Pak J Sci Ind Res 2000 ***43****(3) 162-167*

MINERAL ACID EFFECT ON THE TRACE ELEMENT DETERMINATION IN LICHEN CERTIFIED REFERENCE MATERIAL AND ALGAE BY INDUCTIVELY COUPLED PLASMA ATOMIC EMISSION SPECTROMETRY

F O Bamiro*, Z Benzo, H Schorin, E Marcano and C Gomez

Department of Chemistry, University of Agriculture, Abeokuta, Nigeria

(Received 9 September 1997; accepted 11 November 1999)

A study has been made of mineral acid matrix interferences in trace element determinations and methods are described for the multi-element analysis of lichen and algae (biomonitors) using Inductively Coupled Plasma Emission Spectrometry (ICP-AES). Several calibration strategies were compared employing aqueous standards with and without acids using modified ICP instrumental parameters. There was marked depression in analyte signal intensity in all the measurements. It has been shown that signal depressions of up to 32% can be observed under normal operating conditions. The use of acid matrix-matched standards and internal standard for calibrations substantially reduced signal depression problem. The methods gave elemental recovery from 97.7 to 98.8%. The accuracy of the methods was assessed by analysing the certified lichen material from the International Atomic Energy Agency (IAEA). Accurate results were obtained by using the above calibration strategy with improved precision.

Key words. Acid interference, Certified reference material, Algae, Lichen.

Introduction

Lichen and algae are among the biological materials used as biomonitors in pollution studies. Lichens accumulate air pollutants over long period of time and their use in the highly polluted zones usually furnish data to understand the fluctuations in atmospheric pollution for relatively short periods of time and for small volumes of air. Many investigations have been carried out around smelters, power plants and urban centres using lichens as biomonitors (Steinnes 1980; Bargali *et al* 1987; Gordon *et al* 1995; Dongara and Varrica 1998). The use of other biomonitors like algae, seagrasses, mussels (Phillips 1979; Evans and Hutchinson 1996; Xie *et al* 1996; Roberts *et al* 1998; Palmieri *et al* 1998) have also been widely used to establish variations in the bioavailability of trace elements in the environment.

Atomic Absorption Spectrometry (AAS) and ICP-Mass Spectrometry (ICP-MS) have been extensively used to analyse trace elements in biological samples. Mineral acids are widely used for sample digestion and preservation. A suitable dissolution method for biological sample to yield homogenous solution is a crucial first step in many spectrochemical analyses. The acid digestion under high temperature and high pressure in a sealed vessel has been extensively used for ultratrace analysis. The acid medium is one of the most commonly used matrix in ICP-AES. However, depressive effects resulting from the addition of acids have been reported in ICP-AES (Thompson and Walsh 1989; Montaser and Golightly 1992). The depression in the line intensities has been attributed to: (i) Changes in the rate of sample uptake due to different physical properties of the solution (Green *et al* 1976; Dahlquist and Knoll 1978; Ishii and Satoh 1983; Wandt *et al* 1984; Farino *et al* 1987) (ii) Variation in the aerosol transport efficiency (Maessen *et al* 1982; Ishie and Satoh 1983; Shen and Chen 1983) and (iii) changes in plasma excitation conditions due to the increased energy consumption for acid atomization (Maessen *et al* 1982; Chudinov *et al* 1989; Yoshimura *et al* 1990). Chudinov *et al* (1989) have indicated that the acid effect depends on the type of acid and the depression effect increases in the following order HCl < HNO_3 < $HClO_4$ < H_3PO_4 < H_2SO_4. With respect to cause (ii), Farino *et al* (1987) measured the analyte transport efficiency in natural uptake mode, for different acid concentrations and concluded that, for a given acid concentration, the mass of analyte transported were the same for all the analysis and that this mass was higher for the solutions with no acids.

