from plant tissue surfaces. This is of special importance

Table 1. Concentrations of selected trace metals in various tissues of an individual Scots pine (*Pinus sylvestris* L.) tree and *Hypogymnia physodes* (L.) Nyl. (lichen) thalli from the vicinity of Daleszyce in south-central Holy Cross Mountains [1].

Pine tissues/lichen thalli	Al	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
	$mg \cdot kg^{-1}$									
Root	52	<0.5	<1	4	41	0.066	109	3	14	110
Inner wood. (xylem)	8	<0.5	<1	1	9	0.009	67	2	<5	13
Outer wood (xylem)	10	<0.5	<1	3	12	0.003	73	2	<5	18
Bark	417	<0.5	4	5	470	0.101	40	23	17	75
1-year shoots	158	<0.5	<1	6	50	0.007	188	6	<5	51
3-year needles	492	<0.5	<1	3	104	0.040	1356	4	<5	102
2-year needles	487	<0.5	1	4	120	0.042	1192	6	<5	90
1-year needles	182	<0.5	2	8	104	0.022	368	10	<5	48
Lichen thalli	422	0.8	14	7	997	0.255	70	2	15	101

Table 2. Comparison of sulfur concentrations in various plant bioindicators: (1) ashed and subsequently digested with aqua regia, and (2) digested with concentrated nitric acid in a closed microwave system [1].

Plant bioindicator	1	2
	S (mg·kg^{-1})	
Hypogymnia physodes (lichen) thalli	540	1501
Scots pine (*Pinus sylvestris*) needles	490	1452
Hylocomium splendens (moss) tissues	575*	1072

NOTE: Determinations made with an ICP-OES method in the Central Chemical Laboratory of the Polish Geological Institute in Warsaw, *Mean of two determinations: 570 and 580 mg·kg^{-1}

for plants that lack a protection covering tissue (*e.g.* mosses, liverworts) because washing may lead to removing intracellular soluble elements. However, our view is that the plant samples should be rinsed briefly with deionized water, allowed to drain, and air-dried. This procedure enables to remove outer contamination (pollens, cobwebs, dead insects, *etc.*) and obtain homogenous sample. Pollens, for example, may be a considerable source of trace metals [11]. Another error is grinding that may introduce some contaminants to the sample. However, the degree of contamination may be checked by a blank sample. The uncertainty of mechanical preparation should not exceed 5%.

A substantial error may also be linked to plant ashing preceding acid digestion. This procedure may bring about

Table 3. Methods of chemical sample preparation of grass reference material GR94 used for Interlaboratory Comparison IPE 2004.

Number of laboratories	Methods used for chemical preparation of grass sample						
	HNO_3/HCl or HNO_3	Ashing + HCl or HNO_3	HNO_3/HCl	H_2SO_4	HNO_3	HF/$HClO_4$	Others
	Microwaves	Open vessels	Open vessels	Microwaves	Teflon bomb	Open vessels	
148	32	48	33	1	6	4	24

Table 4. Influence of chemical sample preparation on the analysis of tea leaves (prepared by CLCh for interlaboratory comparison) by instrumental methods performed in Central Chemical Laboratory of the Polish Geological Institute in Warsaw (CLCh) and Laboratory of the Government Chemists, Great Britain (LGC).

Element	True value	Ashing pelleting	Recov.	HNO_3 microwaves	Recov.	HNO_3 microwaves	Recov.	Ashing aqua regia	Recov.	Total digestion	Recov.
		CChL (XRF)		CChL (F-AAS)		CChL (ICP-OES)		CChL (ICP-OES)		LGC (ICP-OES)	
	mg·kg^{-1}		%	mg·kg^{-1}	%	mg·kg^{-1}	%	mg·kg^{-1}	%	mg·kg^{-1}	%
Ba	43.2	30.7	71.1	45.0	104.2	43.0	99.5	39.7	91.9	44.2	97.7
Ca	5370	5270*	98.1	5565	103.6	5982	111.4	5452	102.2	5576	103.8
Cr	1.65	1.22	73.9	2	121.2	1.80	109.1	1.80	109.1	1.91	115.8
Cu	20.9	21.1	99.9	22	105.3	20.9	100.0	19.1	91.4	21.5	97.1
Fe	424	498*	82.5	381	89.9	380	89.6	437	103.1	483	113.9
K	15785	15023*	95.2	15743	99.7	15997	101.3	16034	101.6	16333	103.5
Mg	2239	2219*	99.1	2268	98.7	2297	102.6	2235	99.8	2234	99.8
Mn	1509	1410*	93.3	1520	99.3	1567	103.8	1406	93.2	1563	103.6
Na	24.5	24.7*	99.2	18	73.5	–	–	16	65.3	–	–
Ni	5.48	5.02	91.6	6	90.5	5.85	106.8	5.55	101.3	6.12	111.7
P	2130	2049*	92.2	–	–	2475	116.2	2208	103.7	–	–
Pb	1.77	1.35	76.3	<2	–	–	–	1.80	101.7	1.41	79.7
S	2434	1880*	77.2	–	–	2466	101.3	–	–	–	–
Sr	19.5	17.9	91.8	20	102.6	19.4	99.5	18.3	93.8	20.7	106.2
Zn	34.2	29.9	87.4	37	108.2	36.1	105.6	34.2	100.0	35.2	102.9

