Certified reference material of lichen (CRM 482) for the quality control of trace element biomonitoring

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Abstract

Lichens are currently used as bioindicators to monitor air pollution and to follow changes in pollution patterns. In order to perform reliable biomonitoring, these analyses should give accurate results, which implies that a good quality control system should be applied. One way to achieve accuracy is to use certified reference materials (CRMs). In order to produce a lichen CRM, the BCR-programme (now renamed Standards, Measurements and Testing programme) of the European Commission has conducted a certification campaign involving expert laboratories. A lichen material has been collected on pine trees, oven-dried, homogenised and bottled, and its homogeneity and long-term stability verified. The material (CRM 482) was analysed by a group of 11 selected laboratories using different analytical methods. This paper describes the certification work performed to certify the elements Al, As, Cd, Cr, Cu, Hg, Ni, Pb and Zn. Indicative values are given for Ba, Br, Ca, Co, Fe, K, Mn, Mo, P, S, Sb, Se, Sn and V.

Keywords: Quality control; Certified reference material; Lichen; Biomonitoring; Trace elements

1. Introduction

Lichens are currently monitored to aid the control of air pollution and to follow changes in pollution patterns (Hawksworth, 1971; Herzig, 1993). Lichens are found almost everywhere and accumulate trace elements from the atmosphere; therefore, they are often used as a practical means for biomonitoring pollution (Seaward, 1980; Sloof, 1993). The collection and analysis of lichen material is much easier and cheaper than the use of air filters; furthermore, the lichen monitoring produces data over wide geographical areas. The

quality of lichen analysis is influenced by specific matrix effects which are not matched by the matrix of existing plant reference materials.

About 2000 papers involving lichen analysis have been published in recent years, and reveal high variability of data which may not only reflect different distribution pollution patterns but may also involve analytical errors; furthermore, lichen analyses are often performed for governmental bodies or industries which requires to implement quality control (QC) by demonstrating accurate measurements. A rough estimate has shown that more than 5000 lichen analyses are performed every year within the European Community; this means that consequent economic losses are to be

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expected if the quality assurance (QA) of measurements performed is not verified. In order to improve and control the quality of trace element determinations of lichens, the European Commission (through the BCR programme now renamed Standards, Measurements and Testing programme) has launched a project the first step of which was intended to detect and remove the main sources of error likely to occur in lichen analysis through an interlaboratory study (Quevauviller et al., 1993); the results obtained in this exercise demonstrated that a certification campaign could be contemplated and a candidate reference material of lichen (CRM 482) was prepared for this purpose. This paper presents the results of this certification for a range of trace elements.

2. Background of the project

An intercomparison on trace elements in lichen has been organized within the BCR-programme to identify and remove sources of errors likely occurring in this type of chemical measurements. Two lichen samples, containing low and high trace element contents, have been prepared and distributed to 32 laboratories from 9 EC and 2 EFTA States. The interlaboratory study focused on the following elements: Al, As, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, Pb and Zn. The results of the technical discussions are published elsewhere (Quevauviller et al., 1993). The evaluation could allow us to detect sources of errors and to give recommendations to certifying laboratories prior to the certification. In addition, clear requirements for the preparation of the lichen candidate CRM were expressed, in particular washing was to be avoided as this procedure removes water-soluble and exchangeable ions such as, e.g. K. The participants recommended that the lichen material be dried at room temperature and that all adhering particles be manually removed.

3. Preparation of the candidate certified reference material

The epiphytic lichen species *Pseudevernia furfuracea* was identified as a good candidate for a possible certified reference material. Some 40 g of

lichen was collected manually on pine trees located in the area of Axalp (Switzerland) in September/October 1991. The material was air-dried and coarse particles were removed manually. Following this pre-treatment, the sample was stored in polyethylene bags which were transported at the Joint Research Centre of Ispra (I). The material was oven-dried at 105°C for at least 7 h to achieve a moisture content of about 8%. At this stage, the material appeared rather brittle while oven-warm but the fibres became rapidly elastic while picking-up moisture from the ambient air. The material was ground in a hammer-mill equipped with a 1-mm internal chamber sieve. The fraction <125 µm was collected in a drum flushed at regular intervals with argon. The fraction >125 µm was ground again, using a 500-µm chamber sieve and the resulting fraction <125 µm was added to the drum. The resulting material was homogenized for 14 days under dry argon. During this operation, long fibres aggregated and formed tiny balls with size ranging from >100 µm to some mm; these aggregates were removed by sieving the material again at 125 µm mesh. The fraction >125 µm was discarded and the sieved material was further homogenized for another 2 weeks.