Several methods have been proposed to correct this interference in atomic spectrometry (Shen and Chen 1983; Wandt *et al* 1984; Garden *et al* 1991) the most common being matrix matching, use of internal standard and standard addition methods. Some other correction methods have also been used,

---

* Author for correspondence.

normalizing the signal with respect to the analyte transport rate (Farino *et al* 1987), using mathematical methods (Delijska and Vouchkov 1985), normalizing the signal with respect to the dispersed energy and increasing the RF power by 20-30 watts, while using ionic lines (Budic and Hudnik 1994).

The present work, was carried out in order to assess the interference of the mineral acids used during the digestion of the algae samples for rapid multi-element determination of Zr, Mg, Al, Cu, Cr, Mn and Fe by ICP-AES. Attempts were made to reduce acid interference effects to tolerable level by optimising instrumental parameters. The accuracy of such established method was assessed using the certified reference lichen material from the International Atomic Energy Agency (IAEA-336)

Materials and Methods

A simaltaneous Perkin-Elmer Optima 3000 spectrometer, 40 Mhz, 1.5 KW with a Segmented array Charge coupled-device Detector (SCD) was used for all ICP studies. The standard solutions were prepared by suitable dilution of the stock standard solutions with or without mineral acids (HCl, HNO_3, $HClO_4$, H_2SO_4). All glassware was cleaned in nitric acid prior to use. High purity (99.95%) argon was utilized.

Three algae samples meant for quality control were obtained from the International Atomic Energy Agency (IAEA), Austria, as part of the International Quality Control Scheme. Lichen was used as a standard reference material (IAEA-336) to estimate the accuracy of the analytical method. The algae (IAEA-390) were used as sample for analysis. The algae set consists of three algae which were prepared by the Analytical Quality Control Services of the IAEA in cooperation with the Institute of Microbiology, Academy of Sciences of the Czech Republic in Trebon, during 1995. The set (IAEA—390) consists of (i) IAEA—391, a low level algae grown under reduced levels of toxic elements, (ii) IAEA-392 (environmental level), an algae grown under standard culture conditions and (iii) IAEA—393 (elevated level), an algae grown where some toxic elements were added to the nutrient medium during the production period.

The sample digestion protocol used here for algae was according to methods described by Maher (1983). One g of dry sample was allowed to digest in 8 ml of 65% v/v HNO_3 for 16 h. The digest was heated until the evolution of brown fumes ceased. After cooling, 10ml of a acid mixture containing HNO_3 (65% v/v), $HClO_4$ (70% v/v), and H_2SO_4 (98% v/v) in the ratio of 15:3:2 respectively was added, and the mixture was heated to white fumes. The digest was diluted with 5 ml of deionised water and 1 ml of HCl (30% v/v). The mixture was cooled and the contents were diluted to a final volume of 50 ml with deionised water, which gave a total acid concentration of 31% v/v HNO_3, 2% v/v HCl, 3% v/v $HClO_4$ and 2% v/v H_2SO_4 in the mixture containing the analytes.

The plasma power, the carrier gas flow, the plasma gas flow and observation height could be adjusted independently by automatic means from the computer module of the ICP unit. The optimisation of the ICP parameter was in the order: carrier gas flow, RF power, plasma gas flow and observation height. Aqueous standard solutions of 0.5 μg ml^{-1} of each element were prepared separately without acid. A single solution of multi-element standard containing 0.5 μg ml^{-1} of each element was prepared with acids (HNO_3, HCl, $HClO_4$ and H_2SO_4). The concentration of the acids in the multi-element solution was in the same proportion as was in the samples. Both aqueous and reagent blanks were also prepared similarly. The normal ICP parameters set for multi-element analysis in our laboratory is

Table 1

ICP operational parameters*

Plasma gas flow	12 lit min^{-1}
Auxiliary gas flow	0.2 lit min^{-1}
Operating power	1.3 kw
Observation height above r.f coil	16 mm
Carrier gas flow (argon)	0.65 lit min^{-1}
Pump uptake rate	1.5 ml min^{-1}

* The parameters are set at compromise conditions for all element.

