Uncertainty assessment including sampling for determination of metals in lichen samples using ICP-OES

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Abstract: The uncertainty arising from the lichen sampling, preparation and analysis was calculated according to a new guide by Eurachem/EUROLAB/CITAC/Nordest/AMC, 2007, using the retrospective lichen data set completed in 2000 in Turkey. ICP-OES was used to determine the elements Al, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, V and Zn. The method validation studies included linearity, detection limits, precision and accuracy. Measurement uncertainties were calculated by applying the bottom-up approach. It was observed that the largest contribution to the uncertainty came from the sampling. This approach indicated that, the standard uncertainties of the concentration values could be as large as 31.5%.

1. Introduction

This study covers the data obtained from biomonitoring study conducted in western part of Turkey between 1997 and 1999 [1]. The uncertainty calculations arising from the analysis is well-known topic; however, uncertainty calculations including sampling in environmental studies have not been concerned in detail before. The guide [2] published in April 2007 filled a big gap in this area. In the environmental studies, sampling is the first and a very important step. The homogeneity in sampling is especially important in order to obtain representative and reliable results. In estimating the uncertainty, it has been increasingly apparent that sampling is often the more important contributor to the uncertainty and requires careful management and control. The uncertainty arising from the sampling process requires careful evaluation.

The purpose of this work is to perform the uncertainty calculation of the concentrations of metals determined by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES)

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technique in lichen samples collected in Aegean Sea Region of Turkey. It icludes the uncertainty arising from sampling using the bottom-up method for the first time in the literature.

2. Experimental

2.1. Sample collection and handling

Samples of *Xanthoria parietina* (greenish yellow color) (Figure 1) which is found commonly and extensively in Aegean Region of Turkey, were collected from tree substrates. The region was divided into 10 km x 10 km grids. Samples from the same tree and from nearby 3-7 trees in a grid was combined to yield one representative sample of this grid. During the sampling, sample handling and preparation for analyses, polyethylene gloves were worn.

The lichen thallus together with bark substrate was floated in deionized water in a beaker. Debris and soil particles settled down; after rinsing for 30 seconds, they could be separated from the substrate with plastic tweezers. After separation, the samples were placed in folded filter papers and air-dried in clean glass box approximately for one day at room temperature. The dried samples were kept in the acid-washed polyethylene bags.

Figure 1. Whole thallus of *Xanthoria parietina*

2.2. Certified reference materials and reagents

Accuracy of the analytical method was checked by analyzing the certified reference materials: NIST SRM-1571: Orchard Leaves and IAEA-336: Trace elements in lichen.

The following reagents were used during the study: (1) Nitric acid (65%, w/w), Ultra Pure, Merck. (2) Hydrogen peroxide (35%, w/w), Ultra Pure, Merck. (3) Hydrofluoric acid (38-40%, w/w), Ultra Pure, Merck. (4) 1000 mg L^{-1} standard stock solutions of the metals.

In all sample preparation and analyses, deionized water was used which was purified by using Barnstead nanopure ultrapure deionization unit. The produced deionized water finally had a resistance of 18.2 MΩ.

All PTFE containers and TFM (Tetrafluoromethaxil-Regulated trademark of Hoechst) digestion vessels were cleaned by soaking in 30% (v/v) HNO$_3$ for at least 12 hours and rinsed with deionized water several times to be used for the second experiment.

2.3. Instrumentation and optimization

Milestone Ethos 900 microwave digestion instrument was used to digest the samples. Samples were analyzed by using ICP-OES. A Leeman Labs Direct Reading Echelle Spectrometer equipped with a Hildebrand nebulizer and dual-view (radial and axial viewing) torch was employed.

2.4. Digestion procedures

Microwave digestion procedure was applied to digest the lichen samples and two certified reference materials. A set of digestion blanks was prepared together with each batch of samples. All digested samples were transferred to 125 mL acid-washed polyethylene sample bottles to be stored in the refrigerator at 4 ℃ until analysis.

Approximately 0.2 g of lichen sample was weighed and put in 125 mL TFM digestion vessel. Five of these vessels were used for the digestion of lichen samples and one of them was used for reagent blank. The reagent mixture of 8 mL of HNO_3, 2 mL of H_2O_2 and 0.5 mL of HF was used to digest the lichen samples. The microwave digestion was completed in 25 minutes including 5 minutes for ventilation. The contents in the containers were then transfereed to PTFE containers. They were placed on a hot plate at 140 ± 10 ℃ for the evaporation of excess reagents. When 0.5-1 mL of sample aliquot remained inside, the PTFE container was removed from the hot plate, cooled and the contents were diluted to 50 mL with deionized water.