The material was stored in brown glass bottles with polyethylene inserts and plastic screw caps, each containing approx. 15 g of powder.

4. Homogeneity study

The between bottle homogeneity was verified by the determination of Al, As, Cd, Cr, Cu, Hg, Mo, Ni, Pb and Zn on intakes of 250 mg (200 mg for Hg) taken from 20 bottles which were set aside at regular intervals during the whole period of bottling. The within bottle homogeneity was assessed by ten replicate determinations on the well mixed content of one bottle. The uncertainty of the method of final determination was assessed by ten replicate determinations of one digest. Each bottle was shaken manually for few minutes to eliminate segregation which might have occurred during transport and storage. The determinations of the above mentioned elements were performed by ICP-MS (As, Cd, Mo, Pb), ICPAES (Al, Cu, Zn),

Table 1
Summary of techniques of final determinations

Element	Techniques used in certification
Al	DCP-AES, ETAAS, ICP-AES, ICP-MS, INAA
As	HAAS, ICP-MS, INAA, RNAA
Cd	DPASV, ETAAS, ICP-AES, ICP-MS, IDMS
Cr	ETAAS, IDMS, INAA, RNAA
Cu	DCP-AES, DPASV, ICP-AES, ICP-MS, IDMS, INAA, RNAA
Hg	C.V.AAS, ICP-MS, ID-ICPMS, IDMS, INAA, RNAA
Ni	DPASV, DCP-AES, ETAAS, ICP-AES, ICP-MS, IDMS, INAA
Pb	DCP-AES, DPASV, ETAAS, ICP-AES, ICP-MS, ID-ICPMS, IDMS
Zn	DCP-AES, DPASV, ICP-AES, ICP-MS, IDMS, INAA

CVAAS: Cold vapour atomic absorption spectrometry; CVAFS: Cold vapour atomic fluorescence spectrometry; DCP-AES: Direct current plasma atomic emission spectrometry; DPASV: Differential pulse anodic stripping voltammetry; ETAAS: Electrothermal atomic absorption spectrometry; FAAS: Flame atomic absorption spectrometry; FLUO: Fluorimetry; HAAS: Hydride formation atomic absorption spectrometry; HICP: Hydride formation inductively coupled plasma atomic spectrometry; ICP-AES: Inductively coupled plasma atomic emission spectrometry; ICP-IDMS: ICP-Isotope dilution mass spectrometry; ICP-MS: Inductively coupled plasma mass spectrometry; IDMS: Isotope dilution mass spectrometry; INAA: Instrumental neutron activation analysis; RNAA: Neutron activation analysis with radiochemical separation.

ETAAS (Cr, Ni) and CVAAS (Hg), after HNO_3/HF digestion at 100°C for 14 h and 150°C for 2 h. Chlorine interference in As determination by ICP-MS was avoided by ion-exchange chromatography prior to ICP-MS injection (see abbreviations of methods in the captions of Table 1).

The coefficient of variations (C.V.s) for the different elements are summarized in Fig. 1. An *F*-test at a significance level of 0.05 did not reveal significant differences between the within- and between bottle-variances. In all cases, no significant differences were observed between the estimated C.V. of the method and the C.V. of the within- or between-bottle variances as the overlapping was in most cases within the total uncertainty U_T of the C.V. (the approximation of the uncertainty $U_{C.V.}$ of the C.V. is calculated as follows: $U_{C.V.} \approx \text{C.V.}/\sqrt{2n}$).

Considering the results, it was concluded that the material is suitable for use as a CRM and is homogeneous at least at an analytical portion of 250 mg and above for the certified elements.

5. Stability study

The stability of the trace element content was tested to determine the suitability of this material as a reference material. Bottles were kept at, respectively, -20°C , $+20^\circ\text{C}$ and $+40^\circ\text{C}$ over a period of 12 months and the elements considered for the homogeneity study were determined at regular intervals during the storage period. Tests were made at the beginning of the storage period and after 1, 3, 6 and 12 months. The same techniques as the one described in the section 4 were used.