Table 2

SBR values and % signal depression for 0.5 μg ml^{-1} of each element in solution with and without acid at laboratory working ICP condition (Table 1).

Element	Wavelength (nm)	SBR	% Dep.
Zn	213.856	77	32
Mn	257.610	406	23
Fe	259.94	172	17
Cr	267.716	81	22
Mg	280.270	118	13
Cu	324.754	24	13
Al	396.152	88	10

$$SBR = \frac{\text{Net signal intensity in acid medium}}{\text{Back ground intensity in acid medium}}$$

$$\%\,Dep = \frac{\text{Net signal of element in aqueous — Net signal in acid medium}}{\text{Net signal in aqueous medium}}$$

% Dep = Percentage depression.

as shown in Table 1. The optimisation adopted in this work was based on the possibilities of removal of acid interference on the elements by varying carrier gas flows first, and keeping RF power (1.3 kw), plasma gas flow (12 lit mm^{-1}) and observation height (16 mm) constant (univariate method) with the values in Table 1. The net intensities for each element in aqueous and acidic medium were measured to determine depression in signal from aqueous value. The net signal to background ratio (SBR) of element in acid medium was determined for every set of ICP parameters. The SBR indicates gain or loss in sensitivity for any variation in ICP parameters.

Results and Discussion

The results in Table 2 showed a range of 10-32% signal depression for elements studied under our normal laboratory working ICP conditions shown in Table 1. The effects of the various ICP parameters on the signal to background ratio (SBR) and the signal intensity of each element were investigated as described under optimisation. The results obtained are given in Table 3. The results obtained by varying the power, carrier gas flow, and the observation height in the plasma only affect SBR without any significant improvement on the depression of the emission signals from the elements when compared with results in Table 2 under normal ICP conditions. In view of this, direct determination of elements in algae using strong mineral acids for digestion will show interference (depression) when aqueous standards are used.

Our results also seem to be in accordance with the conclusions of many investigators that related acid effects in ICP-AES to transport mechanisms: reductions in the sample uptake rate due to the change of surface tension (Greenfield *et al* 1976),

Table 3

Effect of ICP parameters on SBR values and % signal depression of 0.5 $\mu g\ ml^{-1}$ of each element in solution with and without acids.

Plasma parameters	Zn		Mn		Fe		Cr		Mg		Cu		Al	
carrier gas flow rate l^{-1} min^{-1}	SBR	% Dep	SBR	% Dep	SBR	% Dep	SBR	% Dep	SBR	% Dep	SBR	% Dep	SBR	% Dep
0.55	57	32	309	28	136	22	68	27	111	16	21	12	74	8
0.65	77	32	406	23	172	17	81	22	118	13	24	13	87	10
0.75	54	35	321	24	177	18	78	22	86	13	27	16	67	13
0.90	35	35	254	33	188	25	36	32	123	29	28	17	61	17
1.0	21	35	159	43	121	31	16	48	105	47	15	19	55	17
Power/KW														
1.10	77	29	312	25	168	19	70	22	82	15	33	10	51	11
1.20	75	32	370	24	172	18	72	21	89	12	27	10	52	9
1.30	79	32	407	23	176	17	81	22	119	13	27	13	88	10
1.40	72	27	432	28	153	22	86	26	127	16	26	10	62	9
1.50	63	29	404	26	149	21	59	25	101	13	26	11	22	7
Plasma gas flow rate/l min^{-1}														
12	76	30	412	21	174	19	79	18	116	14	26	11	82	9
13	75	27	407	24	170	21	81	22	119	16	24	13	87	11
14	73	31	401	26	167	22	77	19	109	18	23	14	79	14
15	70	28	392	25	169	24	74	24	114	20	21	15	77	13
Observation height/mm														
8	62	29	169	31	75	22	64	28	87	19	26	11	75	8
10	62	28	207	29	76	18	78	26	92	17	28	14	76	8
12	78	30	288	29	107	22	85	27	110	17	22	9	81	7
14	82	31	354	27	169	19	87	24	111	14	30	8	93	8
16	98	22	415	26	205	18	89	26	132	17	34	10	97	8

Dep, Depression; Zn (λ = 213.856nm); Mn (λ=257.610nm); Fe(λ=259.9nm); Cr (λ=267.716nm); Mg (λ=280.270nm); Cu(λ=324.754nm); Al(λ=396.152nm) intensity suppressions due to the decrease in the analyte mass transport to the ICP due to viscosity differences which influence the quality of the aerosol and particle dimensions (Farino *et al* 1987).