3. Results and discussion

3.1. Optimization

It is important to optimize all the ICP-OES parameters in the presence of the sample matrix as the appearance time and shapes of the element signals are clearly dependent on the matrix.

In this study, the instrumental parameters, namely, RF Power, nebulizer pressure, auxiliary gas flow rate and sample uptake rate were optimized by using samples with lichen matrix rather than aqueous standard solutions. The first two parameters had the most significant effect on emission intensities and depending of the type of emission line, they were subjected to change. The last two parameters had relatively small effects on emission intensity and were usually adjusted to accommodate a particular sample type, such as organics or high dissolved solids, rather than to improve the best detection limits. The optimum values for all parameters were obtained according to high Signal/Blank ratios at less interfered wavelengths. The best lines (λ) for the elements being determined were listed in Table 1. The optimized values were 1.1 kW for RF power, 45 psi for nebulizer pressure, 0.3 L min^{-1} for auxiliary gas flow, 1.4 mL min^{-1} for sample uptake rate. The default value of 18 L min^{-1} was used for the coolant gas flow rate.

Totally 234 samples were analyzed. For mapping purpose, one homogenized sample was digested and analyzed for one grid and one concentration result that was the average of three replicate measurements from the instrument, was obtained. However, some of the samples were treated differently as explained in the related parts of the method validation and the uncertainty calculation sections.

Table 1. The best analyte lines of elements used in this study for ICP-OES

Element	Best line (nm)
Al (I)	308.215
Ca (II)	393.366
Cd (II)	214.438
Cr (II)	205.552
Cu (I)	324.754
Fe (I)	259.940
K (I)	766.490
Mg (II)	279.553
Mn (II)	257.610
Na (I)	589.592
Ni (II)	216.555
Pb (II)	220.353
V (II)	292.402
Zn (I)	213.856

I: Atomic lines; II: Ionic lines

3.2. Validation

3.2.1. Linearity

Calibration curves were obtained from the standard addition calibration solutions. Linearity was evaluated by the calculation of a four-point linear plot with 9 replicate measurements for each, based on the linear regression and squared correlation coefficient, r^2, which was higher than 0.92. Linearity ranged from 0 to 0.1 mg L^{-1} for Cd, V, Cr, Ni, Cu and Pb; 0 to 0.7 mg L^{-1} for Mn and Zn; 0 to 20 mg L^{-1} for Na and Fe; 0 to 30 mg L^{-1} for Al, Ca and Mg; 0 to 50 mg L^{-1} for K. The results are summarized in Table 2.

Table 2. Calibration parameters

Element	R^2	Linear range (mg L^{-1})
Al	0.9998	0-30
Ca	0.9254	0-30
Cd	0.9979	0-0.1
Cr	0.9999	0-0.1
Cu	0.9952	0-0.1
Fe	0.9996	0-20
K	0.9999	0-50
Mg	0.9732	0-30
Mn	0.9958	0-0.7
Na	0.9998	0-20
Ni	0.9986	0-0.1
Pb	0.9962	0-0.1
V	0.9964	0-0.1
Zn	0.9997	0-0.7

3.2.2. Detection limits and precision

In ICP-OES analyses, the method detection limits (MDL) of the measured elements were defined as the concentration of an element which will give a signal three times higher than the standard deviation of 10 replicate measurements of four reagent blanks belonging to four different sets of the experiments.

The precision of the instrument was determined from the five analyses of a reagent blank (Table 3). Some of them were reanalyzed on another day. The maximum relative standard deviation (RSD) was 2%. The MDL was low enough to determine all elements at the blank level by using ICP-OES. In terms of precision, very good results were obtained even at MDL concentration ranges.