Any change in the content of an element with time indicates an instability provided that a good long-term analytical reproducibility was achieved. Instability would be detected by comparing the contents of different elements in samples stored at different temperatures with those stored at a low temperature at the various occasions of analysis. The samples stored at -20°C were used as reference for the samples stored at $+20^\circ\text{C}$ and $+40^\circ\text{C}$.

Table 2 gives the ratios (R_T) of the mean values (X_T) of three measurements made at $+20$ and $+40^\circ\text{C}$ versus the mean value ($X_{-20^\circ\text{C}}$), from three determinations made at the same occasion of analysis on samples stored at a temperature of -20°C :

$$R_T = X_T/X_{-20^\circ\text{C}}$$

Table 2

Certified contents of Al, As, Cd, Cr, Cu, Hg, Ni, Pb and Zn in CRM 482

	Certified value (mg/kg)	Uncertainty (mg/kg)	Number of accepted sets of results
Al	1103	24	9
As	0.85	0.07	6
Cd	0.56	0.02	8
Cr	4.12	0.15	7
Cu	7.03	0.19	10
Hg	0.48	0.02	8
Ni	2.47	0.07	8
Pb	40.9	1.4	10
Zn	100.6	2.2	13

CRM 482 - Results of the homogeneity study

Fig. 1. Results of the homogeneity study for Al, As, Cd, Cr, Cu, Hg, Ni, Pb, Zn and Mo at the 250 mg level of intake. The length of the bar on each block represents $2 U_{C.V.}$.

The uncertainty U_T has been obtained from the coefficient of variation (C.V.) of three measurements obtained at each temperature:

$$U_T = (C.V._T^2 + C.V._{-20^\circ C}^2)^{1/2} \cdot R_T$$

The Figs. 2a–b shows the ratios of mean values (R_T) and the uncertainty U_T . In the case of ideal stability, R_T should be 1. In practice, however, there are some random variations due to the error on the measurement. In general the value 1 was between $R_T - U_T$ and $R_T + U_T$. Slight variations were attributed to measurement uncertainties. On the basis of the results, it was concluded that no instability could be demonstrated. The material can be stored at ambient temperature.

6. Requirements for certification

Each laboratory that took part in the certification was requested to make a minimum of five independent replicate determinations of each element on at least two different bottles of the CRM on different days. A series of steps was undertaken in order to ensure that no substantial systematic errors were left undetected, in particular:

- (1) In the meeting of the laboratories participating to the certification the sources of error and the measures taken to eliminate them were discussed (e.g. care to avoid contamination, losses, incomplete digestion, etc.). As described in the technical discussion, spectral interferences in ICP-MS

Fig. 2. Results of the stability study after 1, 3, 6 and 12 months for Al, As, Cd, Cr, Cu, Hg, Mo, Ni, Pb and Zn. The length of the vertical bar on each block represents $2 U_T$. (a) Stability study at +20°C. (b) Stability study at +40°C.

rendered this technique unsuitable for the certification of As (except after ion-exchange) and Cr (ICP-MS, AAS). Errors related to other technique of final measurement could not be detected.

(2) The laboratories participating in the certification exercise applied their methods correctly. They used at least the following quality control steps:

(a) The determinations were performed only when the method was under control, i.e. the standard deviations observed in the laboratory were in accordance with the normal practice of the laboratories.

(b) The performance of the method was verified by analysing the reference materials used in the first interlaboratory study on trace elements in lichen.

(3) The results of different methods as applied in different laboratories were compared to detect sources of method error. No method bias was detected (see section 10).

7. Methods used for certification

Eleven laboratories from nine countries participated in the certification (see acknowledgements). All proved that they were working under a good quality control system. Table 1 summarizes the different techniques of final determination for the different elements as used by these laboratories.

The pre-treatment techniques were digestion with combination of acids in a pressurized or atmospheric mode, programmed dry ashing, microwave digestion, and irradiation with thermal neutrons. A detailed description of these methods is given in the certification report (Quevauviller et al., 1996).

8. Technical evaluation of the results

All the results were discussed at a technical evaluation meeting to confirm the accuracy of the methods of analysis. Some comments which arose from the technical discussions are summarized below.