*Ashing + fusion with flux, For abbreviations of analytical methods, see Table 5

escaping of some elements, for example As, Bi, Cd, S, Sb, Se. Table 2 presents, for comparison, concentrations of sulfur in *Hypogymnia physodes* (lichen) thalli, Scots pine (*Pinus sylvestris*) needles and *Hylocomium splendens* (moss) tissues: (1) ashed and subsequently digested with aqua regia, and (2) digested with concentrated nitric acid in a closed microwave system. The levels of sulfur in the ashed samples are two to three times lower. This is the reason why most laboratories use only acid digestion instead of ashing combined with acid digestion of plant samples (Table 3). The uncertainty of digestion should not exceed 10% provided that incomplete digestion is reduced to the minimum. The influence of the chemical sample preparation on the analysis of tea leaves by different instrumental methods is presented in Table 4. The degree of recovery of a given element depends both on the preparation and instrumental methods used, for example, ashing and digestion with aqua regia gives a higher percentage

Table 5. Instrumental methods used by different laboratories for determinations of elements in grass reference material GR94 (Interlaboratory Comparison IPE 2004).

Element concentration ($mg \cdot kg^{-1}$)	Number of laboratories using different instrumental methods					Total number of laboratories
	ICP-OES	F-AAS	ICP-MS	ETA-AAS	Others	
Al (468)	31	1			1 XRF	33
As (0.288)	3		10	3	14 HG-AAS	30
B (9.49)	54		3	2	37 SPF	96
Ba (9.55)	11		2		1 XRF	14
Be (0.022)	3		4	1		8
Ca (137*)	76	40			1 XRF	117
Cd (0.082)		12	10	14		36
Co (0.181	12	2	9	3		26
Cr (1.75)	23	10	7	9		49
Cu (7.49)	67	70	4		2 XRF	143
Fe (510)	69	68	2		1 XRF	140
Hg (0.0105)			3		22 CV-AAS	25
K (1050*)	69	24			38 FE	131
Li (1.050)	3		4			7
Mg (82.3*)	34	29		1	2 XRF	66
Mn (79.4)	67	77	2		2 XRF	148
Mo (1.63)	21	1	7	1		30
Na (144*)	50	17			24 FE, 2 XRF	91
Ni (1.46)	21	13	7	8	1 XRF	50
P (124*)	64	1			71 SPF, 1 XRF	137
Pb (1.18)	14	13	14	16	2 XRF	59
S (103*)	55		1		7 SPF, 2 XRF, 9 neph.	74
Se (0.043)	2		3	1	5 HG-AAS	11
Sr (21.2)	12		3		2 XRF	17
V (1.18)	8		6	1		15
Zn (33.0)	67	70	2		1 XRF	140

*Concentrations in $mmol \cdot kg^{-1}$, Abbreviations: ICP-OES – inductively coupled plasma-optical emission spectrometry, ICP-MS – inductively coupled plasma-mass spectroscopy, F-AAS – flame atomic absorption spectrometry, ETA-AAS – electrothermal atomization atomic absorption spectrometry, HG-AAS – hydride generation atomic absorption spectrometry, CV-AAS – cold vapor atomic absorption spectrometry, XRF – X-ray fluorescence, SPF – spectrophotometry, FE – flame emission, neph. – nephelometry

Table 6. Analytical uncertainty of grass reference material GR94 analyzed in the Central Chemical Laboratory of the Polish Geological Institute in Warsaw by an ICP-MS method (sample digested with concentrated HNO_3 with an admixture of H_2O_2 in a closed microwave system).