Table 3. Detection limit and precision (Relative standard deviation, RSD (%)) of ICP-OES

Element	Detection limit ($\mu g\ L^{-1}$)	RSD (%)	Element	Detection limit ($\mu g\ L^{-1}$)	RSD (%)
K	5.0	0.16	Ni	2.3	0.56
Mn	0.14	0.34	Cr	1.6	1.2
Fe	9.0	1.1	V	0.94	0.34
Ca	21	2.0	Cd	0.22	0.46
Cu	1.4	0.32	Al	18	0.46
Zn	0.76	1.1	Mg	1.0	0.65
Pb	7.9	0.76	Na	30	0.79

3.2.3. Accuracy

The accuracy check of the method was performed using two certified reference materials. Additionally, the accuracy check was realized before starting the each set of measurements and repeated after approximately every 30 samples by checking the intensities.

The t-test was used to find out whether there was statistically significant difference between the means of the found and the certified values at 95% confidence level. The results of this test were shown with the significant level notation (S or NS) in Table 4. As it can be seen in the table, the average concentrations of V, Cr, Cu, Pb, Mn, Al, Ca and Fe determined by ICP-OES were very close to the certified values of IAEA-336 SRM within an error limit of less than 15%. In addition, the t-test also showed that there was no statistically significant difference between the means of both values for these elements: this was denoted as NS in the table. On the other hand, the average concentrations of Cd, Zn, Mg, Na and K were far from the average certified values of IAEA-336 SRM. In addition, the t-test indicated that there was a statistically significant difference between the means of the found and the certified values for the elements Cd, Zn, Na and K: this was indicated by S in the table. One must know that the referred certificate values for Cd and Mg in the SRM were only for information. The measured concentrations of Ni could not be evaluated since there is no recommended or information value for it in IAEA-336 SRM list. In case of Ca and K, significant difference is indicated by t-test at 95% confidence level; in case of Ca the difference is larger. In general our results were in better agreement with NIST-1571 SRM (except for Na) than IAEA-336 SRM. Sometimes concentrations of

some elements in SRMs may not be enough to be detected by ICP-OES. However, the results obtained for real samples by independent analytical techniques (e.g. ICP-OES with INAA) were compared and very good agreement was obtained [3].

Table 4. Accuracy results of the method checked with the SRMs IAEA-336 and NIST-1571 using ICP-OES

Elem.	IAEA-336[§]				NIST-1571[§]			
	Found + s	Cert. value +s	% Error,	S/NS[+]	Found +s	Cert. value +s	% Error,	N/NS[+]
Al	650 ± 60	680 ± 110	4.41,	NS	350 ± 70	320 ± 110	9.38,	NS
Ca	2640 ± 34	2600*	1.54		$1.54 \pm 0.03\%$	$2.04 \pm 0.07\%$	24.5,	S
Cd	0.159 ± 0.074	$0.117 \pm 0.017*$	35.9,	S	0.180 ± 0.072	0.162 ± 0.029	11.1,	NS
Cr	1.03 ± 0.24	$1.06 \pm 0.17*$	2.83,	NS	2.7 ± 0.3	2.6 ± 0.6	4.20,	NS
Cu	3.10 ± 0.60	3.60 ± 0.50	13.9,	NS	11.9 ± 0.2	11.8 ± 0.6	0.85,	NS
Fe	410 ± 50	430 ± 50	4.65,	NS	265 ± 28	280 ± 20	5.36,	NS
K	1410 ± 64	1840 ± 200	23.4,	S	$1.28 \pm 0.08\%$	$1.48 \pm 0.05\%$	13.5,	S
Mg	780 ± 30	610*	27.9		6090 ± 480	6000 ± 400	1.50,	NS
Mn	59 ± 6	63 ± 7	6.35,	NS	86 ± 4	86 ± 3	0.35,	NS
Na	265 ± 21	320 ± 40	17.2,	S	140 ± 23	88 ± 14	59,	S
Ni	1.52 ± 0.56	--	--		1.1 ± 0.5	1.5 ± 0.4	26.7,	NS
Pb	4.93 ± 1.02	$4.90 \pm 0.60*$	0.61,	NS	43 ± 5	44 ± 2	2.27,	NS
V	1.46 ± 0.17	$1.47 \pm 0.22*$	0.68,	NS	0.590 ± 0.100	0.510 ± 0.110	15.7,	NS
Zn	23.8 ± 2.0	30.4 ± 3.4	21.7,	S	22 ± 8	25 ± 2	12.0,	NS

* : Information value, [+] : S:significant difference, NS:no significant difference at 95% confidence level, [§]: unit is mg kg^{-1} unless otherwise is specified.