8.1. Arsenic

The spread of results indicated relatively high

ICP-MS results in comparison to data obtained by RNAA. Effects of ArCl interference on the determination of ^{75}As (particularly with chlorine contents as high as 7000 mg/kg) obviously caused such systematic difference to occur. It was agreed that this risk of systematic error was not acceptable for certification and that the ICP-MS datasets should be withdrawn. Lab.03 used a ion-exchange treatment with a chromatographic column prior to ICP-MS injection to remove the high chlorine contents and the set of results was consequently accepted.

Particular care was also recommended for the digestion of the lichen material. A systematic comparison of three microwave-decomposition techniques (with quartz vessels) using HNO_3 at 170°C demonstrated that a high pressure (85 bar) and an addition of HF was necessary to obtain a complete recovery of As in this material. The use of a high pressure (85 bar) mode and a medium pressure (10 bar) mode without addition of HF led, respectively to As contents 35% and 70% lower than the one found with the high pressure/HF method. It was suspected that an adsorption effect of arsenic on the surface of the quartz vessels could have occurred, which was avoided by HF addition. All other sets of data were accepted for certification.

8.2. Cadmium

Low results obtained by DPASV were suspected to be due to an incomplete decomposition of the organic matter; in addition, losses of volatile fractions were assumed with the dry ashing technique used (450°C , open system, instead of high pressure digestion). The set was consequently withdrawn.

8.3. Chromium

A systematic difference was observed between high values obtained by NAA techniques and ICP-MS and ICP-AES data. It was suspected that in some cases the digestion method used was not sufficiently strong to decompose the matrix; the addition of HF after HNO_3 decomposition is a pre-requisite. The main problem was, however, related to strong interferences occurring at mass 52 with argon. As already mentioned for arsenic,

ICP-MS and ICP-AES were not considered to be suitable for certification and the different sets were consequently withdrawn; ETAAS is clearly more reliable for chromium determination in comparison to ICP-MS.

8.4. Copper

Higher standard deviations in ICP-MS were observed in comparison to ICP-AES results; this difference was due to a dilution step necessary prior to ICP-MS measurement (hence increasing the uncertainty) which was not necessary in ICP-AES; this larger uncertainty was considered to be acceptable for certification and the ICP-MS results were, therefore, kept.

8.5. Lead

Effects of different calibration methods using ICP-MS were investigated by one participant who found comparable results using isotope dilution and standard additions ((37.1 ± 0.2) mg/kg and (37.5 ± 0.3) mg/kg, respectively) whereas higher results were obtained using external calibration ((39.9 ± 0.3) mg/kg). The latter technique was not recommended for certification. Systematic effects related to sample dilution were also observed. Since the ID-ICPMS results overlapped with the set of another laboratory using ICP-MS, they were accepted for certification. Doubts were expressed on a possible incomplete digestion performed by dry ashing and addition of HNO_3/HF , which could explain low results of one participant. Further investigations carried out after the meeting demonstrated that higher values were obtained using another digestion method ($\text{HClO}_4/\text{HNO}_3$ at 170°C). Owing to this discrepancy, the participant withdrew all its sets of data.

8.6. Zinc

An incomplete digestion was suspected for two participants, possibly explaining low results. However, a verification using an alternative method was carried by one of the two laboratories, which did not find significantly different results. The two sets were, therefore, accepted for certification.

9. Statistical evaluation and certified values

For each set of results, the mean value and the standard deviation were calculated. The sets of results have been submitted to the following statistical tests:

- Kolmogorov-Smirnov-Lilliefors tests to assess the conformity of the distributions of individual results and of laboratory means to normal distributions;
- Nalimov test to detect 'outlying' values in the population of individual results and in the population of laboratory means;
- Bartlett test to assess the overall consistency of the variance values obtained in the participating laboratories;
- Cochran test to detect 'outlying' values in the laboratory variances (s_i^2); and
- One way analysis of variance (F -test) to compare and estimate the between- and the within-laboratory components of the overall variance of all individual results.

The sets of results found acceptable on technical and statistical grounds are represented in the form of 'bar-charts' of which an example is given in Fig. 3; the length of a bar corresponds to the 95% confidence interval of the mean. The certified values were calculated as the arithmetic means of laboratory means (taking into account the number of sets accepted for certification after both statistical and technical scrutiny); they are featured as vertical dotted lines on the bar-graphs. The uncertainty is given by the half width of the 95% confidence interval of the mean of laboratory means.