Element	Attest (mg·kg^{-1})	u_{RM} (%)	ICP-MS (mg·kg^{-1})	u_d (%)	u_m (%)	B (%)	U_c (%)	U_e (%)
Al	449	11.8	414	3.09	0.43	7.8	14.2	28.4
B	9.75	6.46	9.8	3.20	0.71	0.5	7.3	14.6
Ba	9.58	5.42	9.5	2.59	1.20	0.8	6.2	12.4
Ca	5530	2.89	5431	2.12	0.21	1.8	4.0	8.0
Cd	82.0	2.44	84.0	3.21	2.20	2.4	5.2	10.4
Co	0.179	6.7	0.2	3.84	1.06	11.7	14.0	28.0
Cu	7.43	2.42	7.3	1.51	0.75	1.8	3.5	7.0
K	25 960	2.56	27 070	0.95	0.67	4.3	5.2	10.4
Na	3310	3.47	3481	1.66	0.69	5.2	6.4	12.8
Mg	1993	4.44	2163	1.57	1.05	8.5	9.8	19.6
Mn	79.7	2.26	87.7	3.02	0.56	10.0	10.7	21.4
Mo	1.60	3.63	1.7	1.53	0.64	6.3	7.4	14.8
Ni	1.46	1.37	1.7	8.13	1.01	16.4	18.3	36.6
Pb	1.19	2.86	1.2	4.01	1.07	0.8	5.1	10.2
Rb	35.04	4.73	34.8	1.35	0.65	0.7	5.0	10.0
Sr	20.6	3.88	21.6	1.86	1.25	4.9	6.7	13.4
Zn	33.3	2.40	30.2	4.46	0.75	9.3	10.6	21.2

u_{RM} – reference material uncertainty, u_d – sample digestion uncertainty (HNO_3 in a closed microwave system), u_m – measurement uncertainty by ICP-MS, B – error of the obtained result relative to the attested value, U_c – composite uncertainty, U_e – extended uncertainty significant at 0.05 probability level (extention coefficient k = 2), $U_c^2 = u_{RM}^2 + u_d^2 + u_m^2 + B^2$, $U_e = 2 \times U_c$

Table 7. Analytical uncertainty of grass reference material GR94 analyzed by an ICP-OES method (sample digested with concentrated HNO_3 in a closed microwave system) in the Central Chemical Laboratory of the Polish Geological Institute in Warsaw for Interlaboratory Comparison IPE 2004.

Element	Attest	u_{RM} (%)	ICP-OES	u_d (%)	u_m (%)	B (%)	U_c (%)
Ca mmol·kg^{-1}	137	3.0	129	2.2	5.8	6.9	13.8
K mmol·kg^{-1}	665	2.0	668	2.0	0.5	2.9	5.8
Mg mmol·kg^{-1}	82.3	2.0	83.0	0.3	0.9	2.2	4.4
Na mmol·kg^{-1}	144	3.5	145	1.0	0.7	3.7	7.4
P mmol·kg^{-1}	124	3.0	121.5	0.6	2.8	4.2	8.4
S mmol·kg^{-1}	103	3.0	103	0.0	0.0	3.0	6.0
Al mg·kg^{-1}	448	11.2	452	4.1	0.9	12.0	24.0
B mg·kg^{-1}	9.49	0.44	9.05	2.3	4.6	5.2	10.4
Ba mg·kg^{-1}	9.55	0.43	9.3	0.9	2.6	2.8	5.6
Cu mg·kg^{-1}	7.49	0.21	7.75	1.2	3.5	3.7	7.4
Fe mg·kg^{-1}	510	2.9	535	2.6	4.9	6.3	12.6
Mn mg·kg^{-1}	79.4	1.80	75.6	0.4	4.8	5.1	10.2
Sr mg·kg^{-1}	21.2	0.55	19.85	0.9	6.4	6.5	13.0
Zn mg·kg^{-1}	33.0	1.0	33	1.7	0.0	2.0	4.0

For symbol explanation, see Table 6

of recovery for Ca, Fe, Mg, Ni, P and Zn than digestion with concentrated HNO_3 in a microwave system, using the same ICP-OES method.

Instrumental Methods and Techniques

This stage is another source of uncertainty including: (1) instrumental measurement, (2) quality of instrument calibration, (3) calibration solutions, and (4) precision and accuracy of measurement.