3.3. Estimation of Uncertainty

3.3.1. Identification of uncertainty sources in the analyses

The analyte concentration in the sample, expressed in mg kg^{-1}, was obtained from the equation 1:

$$\text{Concentration} = \frac{(C_A)(V_{final})}{m_{sample}} \tag{1}$$

Where C_A is the analyte concentration obtained from the calibration (in mg L^{-1}); V_{final} is the final volume (0.050 L) and m_{sample} is the weight (0.20x10^{-3} kg) of the investigated sub-sample.

3.3.2. Identification of standard uncertainties associated to each step

For the identification of the uncertainty sources, the use of the so-called "Cause and Effect diagram" is suggested. The diagram (Figure 2) helps to prevent an uncertainty contribution being incorporated into the budget more than once. Subsequently, it is possible to decide which are more significant and which are negligible. All repeatability parameters under each step are collected in one repeatability source.

The concentration results of metals were mainly affected by the following sources.
Standard solution preparation and its repeatability (including calibration curve (C_A))
Final volume of the sample digest (V_{final})
Sample weight (dry weight) (m_{sample})
Repeatability
Sampling

The combined uncertainty (in terms of relative uncertainty, $u_{rel}=u(X)/X$ can be calculated using the equation 2:

$$u_{rel}(CON) = \sqrt{u^2_{rel}(C_A) + u^2_{rel}(V_{final}) + u^2_{rel}(m_{sample}) + u^2_{rel}(Re\,p) + u^2_{rel}(Sampling)} \tag{2}$$

3.3.2.1. Estimation of the uncertainty derived from the estimation of the analyte concentration, u(C$_A$)

This is a combination of the uncertainties associated with the preparation of the stock solution and calibration curve. The uncertainty of stock solution (10 mg L^{-1}) preparation is also a combination of concentration uncertainty of element given in the certificate by the manufacturer at 95% confidence level (k=2), $u_{element}$, and uncertainty arising from the volumetric flasks and pipettes was used for dilution, u_V. The uncertainty of calibration standards are given as 1000 ± 3 (k=2, 95 % confidence level) for each element. The equation 4 was used to calculate the uncertainty arising from the volumetric containers. The standard deviation of 100 mL volumetric flask is 0.0144 mL. The volumetric calibration standard uncertainty of 1 mL automatic pipette, u_{vcal}, was calculated from the manufacturer reported CV value of 0.07 (i. e. u_{vcal}=(0.07/100)*1= 0.0007 mL). The uncertainty of the temperature effect was also included in the calculation of u_v. The calculations were performed using the equation 5 and the numeric results were given in Table 5 and Table 6.

$$u_{std} = \sqrt{(u_V)^2 + (u_{element})^2} \tag{3}$$

Table 5. Uncertainty calculation of dilution of stock solution with 100 mL volumetric flask.

Description	Value (X)	Uncertainty type	Factor	u(X)
Calibration	0.0144	standard deviation	1	0.0144
Temperature ($3*100*2.1 \times 10^{-4}$)	0.063	rectangular	1.73	0.0364
Combined uncertainty (u_v)				0.0386

Table 6. Uncertainty calculation of preparation of 10 mg L^{-1} stock solution

Description	Value (X)	u(X)	u(X)/X
100 mL	100	0.0386	0.000386
1 mL	1	0.00007	0.00007
Stock solution	1000	1.50	0.0015
Relative uncertainty			0.00155
Concentration (mg L^{-1})	10		
Standard uncertainty (u_{std})		0.016	

