

## Determination of Elemental Concentrations in Lichens Using ICP-AES/MS

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**[Abstract]** Lichens are good biomonitors for air pollution because of their high enrichment capability of atmospheric chemical elements. To monitor atmospheric element deposition using lichens, it is important to obtain information on the multi-element concentrations in lichen thalli. Because of serious air pollution, elemental concentrations in thalli of lichens from North China (especially Inner Mongolia, Hebei, Shanxi and Henan province) are often higher than those from other regions, therefore highlighting the necessity to optimize ICP-AES/MS (Inductively coupled plasma-atomic emission spectroscopy/mass spectrometry) for analyzing lichen element content. Based on the high elemental concentrations in the lichen samples, and the differences in the sensitivity and detection limits between ICP-MS and ICP-AES, we propose a protocol for analyzing 31 elements in lichens using ICP-AES/MS. Twenty-two elements (Cd, Ce, Co, Cr, Cs, Cu, K, La, Mo, Na, Ni, Pb, Rb, Sb, Sc, Sm, Sr, Tb, Th, Tl, V and Zn) can be identified by using microwave digestion- ICP-MS, and 9 elements (Al, Ba, Ca, Fe, Mg, Mn, P, S and Ti) by using ashing-alkali fusion digestion- ICP-AES.

**Keywords:** Element content, Heavy metal, ICP-AES, ICP-MS, Lichens, Microwave digestion, Ashing-alkali fusion

**[Background]** Lichens have been widely used in biomonitoring of air pollution in many regions of the world, including China that experiences heavy-metal atmospheric pollution in some areas. Recent studies found that the element concentrations in lichens from North China are higher than or at the upper range of the literature values from other regions (Liu *et al.*, 2016a; 2016b and 2016c), due to the severe air pollution in this region.

Compared with other techniques (for example spectrophotometric methods, atomic absorption spectrometry and atomic fluorescence spectrometry), ICP-AES/MS is a multi-element analysis method involving simple procedure with relatively low detection limits. This method is widely used in the quantitative analysis of lichen elements outside China. However, to date, there have been no reports on the application of ICP-AES/MS to analyze elements in lichens of North China. Specific procedures for the sample preparation of the lichens are required due to the high loadings of elements with great variation in concentration. Therefore, to facilitate the biomonitoring of atmospheric elemental deposition with lichens in China, we propose a protocol for lichen elemental concentration analyses using ICP-AES/MS.

## **Materials and Reagents**

1. Paper bags
2. 10 mesh nylon standard inspection sieve (pore size 2 mm) (Shangyu Huafeng Hardware Instrument)
3. 50 ml plastic centrifuge tube (SARSTEDT, catalog number: 62.559.001)
4. National certified reference materials: GBW10010 (rice), GBW10014 (cabbage) and GBW10015 (spinach; All materials mentioned above were provided by the Institute of Geophysical and Geochemical Exploration, Chinese Academy of Geological Sciences). Lichen material IAEA-336 (International Atomic Energy Agency)
5. Lichens: *Xanthoria mandschurica* and *Xanthoparmelia mexicana*, collected from Taihang Mountains, Hebei Province, China
6. H<sub>2</sub>O<sub>2</sub>, > 30% (w/v) (Beijing Institute of Chemical Reagents, catalog number: 160215)
7. <sup>103</sup>Rh: 1.00 mg/ml (GSB-G62037-90, National Analysis Center for Iron & Steel, Beijing, China)
8. HCl, 35-37% (w/v) (Beijing Institute of Chemical Reagents, catalog number: 160718)
9. Standard stocking solutions: 1.00 mg/ml Al (GBW[E]086219), Ba (GBW[E]080243), Ca (GBW[E]080261), Cd (GBW08612), Ce (GSB04-1775), Co (GBW08613), Cr (G130008614), Cs (GSB04-1724), Cu (GSB04-1725), Fe (GBW08616), K (GBW[E]080125), La (GBW08651), Mg (GBW[E]080126), Mn (GSB04-1736), Mo (GBW[E]080218), Na (GBW[E]080127), Ni (GBW08618), P (GBW[E]080186), Pb (GBW08619), Rb (GSB04-2836), S (GBW[E]080266), Sb (GBW[E]080545), Sc (GBW[E]3141), Sm (GSB64-1778), Sr (GSB04-1754), Tb (GSB04-1781), Th (GBW[E]080174), Ti (GBW3041), Tl (GSBG62070-90), V (GBW[E]080243), and Zn (GBW[E]080607) (National Research Center for standard materials, Beijing, China)
10. Standard intermediate solutions (see Recipes; Table 8)
11. Standard working solutions (see Recipes; Table 8)