The certified values (unweighted mean of P accepted sets of results) are given in Table 2 as mass fractions (based on dry mass) in mg/kg, along with their calculated uncertainties (half width of the 95% confidence intervals).

10. Comparison of methods

In the certification exercise of some elements two or more laboratories applied the same technique of final determination. In such a case it was possible to compare the results per technique. A grand mean of the means of all the three or more laboratories applying the same technique of final

BAR-GRAPHS FOR LABORATORY MEANS AND 95% CI

Fig. 3. Example of bar-graph corresponding to the sets of data accepted after the technical and statistical evaluations (copper). The laboratory codes are indicated with the methods used (see abbreviations in Table 1). The results plotted for each laboratory correspond to five replicate determinations.

determination was calculated. The obtained grand means were then compared to investigate whether a particular bias could be attributed to any method.

The graphs present the grand mean per technique of final measurement (for those elements where this was possible) together with the standard deviation of the set.

Table 3
Results of the evaluation of consistency of the methods used

Element	Techn. of final determination	C.V. (%) between means of lab. with the same technique	nr of sets of results	C.V. (%) between means of diff. techniques
Aluminium	INAA	2.8	2	2.3
	ICP-AES	2.2	4	
Arsenic	INAA	5.4	2	9.2
	RNAA	7.1	2	
Chromium	INAA	2.2	2	2.2
	RNAA	4.7	3	
Copper	ICP-AES	4.2	3	0.7
	ICP-MS	2.4	2	
Mercury	ICP-MS	3.4	3	1.2
	CVAAS	0.3	2	
Zinc	INAA	2.8	2	3.8
	ICP-MS	1.8	3	
	ICP-AES	3.3	4	

Table 4
Indicative values in CRM 482 (in mg/kg) P: sets of results (each of five replicates)

Element	mean	S.D.	P	Techniques used
Ba	14.9	2.4	2	INAA, ICP-AES
Br	25.5	0.3	2	INAA
Ca	2624	180	2	ICP-AES, XRF
Co	0.32	0.03	3	DPASV, ICP-MS, INAA
Fe	804	160	3	ICP-AES, INAA, XRF
K	3900	224	3	ICP-AES, INAA, XRF
Mn	33.0	0.5	2	ICP-AES, INAA
Mo	0.85	0.01	2	ICP-MS, INAA
P	690	10	2	ICP AES
S	2166	292	2	ICP-AES, XRF
Sb	0.35	0.09	3	ICP-MS, INAA
Se	0.6	0.2	4	ICP-MS, INAA, ETAAS
Sn	1.31	0.10	2	ICP-MS
V	3.74	0.61	2	ICP-MS, INAA

Table 3 presents the results of the evaluation. As shown, the C.V.s within one method are generally of the same order of magnitude than those between different techniques.

11. Indicative values

During the course of the certification some laboratories individually determined other elements. These results are given in Table 4 together with the

Table 5
Comparison of the certification results with the results of the interlaboratory study

Element	C.V.s between Labs. TP24*	C.V.s between Labs. TP25*	C.V.s between Labs. CRM 482
Al	6.3	7.3	2.8
As	13.1	13.1	7.8
Cd	24.1	23.8	2.6
Cr	19.1	14.0	3.9
Cu	13.3	11.7	3.6
Hg	18.2	15.5	3.1
Ni	18.8	25.1	3.1
Pb	20.0	7.6	4.5
Zn	13.1	10.2	3.6

The results listed in this table correspond to the C.V.s of the mean of laboratory means after technical scrutiny. TP24 and TP25 are the samples which were used in the intercomparison (*form Quevauviller et al., 1993).

standard deviation obtained, the method(s) used and the number of sets of results. It is emphasized that the values of this table are not certified.

12. Conclusions

Table 5 summarizes the results of the certification (C.V.s of the means of laboratory means) in comparison with the results obtained in the intercomparison (Quevauviller et al., 1993); as shown in the table, the C.V.s obtained in the certification decreased drastically which illustrate that a considerable improvement could be achieved.

The CRM 482 is now available for quality control of trace element biomonitoring; the material can be purchased at the Institute for Reference Materials and Measurements (IRMM), Management of Reference Materials Unit, Retiesweg, B-2440 Geel (Belgium). Each bottle is accompanied by a certificate and a report describing the work performed (preparation of the material, homogeneity and stability studies, analytical methods used in the certification, results of the technical and statistical evaluation, and individual results).

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