The uncertainty arising from the calibration curve was calculated by using the formula given in EURACHEM/CITAC guide [4]. The four calibration standarts were prepared and measured as 9 replicates.
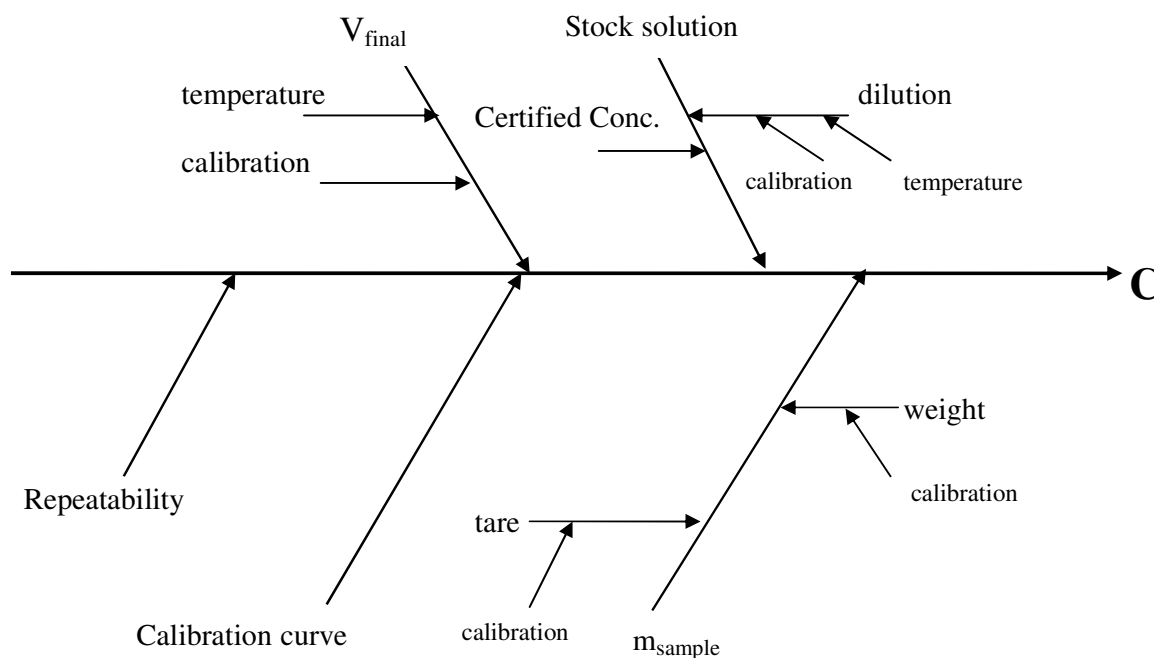
Figure 2. Cause and effect diagram for the analyses of elements in lichens

3.3.2.2. Estimation of the uncertainty derived from the dilution of the sample digest, u(V$_{final}$)

The uncertainty arising from dilution of sample is combination of both the temperature effect and the uncertainty in the calibration of volumetric flask.
The volumetric calibration standard uncertainty of 50 mL volumetric flask, u$_{vcal}$, was calculated from the standard deviation as 0.023 mL from manufacturer report.

The temperature effect describes the dispersion produced by a variation of liquid temperature, through a rectangular distribution [4], within ±3 °C around the calibration temperature.

$$u_{vtemp} = \frac{3(V)(Q)}{1.73} \quad (4)$$

Where, V is the measured volume (50 mL) and Q is the coefficient of volume expansion of the water (Q$_{water}$= 2.1x 10^{-4} °C^{-1}).
These sources were combined in the equation 4 and the calculations were summarized in Table 7:

$$u_V = \sqrt{(u_{vcal})^2 + (u_{vtemp})^2} \quad (5)$$

Table 7. Uncertainty calculation of dilution of sample digest with 50 mL volumetric flask.

Description	Value (X)	Uncertainty type	Factor	u(X)
Calibration	0.023	standard deviation	1	0.023
Temperature (3*50*2.1x10^{-4})	0.0315	rectangular	1.73	0.018
Combined uncertainty (u$_v$)				0.029

3.3.2.3. Estimation of the uncertainty derived from the sample weight, u(m$_{sample}$)

The balance uncertainty was obtained from the calibration certificate. The uncertainty was given as 0.0011mg at 95% (k=2) confidence level. This source of uncertainty is considered twice as the weighing process involves a difference. The numeric calculation results were given in Table 8.

Table 8. Uncertainty calculation arising from the sample weighing

Description	Value (X)	Uncertainty type	Factor	u(X)
Weight	0.0011	Normal	2	0.00055
Tare	0.0011	Normal	2	0.00055
Combined uncertainty (u(m$_{sample}$))				0.000778

3.3.2.4. Estimation of the uncertainty derived from the repeatability

The uncertainty from the repeatability covers the contributions from purity of acids, losses from digestion procedure, precision of calibration, stability of the instrument etc. Whole sample

preparation and analysis procedure were applied to five subsamples and the results of them were used in the calculations. Standard uncertainty for the repeatability was calculated by the formula of $RSD/\sqrt{n}$.