## **Equipment**

1. Inductively coupled plasma mass spectrometer (ICP-MS) (Agilent Technologies, model: Agilent 7700X ICP-MS)
2. Inductively coupled plasma optical emission spectrometer (ICP-AES) (Varian, model: Varian Vista MPX)
3. Microwave digestion system (equipped with Teflon digestion vessels) (CEM Corporation, Matthews, model: MARS X-press)
4. Grinding mill equipped with Tungsten Carbide jars (Retsch, model: Retsch MM400)
5. Stereo microscope (Motic, model: Motic SMZ-140)
6. Muffle furnace (Yiheng, model: SX2-4-10TP)
7. Oven (50-120 °C)
8. Tweezers

9. 50 ml volumetric flask
10. 30 ml nickel crucible (Jiangsu Plaza Premium Electric Instrument, catalog number: 30 ml crucible)
11. Deionized water: resistivity  $\geq 18.0$  M $\Omega$  cm (Aquapro International, model: Aquaplore3)

## **Procedure**

### A. Sample preparation

1. Sample preparation for microwave digestion – ICP-MS
  - a. The lichen samples (> 200 g per sample, fresh weight) are air-dried at room temperature in paper bags for at least 5 days. Carefully remove soil particles and other debris on lichen thalli under a stereo microscope using clean tweezers.
  - b. The cleaned samples are oven-dried at 70 °C for 72 h to constant weight (determined by weighting each sample at an interval of 8-12 h). Cool down the samples to room temperature and ground each sample to powder in a grinding mill, then pass each sample through a 10 mesh nylon sieve.
  - c. Transfer an accurately weighted portion of each sample (0.3000 g) to a Teflon digestion vessel. Add 4.0 ml HNO<sub>3</sub> to the vessel and incubate for 4 h at room temperature. Then add 2.0 ml H<sub>2</sub>O<sub>2</sub>, and transfer the digestion vessel to a microwave digestion system (MARS-X), applying the following temperature control procedure (Table 1).

**Table 1. Temperature control procedure for microwave digestion**

Step	Temperature rising time (min)	Temperature (°C)	Time duration (min)
1	10	110	10
2	5	160	8
3	10	190	10

- d. After digestion, when the pressure inside the vessel lowered to normal pressure and the vessel is cooled down to room temperature, open the vessel and wash the sample into a 50 ml volumetric flask by using deionized water (20-40 ml). Dilute the sample to 50 ml with deionized water for ICP-MS testing.
- e. Prepare blank samples and certified reference material samples (IAEA-336, GBW10010, GBW10014 and GBW10015) according to the above mentioned procedures.
2. Sample preparation for dry ashing-alkali fusion digestion – ICP-AES
  - a. Transfer an accurately weighted portion of each sample (1.0000 g) of the lichen (obtained in step A1b) to a nickel crucible and then all samples are heated to 500 °C until they turn grayish white (and therefore are fully ashed).
  - b. Add 1.5 g of NaOH to decompose each sample at 700 °C for 10 min. Cool down all samples to room temperature and wash each sample into a 50 ml plastic centrifuge tube using hot deionized water (70-80 °C, 20-30 ml). To ensure complete transfer of each

sample, also wash the nickel crucible according to the method mentioned above. Dilute the solution in the tube to 50 ml and shake it well before ICP-AES testing.

- c. Prepare blank samples and certified reference material samples (IAEA-336, GBW10010, GBW10014 and GBW10015) according to the procedures mentioned above.

**B. Measurement of element concentrations in lichens**

**1. ICP-MS analysis**

- a. Set the optimal operating conditions for ICP-MS (Table 2).

**Table 2. Optimal operating conditions of ICP-MS**

Parameter	Set value	Parameter	Set value
Power	1,300 w	Lifting rate	1.0 ml/min
Cooling gas flow rate	14 L/min	Sample depth	6.9 mm
Carrier gas flow rate	1.15 L/min	Integral time	0.5 sec
Compensated gas flow rate	0.55 L/min	Collection times	3 times

- b. Measure twenty-two elements (Cd, Ce, Co, Cr, Cs, Cu, K, La, Mo, Na, Ni, Pb, Rb, Sb, Sc, Sm, Sr, Tb, Th, Tl, V and Zn) in samples obtained in step A1d using ICP-MS. Select the isotopes of the 22 elements for analysis to remove isotopic interference (Table 3).