Of the different instrumental methods, inductively coupled plasma-optical emission spectrometry (ICP-OES), flame atomic absorption spectrometry (F-AAS) and electrothermal atomization atomic absorption spectrometry (ETA-AAS) are commonly used for determining trace elements in plants [21–23]. ETA-AAS is now more often replaced by inductively coupled plasma-mass spectros-

Table 8. Extended uncertainty (U_e) of plant sample analysis with ICP-OES and CV-AAS methods *versus* concentrations of elements.

Element	Method	Range ($mg \cdot kg^{-1}$)	Uncertainty U_e (%)
Al	ICP-OES	0.2 – 20 20 – 500	15 7.7
As	ICP-OES	0.02 – 0.5 0.5 – 15	19 15
Ca	ICP-OES	1 – 10 10 – 500000	8 7
Cd	ICP-OES	0.01 – 0.2 0.2 – 20	18 8.5
Co	ICP-OES	1 – 10 10 – 500	14 7.5
Cr	ICP-OES	0.2 – 2 2 – 50	14 9.5
Cu	ICP-OES	0.05 – 0.5 0.5 – 2	16 8
Fe	ICP-OES	0.5 – 5 5 – 5000	13 6
Hg	CV-AAS	0.002 – 0.05 0.05 – 0.5 0.5 – 5	13 8.8 7
K	ICP-OES	20 – 500 500 – 800000	9.8 5
Mg	ICP-OES	0.1 – 1 1 – 10	13 8
Mn	ICP-OES	0.1 – 1 1 – 50	8 5
Mo	ICP-OES	0.2 – 5 5 – 500	24 8.5
Na	ICP-OES	20 – 500 500 – 700000	14 6.8
Ni	ICP-OES	1 – 10 10 – 500	19 10
P, PO_4	ICP-OES SPF	50 – 500 500 – 500000	22 8.2
Pb	ICP-OES	0.05 – 0.2 0.2 – 50	23 8.5
S, SO_4	ICP-OES gravimetric analysis	1 – 100 100 – 500000	10 5
Sb	ICP-OES	0.05 – 0.5 0.5 – 50	28 13
Se	ICP-OES	0.01 – 0.1 0.1 – 2	28 20
Sn	ICP-OES	1 – 20 20 – 500	25 14
Zn	ICP-OES	0.5 – 10 10 – 100000	10 6.8

copy (ICP-MS). The latter is used for determining As, Sb, Se and Hg commonly determined by hydride generation atomic absorption spectrometry (HG-AAS) and cold vapor atomic absorption spectrometry (CV-AAS), respectively. The scope of modern instrumental methods used for some routine plant analyses is presented in Table 5.

The uncertainty of a given analytical method (U_{method}) may be easily assessed by analyzing reference material (RM) of the same matrix as the sample examined and the same amount and form of analyte. The U_{method} value can be presented as a total uncertainty of the attested RM (U_{RM}) and the result of RM analysis (U_R) in a given laboratory [24]:

$$U_{method} = \sqrt{U_{RM}^2 + U_R^2}$$

The uncertainty of the result also includes other partial uncertainties, *i.e.* chemical sample preparation, precision and accuracy of measurement, and relative error [25].

The analytical uncertainty of grass reference material GR94 was calculated in the Central Chemical Laboratory of the Polish Geological Institute in Warsaw using an ICP-MS method (quadruple spectrometer Perkin Elmer DRC II) (Table 6). The sample was digested with concentrated HNO_3 with an admixture of H_2O_2 (microwave system Milestone ETHOS D). The distinct extended uncertainty (U_e) for Ni, Al, Mn, Zn and Mg results from the lack of interference compensation for these elements. The high uncertainty for Co (U_e = 28%) is associated with a low concentration of this element in the grass examined. The analytical uncertainty of the same grass sample was also determined with an ICP-OES method (spectrometer Jobin-Yvon model JY 70 PLUS with vertical plasma). Of the elements determined, Al, Ca, Sr, Fe, Mn and B show the most distinct extended uncertainty exceeding 10% (Table 7). The extended uncertainty of plant sample analysis with an ICP-OES method *versus* concentration ranges of selected elements is presented in Table 8. It is interesting to note that the analytical uncertainty drops with the increase in element concentration ranges.

Table 9. Uncertainty of plant analysis with an ICP-MS method (including sampling uncertainty) based on the concentration level 20×MDL.