The numeric calculation results arising from analysis of Cd were given in Table 9.

Table 9. Uncertainty calculation arising from analyses of Cd

Parameter	Value (X)	u(X)	u(X)/X
Standard solution (mg L^{-1})	10	0.016	0.0016
Weight, m$_{sample}$ (mg)	200	0.000778	0.00000389
Sample volume, V$_{final}$ (mL)	50	0.029	0.00058
Repeatability	1	0.069	0.069
Calibration curve	30	0.773	0.026
Relative combined uncertainty			0.074
Measurement result (mg kg^{-1})	0.30		
Standard combined uncertainty		0.022	
Expanded uncertainty (k=2)		0.044	
Relative uncertainty (%)		14.7	

3.3.3. Identification of uncertainty sources derived from sampling, preparation and analysis

The input quantities of the sampling effects are not constituent parts of the model equation from which the measurement result was calculated. The new model equation for the overall process can be rewritten by putting the respective nominal correction factors on the analytical result (Figure 3):

$$x_{site} = \bar{x}_{analy} * f_{strategy} * f_{size} * f_{tree} * f_{morph} * f_{dry} \qquad (6)$$

x_{site}= measurement result
$\bar{x}_{analy}$ = mean from the analysis of sample
$f_{strategy}$= correction factor for bias due to sampling strategy
f_{size}= correction factor for lichen size effect
f_{morph}= correction factor for lichen morphology (apotechia, leafy part)
f_{tree}= correction factor for deviations from different tree barks
f_{dry}= correction factor for deviation of moisture content

3.3.3.1. Sampling strategy

Three sub-samples from three different grids were analyzed and the mean of the standard deviations was considered as standard uncertainty of the sampling strategy.

$$u_{strategy} = \bar{s}_{strategy} \qquad (7)$$

3.3.3.2. Lichen morphology

        Xanthoria parietina has an apparent morphology [5] as shown in Figure 1. In some of the lichen thallus, the leafy part of the lichen thallus is dominant, and in some of the lichen thallus, the apothecia part which are the cup shaped (or dishlike) fruiting body cells, in which fungus releases spores to find a free living algal cell and form a new lichen, is dominant. The same thallus was divided into two parts namely the apothecia and the rest of the thallus (leafy part). The whole thallus (mixture of two parts) and these two parts were digested and analyzed separately. Thus, for each grid, the CV was calculated using the mean and the standard deviation of three parallel measurements. Lichens collected in five grids were treated as mentioned to find the morphology effect. As a simple approximation, the mean of the standard deviations was applied as the standard uncertainty of the morphology effect.
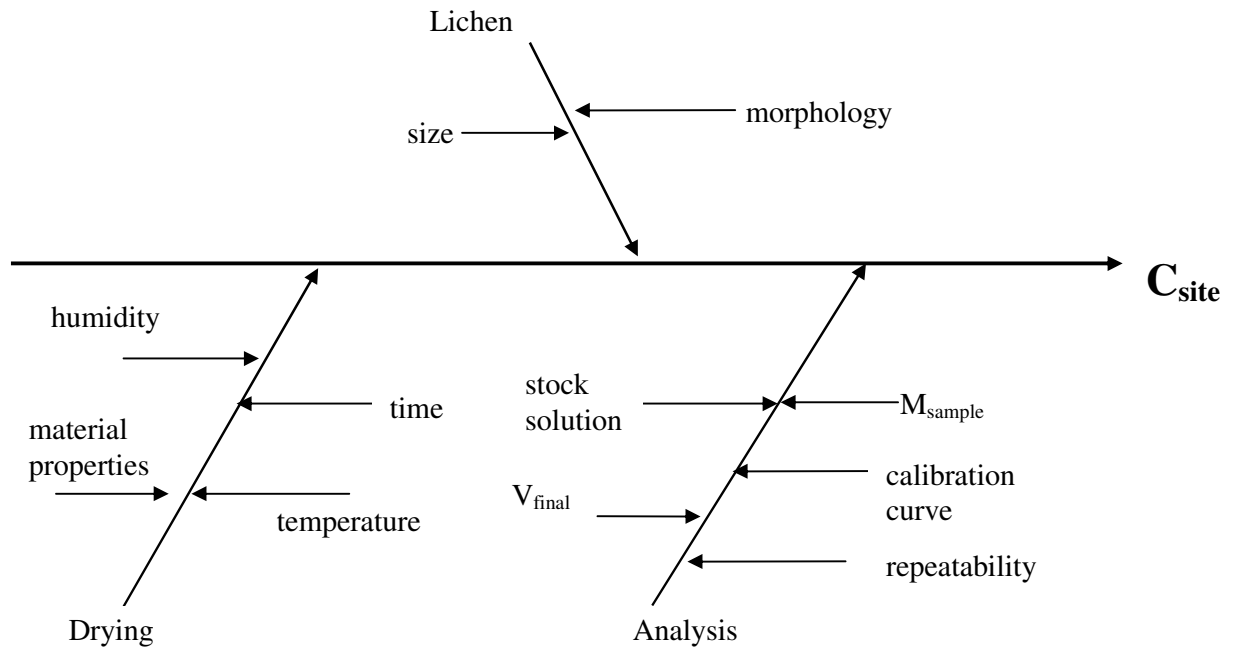
$$u_{morph} = \bar{s}_{morph} \tag{8}$$