**Table 3. Isotope of 22 elements for ICP-MS analysis**

Element	Mass number	Element	Mass number	Element	Mass number	Element	Mass number
Cd	114	K	23	Rb	85	Th	232
Ce	140	La	139	Sb	121	Tl	205
Co	59	Mo	95	Sc	45	V	51
Cr	52	Na	39	Sm	147	Zn	66
Cs	133	Ni	60	Sr	88		
Cu	63	Pb	208	Tb	159		

- c. Add 5 µg/L of the internal standard <sup>103</sup>Rh into each sample to remove physical interference in the ICP-MS analysis.
- d. The linear regression equation and correlation coefficient are determined using standard working solution series (see Recipes; Table 8), and are given by the machine (Table 4). The concentration (µg/ml) for each element is also given by the machine.

**Table 4. Parameters of the standard curves and detection limits for elements measured by ICP-MS**

Element	Linear regression equations	Correlation coefficients	Linear ranges (µg/ml)	Detection limits (µg/ml)
Cd	$Y=6.025 \times 10^6X + 1.194 \times 10^3$	0.9999	0-0.1	0.00007
Ce	$Y=2.131 \times 10^7X + 2.343 \times 10^3$	0.9995	0-1	0.00002
Co	$Y=2.082 \times 10^7X + 3.094 \times 10^3$	1.0000	0-0.1	0.00010
Cr	$Y=1.639 \times 10^7X + 1.610 \times 10^4$	0.9997	0-1	0.00017
Cs	$Y=2.016 \times 10^7X + 1.041 \times 10^4$	0.9999	0-0.1	0.00006
Cu	$Y=1.235 \times 10^7X + 2.379 \times 10^4$	0.9993	0-1	0.00038
K	$Y=3.140 \times 10^7X + 2.307 \times 10^6$	0.9990	0-100	0.0052
La	$Y=2.206 \times 10^7X + 2.702 \times 10^3$	0.9995	0-0.1	0.00003
Mo	$Y=3.224 \times 10^6X - 2.020 \times 10^1$	0.9997	0-0.1	0.00093
Na	$Y=1.450 \times 10^8X + 8.746 \times 10^5$	0.9993	0-30	0.0051
Ni	$Y=5.076 \times 10^6X + 1.865 \times 10^3$	0.9999	0-1	0.00007
Pb	$Y=3.680 \times 10^7X - 9.256 \times 10^2$	0.9997	0-1	0.00031
Rb	$Y=1.335 \times 10^7X + 1.228 \times 10^2$	0.9999	0-30	0.00003
Sb	$Y=6.410 \times 10^6X + 8.018 \times 10^2$	0.9999	0-0.1	0.00008
Sc	$Y=1.591 \times 10^7X - 8.921 \times 10^2$	0.9995	0-0.1	0.00009
Sm	$Y=3.954 \times 10^6X + 3.806 \times 10^2$	0.9996	0-0.1	0.00004
Sr	$Y=1.814 \times 10^7X + 2.331 \times 10^4$	0.9997	0-30	0.00011
Tb	$Y=3.002 \times 10^7X + 3.621 \times 10^3$	0.9996	0-0.1	0.00004
Th	$Y=2.953 \times 10^7X - 1.085 \times 10^4$	0.9997	0-0.1	0.00004
Tl	$Y=2.666 \times 10^7X - 4.871 \times 10^3$	0.9999	0-0.1	0.00008
V	$Y=1.800 \times 10^7X - 1.130 \times 10^4$	0.9997	0-1	0.000052
Zn	$Y=3.165 \times 10^6X + 4.528 \times 10^3$	0.9995	0-5	0.00095

2. ICP-AES analysis

- a. Set the optimal operating conditions for ICP-AES (Table 5).
- b. Measure nine elements (Al, Ba, Ca, Fe, Mg, Mn, P, S and Ti) in the samples obtained in step A2c using ICP-AES. The analytical spectral lines for these elements are listed in Table 6.
- c. The linear regression equation and correlation coefficient are determined using standard working solution series (see Recipes; Table 8), and are given by the machine (Table 7). The concentration (µg/ml) for each element is also given by the machine.