Stages of sample treatment/uncertainty sources	Relative assessment of uncertainty (%)	
	best	worst
1) Sampling (u_s) - contamination - loss of elements - representative sample of the population - durability	10*	30*
2) Sample preparation for analysis (u_p) - grinding (blank sample) - homogeneity - digestion (blank sample) - recovery - distribution repeatability	7	10
3) Calibration u_k ($u_{RM} + u_R$) - repeatibility of calibration curve - calibration method - sample and reference material adjustment (matrix interferences) - certified concentrations in reference materials - instrumental blank sample	1	2.5
4) Spectrometer (u_m) - mass spectrometry system (selection of isotope, thermal stability) - nebulizer system (nebulizer and cloud chamber cleanliness) - induction system (burner cleanliness) - sample introduction system (cone cleanliness) - measurement system (interferences) - mass interferences - argon flow - argon purity	7	12
5) Calculation of results (u_d) - mathematical model - calculations - presentation of results	0.5	1
Total uncertainty of analysis (U_c)	10	15.9
Total uncertainty of sampling and analysis (U_c*)	14.1	33.9

The total uncertainty of plant analysis (including sampling) with an ICP-MS method based on the concentration level 20×MDL is summarized in Table 9. The total uncertainty of plant analysis by this method may reach even 16%, whereas that of plant sampling and analysis nearly 34%.

Considering the plant sample treatment stages, sampling is a source of the highest uncertainty, varying from 10 to 30%. In general, the analytical uncertainty is estimated to be in the range of 7–12% and is linked to the sample type, applied analytical method and detection limit.

One of the most important tests for laboratories analyzing plant samples is the participation in the reputable International Plant-Analytical Exchange (IPE) organized by the Department of Soil Science and Plant Nutrition, Wageningen Agricultural University in Holland. Each of 218 participants receives four plant samples every three months for element determinations. The results submitted are published in quarterly and annual reports along with the sample preparation and analytical methods used by individual laboratories. The concentrations of Cd, Cr and S in the grass sample GR94 derived from the Interlaboratory Comparison IPE 2005.3 are presented in Tables 10, 11 and 12. It is interesting to note that concentrations of sulfur by an ICP-OES method vary from 91 to 128 $mg \cdot kg^{-1}$.

Conclusions

From the biogeochemical studies and interlaboratory comparisons we conclude that:

1. The element concentrations in plants depend on many environmental and biological factors that may greatly influence data evaluation. This is the main reason why these factors should be identified and assessed during pre-sampling reconnaissance.
2. Of the different stages of the plant sample treatment, sampling has a decisive influence on the results obtained. The sampling uncertainty may reach 20%.
3. Based on the concentration level 20×MDL (method detection limit), the uncertainty of plant material analysis by instrumental methods should not exceed 15%. However, it should be stressed that the measuring technique used, analytical experience of chemist, validation quality of the method used, and adequate quality control play a more significant part on the result than the instrumental method used.
4. The total uncertainty including plant sampling, sample preparation and analysis should not exceed 35% excluding interpretation.

Table 10. Concentrations of cadmium in grass reference material GR94 derived from Interlaboratory Comparison IPE 2005.3 [26].

Cadmium ($mg \cdot kg^{-1}$)	Analytical method	Sample preparation method
0.117	ICP-OES	microwaves/aqua regia
0.115	ICP-OES	microwaves/aqua regia
0.100	F-AAS	HNO_3
0.098	2x ICP-OES	microwaves/aqua regia
0.097	ICP-OES	ashing/HNO_3
0.095	F-AAS	ashing/HNO_3
0.091	ICP-MS	microwaves/aqua regia
0.090	F-AAS	HNO_3
0.089	ETA-AAS	HNO_3
0.086	2x ICP-MS, ICP	microwaves/aqua regia
0.084	ICP-MS, 2x ETA-AAS, F-AAS	teflon bomb/HNO_3, microwaves/aqua regia, 2x HNO_3
0.083	2x ICP-MS	microwaves/aqua regia, HNO_3
0.082±0.0019	ICP-MS, 3x ETA-AAS, F-AAS	HNO_3, 2x microwaves/aqua regia, 2x ashing/HNO_3
0.081	ICP-MS, 3x EAT-AAS	2x HNO_3, ashing/HNO_3, microwaves/aqua regia
0.080	ICP-MS, 6x F-AAS, ICP-OES	6x ashing/HNO_3, microwaves/aqua regia, HNO_3
0.077	ICP-MS	microwaves/aqua regia
0.065	ETA-AAS	microwaves/aqua regia
0.063	F-AAS	ashing/HNO_3

NOTE: Attested value in boldface, For abbreviations of analytical methods, see Table 5

Table 11. Concentrations of chromium in grass reference material GR94 derived from Interlaboratory Comparison IPE 2005.3 [26].