Figure 3. Cause and effect diagram for the measurement at the site

3.3.3.3. Lichen size

        The lichen species, which were collected as a thallus in seven grids, were chosen for this purpose. After investigating of them it was seen that they can be grouped into two parts considering the diameters of the whole thallus [5]. The whole thallus diameter smaller than 2.5 cm (1 cm-2.5 cm) and whole thallus diameter greater than 2.5 cm (2.5 cm-4 cm) were cleaned, digested and analyzed separately. The lichen thalli of the 2 size ranges together with the mixed sample (combination of 2 sizes) were used for CV calculation. The equation 9 was used to calculate the uncertainty.

$$u_{size} = \bar{s}_{size} \tag{9}$$

3.3.3.4. Between-tree effect

The most common tree species from which lichens were collected was olive as olive production is very common in Aegean Region. The lichen samples in the same sampling point from different olive trees were analyzed separately in order to investigate the substrate effect. Lichens collected in four grids from olive trees were analyzed twice. Relative standard deviations between the duplicates of each of the four grids were calculated. The mean of the standard deviations was considered as the standard uncertainty of the between-tree effect.

$$u_{tree} = \bar{s}_{tree} \tag{10}$$

3.3.3.5. Drying

Lichens were air-dried after the sampling. In order to determine the drying effect, moisture content between 1 and 4 % has been found. No correction was required for the concentration measurements. However, a range of $\Delta x_{dry} = 3$ % difference in moisture content was considered for the uncertainty calculations. Assuming the rectangular distribution across this range, the standard uncertainty for all analytes was estimated as:

$$u_{dry} = \frac{\Delta x_{dry}/2}{\sqrt{3}} = 0.87 \text{ \%} \tag{11}$$

3.4. Interpretation of uncertainty results

The sample computation of uncertainty of Cd concentration at site (including sampling, preparation and analyses) was given in Table 10. The calculated expanded uncertainties U (k=2) of concentrations of elements arising from analyses and at site (sampling, preparation and analysis) are presented in Table 11 and the relative uncertainty of each source was shown in Figures 4 and 5. The relative standard uncertainties arising from sample volume, standard solution preparation and sample weight were not included in Figure 4 since they had the same values for all elements and the contributions from them were insignificant; as the repeatability and then the calibration curve significantly contributed to the uncertainty. If we include the uncertainties coming from the sampling then all sampling steps make the more important contributions to the uncertainty (Figure 5). Thus, the contributions from the analysis became the less important. Unfortunately, we can not reach the same conclusion for the element Cu, Pb and Ni due to the lack of data in the sampling strategy, lichen morphology and lichen size experiments (Figure 5).

Table 10. Uncertainty calculation arising from sampling, preparation and analyses for Cd

Parameter	Value (X)	u(X)	u(X)/X
f_{dry}	100	0.87	0.0087
$\overline{X}_{analy}$	100	7.4	0.074
f_{size}	100	13	0.13
f_{tree}	100	6.4	0.064
$f_{strategy}$	100	11.7	0.117
f_{morph}	100	15	0.15
Relative combined uncertainty			0.25
Measurement result (mg kg^{-1})	0.30		
Standard combined uncertainty		0.075	
Expanded uncertainty (k=2)		0.15	
Relative uncertainty (%)		50	