**Table 5. Optimal operating conditions of ICP-AES**

Parameter	Set value	Parameter	Set value
Power	1.2 kW	Integral time	10 sec
Plasma gas flow rate	15.0 L/min	Washing time	20 sec
Auxiliary gas flow rate	1.5 L/min	Sample delay time	15 sec
Nebulizer pressure	200 kPa	Instrument stabilization delay	10 sec
Observation height	10 mm	Reading times	3 times

**Table 6. Analytical spectral lines of the elements**

Elements	$\lambda$ /nm	Elements	$\lambda$ /nm
Al	394.401	Mn	257.609
Ba	233.527	P	213.617
Ca	317.933	S	180.731
Fe	259.940	Ti	334.94
Mg	279.079		

**Table 7. Parameters of the standard curves and detection limits for elements measured by ICP-AES**

Element	Linear regression equations	Correlation coefficients	Linear ranges ( $\mu\text{g/ml}$ )	Detection limits ( $\mu\text{g/ml}$ )
Al	$Y=3.013 \times 10^7X + 9.316 \times 10^4$	0.9998	0-100	0.0054
Ba	$Y=2.609 \times 10^6X + 3.611 \times 10^3$	0.9996	0-5	0.0004
Ca	$Y=8.541 \times 10^4X + 8.143 \times 10^2$	0.9994	0-100	0.0053
Fe	$Y=2.032 \times 10^7X + 8.088 \times 10^5$	0.9992	0-100	0.0040
Mg	$Y=1.756 \times 10^7X + 4.225 \times 10^5$	0.9994	0-30	0.0049
Mn	$Y=1.945 \times 10^7X + 2.161 \times 10^4$	0.9997	0-5	0.00014
P	$Y=1.539 \times 10^6X + 1.424 \times 10^4$	0.9996	0-30	0.013
S	$Y=8.504 \times 10^4X + 1.958 \times 10^5$	0.9993	0-30	0.0053
Ti	$Y=1.369 \times 10^6X + 9.683 \times 10^2$	0.9991	0-30	0.00060

### C. Evaluation of the optimized methods

#### 1. Linear range and detection limit of the optimized methods

Dilute the standard working solution for each element by 8% HNO<sub>3</sub> using serial dilution (see Recipes). Detection limits were determined by 8 technical replicates of blank solutions.

The linear regression equation, linear correlation coefficient, linear range and detection limit of 22 elements (Cd [linear ranges, 0-0.1  $\mu\text{g/ml}$ ; detection limits, 0.00007  $\mu\text{g/ml}$ ], Ce [0-1; 0.00002], Co [0-0.1; 0.0001], Cr [0-1; 0.00017], Cs [0-0.1; 0.00006], Cu [0-1; 0.00038], K [0-100; 0.0052], La [0-0.1; 0.00003], Mo [0-0.1; 0.00093], Na [0-30; 0.0051], Ni [0-1; 0.00007], Pb [0-1; 0.00031], Rb [0-30; 0.00003], Sb [0-0.1; 0.00008], Sc [0-0.1; 0.00009], Sm [0-0.1; 0.00004], Sr [0-30; 0.00011], Tb [0-0.1; 0.00004], Th [0-0.1; 0.00004], Tl [0-0.1; 0.00008], V [0-1; 0.000052], and Zn [0-5; 0.00095]) analyzed by ICP-MS can be seen in Table 4, of 9 elements (Al [Linear ranges, 0-100; detection limits, 0.0054], Ba [0-5; 0.0004], Ca [0-100; 0.0053], Fe [0-100; 0.004], Mg [0-30; 0.0049], Mn [0-5; 0.00014], P [0-30; 0.013], S [0-30; 0.0053], and Ti [0-30; 0.0006]) analyzed by ICP-AES can be seen in Table 7. The results show that both methods had low detection limits and wide linear ranges.

#### 2. Accuracy and precision of the methods

Prepare and decompose the certified reference materials (IAEA-336, GBW10014 and GBW10015) followed steps A1 and A2. The sample is analyzed with 8 technical replicates using ICP-AES/MS under optimal operating conditions (Tables 2 and 5). A comparison of the measured values with certified values is shown in Tables S1 and S2 for ICP-MS and ICP-AES analysis, respectively. The results show that both methods have a good accuracy (relative error < 10%) and precision (relative standard deviation ranging from 1.56% to 9.55%).

## **Data analysis**

Accuracy and precision of the protocol for each element are obtained using Equation 1 and 2 (based on 8 technical replicates), respectively.

$$\text{Calculation of accuracy } RE = \frac{C_j - C_s}{C_s} \times 100 \quad (\text{Equation 1})$$

$$\text{Calculation of precision } RSD = \frac{\sqrt{\frac{\sum_{i=1}^n (C_i - C_j)^2}{n-1}}}{C_j} \times 100 \quad (\text{Equation 2})$$

In both equations,  $C_j$  denotes the mean of certified reference material values based on  $n$  technical replicates;  $C_s$  denotes certified values of the certified reference material;  $C_i$  denotes the measured value of the certified reference material.