Chromium ($mg \cdot kg^{-1}$)	Analytical method	Sample preparation method
3.63	ICP-OES	microwaves/aqua regia
2.41	ICP-OES	ashing/HNO_3
2.38	ICP-OES	teflon bomb/HNO_3
2.37	F-AAS	ashing/HCl
2.19	ICP-MS	HNO_3
2.10	ICP-MS	microwaves/aqua regia
2.07	ICP-MS	HNO_3
2.06	ETA-AAS	HNO_3
1.94	ICP-MS	microwaves/aqua regia
1.90	ICP-OES	HNO_3
1.88	ICP-MS	HNO_3
1.87	ICP, ETA-AAS	microwaves/aqua regia, HNO_3
1.83	ICP-OES	teflon bomb/HNO_3
1.80	ICP-OES	HNO_3
1.79	F-AAS	HNO_3
1.78	ICP-OES, ETA-AAS, 2x F-AAS	2x HNO_3, ashing/HNO_3, $HClO_4$
1.775±0.015	ICP-OES, F-AAS	ashing/HNO_3, HNO_3
1.76	ICP-MS, ETA-AAS, 2x F-AAS	2x microwaves/aqua regia, 2x ashing/HNO_3
1.75	F-AAS	ashing/HNO_3
1.72	F-AAS	ashing/HNO_3
1.70	ICP-OES	ashing/HNO_3
1.68	ICP-MS	microwaves/aqua regia
1.65	ETA-AAS, ICP-OES	teflon bomb/HNO_3, HNO_3
1.59	ICP-OES	HNO_3
1.40	ICP-OES	HNO_3

NOTE: Attested value in boldface, For abbreviations of analytical methods, see Table 5

Table 12. Concentrations of sulfur in grass reference material GR94 derived from Interlaboratory Comparison IPE 2005.3 [26].

Sulfur ($mg \cdot kg^{-1}$)	Analytical method	Sample preparation method
128	ICP-OES	microwaves/aqua regia
127	ICP-OES	HNO_3
119	2x ICP-OES	microwaves/aqua regia
117	ICP-OES	ashing/HNO_3
113	ICP, IRS	ashing/HNO_3, HNO_3
112	2x ICP-OES	$HClO_4$, HNO_3
110	ICP-OES, SPF	$HClO_4$, HNO_3
109	ICP-OES, ICP-MS	HNO_3, microwaves/aqua regia
108	ICP-OES	microwaves/aqua regia
107	ICP-OES	$HClO_4$
106	3x ICP-OES, IR, turbidimetry	teflon bomb/HNO_3, 2x $HClO_4$, microwaves/aqua regia, ashing/HNO_3
105	ICP-OES, turbidimetry	$HClO_4$, ashing/HNO_3
104	5x ICP-OES	2x HNO_3, teflon bomb/HNO_3, microwaves/aqua regia, ashing/HNO_3
103±3	4x ICP-OES, SPF	$HClO_4$, 2x HNO_3, teflon bomb/HNO_3, microwaves/aqua regia
102	3x ICP-OES, turbidimetry	2x microwaves/aqua regia, $HClO_4$, HNO_3
101	ICP-OES, 3x SPF	$HClO_4$, HNO_3, microwaves/aqua regia, ashing/HNO_3
100	2x ICP-OES, turbidimetry	2x HNO_3, ashing/HNO_3
99	2x ICP-OES	$HClO_4$, microwaves/aqua regia
98	2x ICP-OES	microwaves/aqua regia, ashing/HNO_3
97	ICP-OES	microwaves/aqua regia
94	turbidimetry	teflon bomb/HNO_3
93	2x ICP-OES	$HClO_4$, microwaves/aqua regia
91	ICP-OES, SPF	HNO_3, microwaves/aqua regia
80	turbidimetry	$HClO_4$
60	turbidimetry	teflon bomb/HNO_3
30	turbidimetry	$HClO_4$

NOTE: Attested value in boldface, IRS – infrared spectrophotometry; for other abbreviations, see Table 5

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