Table 11. The expanded uncertainties of all elements (k=2, 95% confidence level)

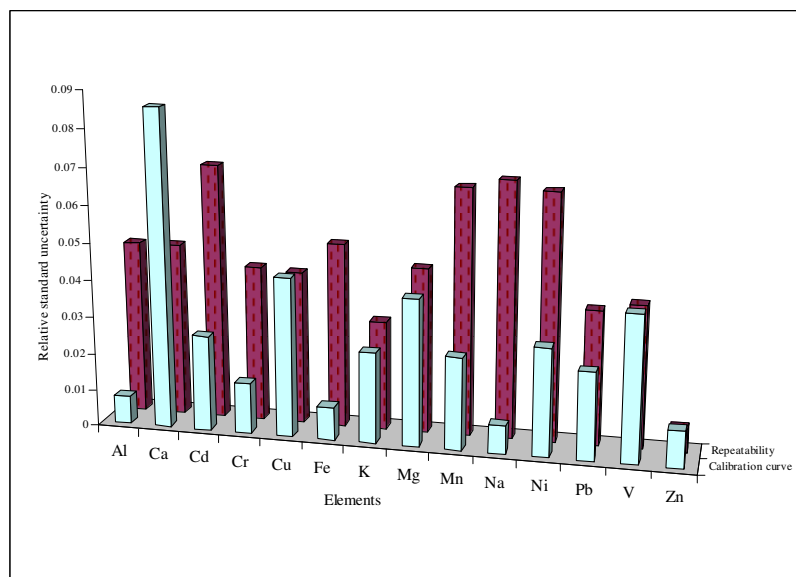| Element	Arising from analyses Concentration (mg kg^{-1}) with expanded uncertainties	Arising from sampling, preparation and analyses Concentration (mg kg^{-1}) with expanded uncertainties
Al	2000±190	2000±900
Ca	1500±290	1500±820
Cd	0.30±0.04	0.30±0.15
Cr	5.0±0.4	5.0±3.1
Cu	6.0±0.7	6.0±0.8
Fe	1200±120	1200±470
K	2500±190	2500±860
Mg	1500±180	1500±650
Mn	40±6	40±15
Na	200±80	200±80
Ni	4.0±0.6	4.0±0.6
Pb	9.0±0.8	9.0±0.9
V	4.0±0.4	4.0±2.1
Zn	40±2	40±14

Figure 4. Diagram of relative standard uncertainty of elements in the analysis
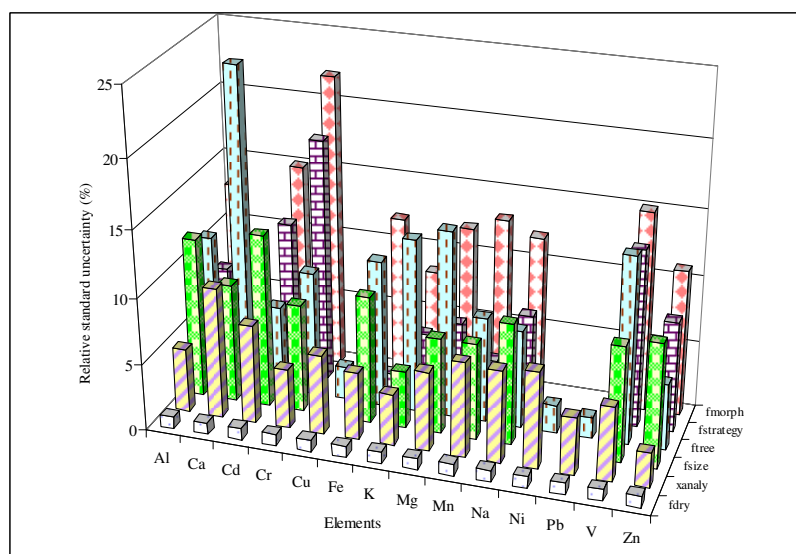

Figure 5. Diagram of relative standard uncertainty (%) of elements for the measurement at site

4. Conclusions

The application of the calculation of uncertainty arising from the sampling to the lichen data set which was completed in 2000 [1] showed that the sampling is the most curicial contributor to the uncertainty budget. Therefore, the uncertainty calculations involving the sampling and the sample preparation should be treated carefully. The sampling designs in the new guide [2] should be regarded and applied in designing the environmental sampling strategy from now on.

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