## **Notes**

1. Lichen samples should be carefully cleaned to remove the attached particles on the surface of the lichen thalli to avoid inaccuracy in the determination of the element concentration.
2. Sample preparation is essential in this protocol. The classic preparation methods for plant samples are dry ashing, wet digestion, and microwave digestion. The wet digestion method is less suitable for our samples, because of the cumbersome, time-consuming operations, high risk of contamination, and high blank values leading to low precision. Although all elements can be analyzed by using both microwave digestion- ICP-MS and dry ashing-alkali fusion digestion- ICP-AES, we propose that it is better to analyze 22 elements (Cd, Ce, Co, Cr, Cs, Cu, K, La, Mo, Na, Ni, Pb, Rb, Sb, Sc, Sm, Sr, Tb, Th, Tl, V and Zn) using ICP-MS and 9 elements (Al, Ba, Ca, Fe, Mg, Mn, P, S and Ti) using ICP-AES. Microwave digestion with the  $\text{HNO}_3\text{-H}_2\text{O}_2$  system is effective for most refractory elements, and the closed vessel helps in preventing losses of the volatile elements. Although the dry ashing method can lead to a lower result due to the loss of some volatile elements at high temperature, it can be used to prepare samples for analyzing 9 elements (Al, Ba, Ca, Fe, Mg, Mn, P, S and Ti) using ICP-AES analysis.
3. Because of the high content of metals in lichens, the lichen samples should be soaked in  $\text{HNO}_3$  for 4 h, and then be digested by adding  $\text{H}_2\text{O}_2$ . If the sample is difficult to be completely digested, the sample size should be reduced and the soaking duration should be prolonged to overnight.
4. This protocol is suitable for lichens collected from North China, particularly Inner Mongolia, Shanxi, Hebei and Henan Provinces which are characterized by heavy air pollution.

## Recipes

1. The standard intermediate solutions and standard working solutions for the measured elements are listed in Table 8.

**Table 8. The standard intermediate solutions and standard working solutions for the measured elements**

Element	ICP-MS								
	Standard intermediate solution ( $\rho$ , $\mu\text{g/ml}$ )			Standard working solution ( $\rho$ , $\mu\text{g/ml}$ )					
	A	B	C	1	2	3	4	5	6
Cd	100	10	1	0	0.001	0.01	0.05	0.10	-
Ce	100	10	1	0	0.001	0.01	0.05	0.10	-
Co	100	10	1	0	0.001	0.01	0.05	0.10	-
Cr	100	10	1	0	0.01	0.1	0.5	1.0	-
Cs	100	10	1	0	0.001	0.01	0.05	0.10	-
Cu	100	10	1	0	0.01	0.1	0.5	1.0	-
K	-	-	-	0	5	10	20	40	60
La	100	10	1	0	0.001	0.01	0.05	0.10	-
Mo	100	10	1	0	0.001	0.01	0.05	0.10	-
Na	-	-	-	0	1	5	10	20	30
Ni	100	10	1	0	0.01	0.1	0.5	1.0	-
Pb	100	10	1	0	0.01	0.1	0.5	1.0	-
Rb	100	10	1	0	0.01	0.1	0.5	1.0	-
Sb	100	10	1	0	0.001	0.01	0.05	0.10	-
Sc	100	10	1	0	0.001	0.01	0.05	0.10	-
Sm	100	10	1	0	0.001	0.01	0.05	0.10	-
Sr	100	10	1	0	0.01	0.1	0.5	1.0	-
Tb	100	10	1	0	0.001	0.01	0.05	0.10	-
Th	100	10	1	0	0.001	0.01	0.05	0.10	-
Tl	100	10	1	0	0.001	0.01	0.05	0.10	-
V	100	10	1	0	0.01	0.1	0.5	1.0	-
Zn	100	10	1	0	0.01	0.1	0.5	1.0	-
Element	ICP-AES								
	Standard intermediate solution ( $\rho$ , $\mu\text{g/ml}$ )			Standard working solution ( $\rho$ , $\mu\text{g/ml}$ )					
	A	B	C	1	2	3	4	5	6
Al	-	-	-	0	5	10	20	40	60
Ba	100	-	-	0	0.5	1.0	2.0	4.0	6.0
Ca	-	-	-	0	5	10	20	40	60
Fe	-	-	-	0	5	10	20	40	60
Mg	-	-	-	0	1	5	10	20	30
Mn	100	-	-	0	0.5	1.0	2.0	4.0	6.0
P	-	-	-	0	1	5	10	20	30
S	-	-	-	0	1	5	10	20	30
Ti	-	-	-	0	1	5	10	20	30

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