Two solutions of each element containing 0.5µg ml^{-1} each were prepared with and without acids. The acids are present in the exact concentration for the digestion of algae sample. Another solution was prepared containing 0.5 g l^{-1} of each element present as acidified multi element solution. Analyte signal recovery for each element in multi element solution was calculated as a percentage of the response obtained for aqueous and acidified analyte solutions respectively. The results for the elements are presented in Table 4. From these results, it can be seen that the recovery of the elements ranged from 76.2 to 93.8%, when aqueous solution was used. The recovery of the elements generally improved and increased within the range of 97.7 to 98.8% when acidified solution was used. It is clear that the procedure involving acid matrix matched standard resulted in a considerable improvement in all the element signal recovery and precision. However, it was noted that although, values of recovery increased to 98% of the expected value, there was still a deficit of 2%, which may be caused by some additional factor or achieved with higher concentration of analyte rather than 0.5µg ml^{-1} used in this experiment.

The acid digested lichen sample was analysed using matrix matched calibration standard solutions. The normal working conditions of the ICP in Table 1 were retained and employed for the lichen reference material along with the certified values as shown in Table 5. The results obtained were lower than the certified values for Fe, Mg, Mn and Zn, while Cr and Cu were close to the lower limit of the certified range. Only Al concentration was within the certified range. This is envisaged to be due to the wet-ashing of the lichen sample, where acids were added but not heated. This procedure might result in incomplete digestion of the lichen sample, hence lower values obtained in some elements.

In view of the poor results obtained for lichen (Table 5) using wet-ashing, it was decided to explore acid matrix matched aqueous calibration standards and dry-ashing (with heating) the lichen sample. The results obtained are shown in Table 6, whereby a good correlation was found between the experimental and the certified ranges for all the elements.

One of the methods proposed to compensate for the effects of acids on the analyte signal is to use appropriate internal standard (Garden *et al* 1991). Internal standardization has also been used to improve the precision (Schmidt and Slavin 1982). Even when some authors have reported that a single internal standard would not compensate for these variations

Table 4

Analyte signal recovery of each element in multi-element solution against each element in aqueous and acidified solutions respectively (using ICP conditions in Table 1)

Element	% Recovery of each element in aqueous solution	%RSD* intensity	%Recovery of each element in acidifed solution	%RSD* intensity
Zn	76.2	2.4	98.2	0.5
Mn	77.9	2.0	97.9	0.4
Fe	88.3	2.5	98.8	0.3
Cr	81.1	2.4	98.4	0.5
Mg	91.4	1.6	98.5	0.4
Cu	93.4	2.1	97.7	0.3
Al	93.8	2.6	98.3	0.5

*The RSD quoted is that of the element emission intensity and not the RSD of the recovery.

Table 5

Comparison of the results for the wet-ashing lichen certified reference material using acidified aqueous material.

Element	Certified range µg g^{-1}	Average recommended values µg g^{-1}	Found* µg g^{-1}	RSD(%)
Al	570-780	680	578	2.9
Cr	0.8-1.17	1.03	0.83	14.3
Cu	3.09-4.01	3.55	2.94	4.8
Fe	380-472	426	329.0	3.6
Mg	500-710	610	484.0	3.1
Mn	57.0-71.0	64	41.8	2.6
Zn	28.1-35.10	31.6	21.8	5.1

*Mean of three replicate runs.

Table 6

Comparison of the analytical results for the dry-ashing lichen certified reference material using acidified aqueous standards

Element	Found* µg g^{-1}	RSD(%)	Certified range µg g^{-1}
Al	605.0	1.2	570-780
Cr	1.03	5.30	0.8-1.17
Cu	1.03	5.30	0.8-1.17
Fe	409.0	4.00	3.09-4.01
Mg	589	2	500-710
Mn	66.17	1.50	57.0-71.0
Zn	32.0	4.6	28.1-35.10

* Mean of three replicate runs

(Dahlquist and Knoll 1976), some others (Schmidt and Slavin 1982; Garden *et al* 1991), have found this method useful using high concentration of acids. For comparison purpose, the internal standardisation method was also employed for the analysis of the lichen sample. Lanthanum (La) was used as the internal standard on the basis that the line 408.672nm does not interfere with the spectral lines of the analytes under study (Table 7).

The results show that all the elements fall within the concentration range. Hence, under plasma operating condition in this work and the digestion procedure adopted, the use of internal standardisation was found to be useful to compensate for acid interference.

Analysis of the algae samples. The internal standard or matrix matched method could successfully be used for the analysis of algae as achieved in this work. The analysis of algae was carried out using the later method. The results obtained in the three sets of algae are presented in Table 8. The algae sample results follow the trend of increasing trace elemental concentrations from algae 391 to 393, with the exception of copper and Mg which differ.

Table 7

Analysis of lichen certified reference material using La=408.672nm as the internal standardization

Element	Found* μg g^{-1}	RSD(%)	Certified range μg g^{-1}
Al	708	3	570-780
Cr	0.91	16	0.8-1.17
Cu	--	--	3.09-4.01
Fe	428	3	380-472
Mg	620	3	500-710
Mn	71	3	57.0-710
Zn	31	3	28.1-35.10

* Mean of three replicate runs

Table 8

Analytical results (μg g^{-1}) for the algae samples

Element	IAEA 391		IAEA 392		IAEA 393	
	Found*	RSD(%)	Found*	RSD(%)	Found*	RSD(%)
Al	10.52	5.24	26.3	3.16	69.60	3.58
Cr	1.67	8.80	4.19	5.80	311	2.86
Cu	46.22	3.27	21.44	2.13	11.92	8.60
Fe	300.5	1.09	497	6.44	1477	7.23
Mg	8188	0.13	2252	1.66	3831	8.67
Mn	32.23	3.48	59.22	2.67	154.82	3.11
Zn	44.35	0.92	139	7.7	207	8.02

* Mean of three replicate runs

Conclusion

This work has highlighted the depth of acid interference which gave 10-32% analyte emission signal depression for elements studied in lichen and algae. The alteration of plasma conditions was not found to reduce the level of interference effects. Application of acid matrix-matched standard method or the use of internal standardisation was established to be suitable for the determination of elements in lichen and algae. The methods of standard addition, signal normalization with respect to the uptake rate or analyte transport rate may be tedious and time consuming especially for multi element analysis.

A direct method has been established for the multi element determination in algae (biomonitors) which is simple and rapid, requiring dry ashing of the sample in acid and using acid matrix matching standard solutions for calibration. Using this technique, an analytical throughput of about 20 samples per hour could be achieved for multi element analysis with the same optimum conditions of the plasma.

References

Bargagli R, Josco F P, Barghigiani C 1987 Assessment of mercury dispersal in abandoned mining area by soil and lichen analysis. *Water Air Soil Pollut* **36** 219.

Budic B, Hudnik V 1994 Effect of potassium chloride and phosphoric acid in ICP-AES. *J Anal Atom Spectrom* **9** 53.

Chudinov E G, Ostroukhova I I, Varvanina G V 1989 Acid effects in ICP-AES. *Fresenius Z Anal Chem* **355** 25.

Dahlquist R L, Knoll J W 1976 *An interium Discussion of Acid Effects*. Hasler Research Centre, Applied Research Laboratories Report, California, 1 September.

Dahlquist R L, Knoll J W 1978 ICP-AES Analysis of biological materials and soils for major trace and ultra-trace elements. *Appl Spectrosc* **32** 1.

Delijska A, Vouchkov M 1985 Influence of solution uptake rate on signals and interference in ICP-OES. *Frezenius Z Anal Chem 321,* 448.

Dongara G, Varrica D 1998 The presence of heavy metals in air particulate at Vislcano Island (Italy) using lichen samples as biomonitors. *Sci Total Environ* **212** (1) 1-9.

Evans C A, Hutchinson T C 1996 Mercury accumulation in transplanted moss and lichens at high elevation sites in Quebec. *Water Air Soil Pollut* **90** (3-4) 475-486.

Farino J, Miller J R, Smith D D, Browner R F 1987 Possibilities for correcting the influence of mineral acids and of drift in ICP emission spectral analysis.*Anal Chem* **59** 2303.

Garden L M, Marshall J, Littlejohn D 1991 Correction of mineral acid interferences in ICP-OES on Cu and Mn using internal standardisation. *J Anal Atom Sepctrom* **6** 159.

Gordon C A, Herrera R, Hutchinson T C 1995. *J Trop Ecol* **2** (1) 1 1-26.

Greenfield S, Mc Geachin, Mc D H, Smith P B 1976 Nebulisation effects with acid solutions in ICP spectrometry. *Anal Chim Acta* **84** 67.

Ishii H, Satoh K 1983 Determination of rare earth in Lanthanum oxide by ICP emission derivative spectrometry without pretreatment. *Talanta* **30** 111.

Maher W A 1983 Determination of selenium in marine organisms using hydride generation and electrothermal atomic absorption spectroscopy. *Anal Lett* **16** 801-810.

Maessen F J, Balke J, De Boen J L 1982 Preservation of accuracy and precision in the analytical practice of low power ICP-AES. *Spectrochim Acta* **37B** 517.

Montaser A, Golightly D W 1992 *Inductively Coupled Plasma in Analytical Atomic Spectrometry,* 2nd ed, VCH Publisher Inc, USA, pp 278.

Palmieri F, Neri R, Benco C, Serracca L 1998 Lichens moss as biomonitors and bioaccumulators in air pollution monitoring. *J Environ Pathol Toxicol Oncol* **16** (2-3) 175-190.

Phillips D J H 1979 Trace metals in the common mussel, *Mytilus edulis* (L), and in alga *Fucus vesiculosus* (L) from the region of the sound (ORESUND). *Environ Pollut* **18** 31.

Roberts D E, Smith A, Ajani P, Davis A R 1998 Rapid changes in encrusting marine assemblages exposed to anthropigenic source pollution. *Mar Ecol Prog Ser* **163** 213-224.

Schmidt G J, Slavin W 1982 ICP emission spectrometry with internal standardisation and substraction of plasma background fluctuations. *Anal Chem* **54** 2491.

Shen X-e, Chen Q-I 1983 Effect of small amount of phosphoric acid in ICP-AES. *Spectrochim Acta* **38B** 115.

Steinnes E J 1980 Atmospheric deposition of heavy metals in Norway studied by the analysis of moss samples. *Radioanal Chem* **58** 387.

Thompson M, Walsh J N 1989 *Handbook of Inductively Coupled Plasma Spectrometry*, 2nd ed. Blackie Chapman and Hall, USA, pp 155-157.

Wandt M A, Pouget M A, Rodgers A 1984 Determination of Ca, Mg and P in human stones by ICP-AES. *Analyst* **109** 1071.

Xie J Z, Chang H L, Kilbane J J 1996 Removal and recovery of metal ions from waste-water using bio-sorbent and chemically biosorbent. *Bioresour Technol* **57** (2) 127-136.

Yoshimura E, Suzuki H, Yamazaki S, Toda S 1990 Interference by mineral acids in ICP-AES. *Analyst* **115** 167.