STUDIA UNIVERSITATIS BABEŞ-BOLYAI, CHEMIA, XLV, 1-2, 2000

TRACE ELEMENT MEASUREMENT BY ICP-AES FOR THE ROUTINE MULTI-ELEMENT ANALYSIS OF LICHEN AND SOIL SAMPLES FOR ENVIRONMENTAL POLLUTANT STUDIES

ANA-MARIA RUSU[1], KATALIN BARTOK[2], VICTOR DIN[3], WILLIAM PURVIS[4] and WILLIAM DUBBIN[3]

[1] Department of Chemistry, Babes-Bolyai University, Cluj-Napoca, Romania
[2] Department of Biology, Babes-Bolyai University, Cluj-Napoca, Romania
[3] Department of Mineralogy, The Natural History Museum, London, UK
[4] Department of Botany, The Natural History Museum, London, UK

ABSTRACT. Inductively-coupled plasma atomic emission spectrometry (ICP-AES) is a very important technique for the determination of trace element concentrations in environmental samples. Lichen and soil samples were collected from different polluted sites around Zlatna town, Romania. Contaminant elements in the samples were determined by ICP-AES after extraction from the soils using DTPA and TEA mixture and digestion of the lichens in HNO_3 + H_2O_2 mixture. Copper, Pb, Zn, Ni, Fe, Cd and S were determined in both soils and lichens. Mean and extreme values of the elements determined are listed. A certified reference material, BCR CRM 482 lichen, provided comparative data for the lichen samples. The methods described are rapid and easy to use for environmental pollution studies.

INTRODUCTION

Inductively-coupled plasma atomic emission spectrometry (ICP-AES) is widely recognised as a suitable technique for the determination of trace elements in environmental samples, particular because of its multi-element capability, large dynamic range and effective background correction. The technique is important for the determination of trace metal concentrations in environmental samples [1].

Airborne contaminants are usually measured with specialised instruments but, during the last two decades, lichens have increasingly been used as bioindicators of metal contaminants. Most work on the accumulation of heavy metals by lichens has focused on pollution from smelters, power plants, busy roads, urban sites and rural areas. Puckett [2] pointed out that lichens and bryophytes have certain characteristics which meet several requirements of ideal biological monitors. For example, they are geographically widespread, growing everywhere from rocky coasts to mountain summits, from Polar Regions to the tropics, on rocks, trees, soil, buildings, etc. This allows comparison of the concentrations of pollutant metals from diverse regions; they form a more-or-less constant, perennial body enabling accumulation to occur throughout the year. Puckett also mentioned the ability of lichens and bryophytes to accumulate metals to concentrations far above their expected physiological needs. Other characteristics that make lichens suitable as biomonitors are their very slow growth rates, their longevity and their rapid uptake and accumulation of cations and metal particulates [3].

The town of Zlatna, in the Apuseni Mountains of Romania, is built around a mineral processing plant and smelter. The plant processes a variety of complex, ores (mainly sulphides) for Cu, with Au, Ag, Pb, Zn, S, Bi, Cd and Mo as major by-products. Release of SO_2 and metals via the vapour phase and as fine particles of slag causes acid precipitation and metal contamination for more than 30 km down wind from the smelter [4]. On previous studies in Zlatna area, Bartok and co-workers [5] determined Pb, Mn, Cu and Zn in transplanted macrolichens; Purvis and co-workers [6] used microanalysis techniques for measuring Pb bioaccumulation in *Acarospora smaragdula* collected from the same area.

Environmental analyses are commonly carried out using established classical methods. In this paper, we would like to propose two rapid and easy methods for the determination of pollutant elements in lichen and soil samples using an ICP-AES instrument. The main aim of this work is to present methods for the analysis of environmental samples, which fulfil the following demands:

(i) many elements can be determined in the same digest
(ii) the sample preparation is simple and fast
(iii) the minimum volume of acid is used
(iv) calibration is preferably made by direct methods, not by the standard addition method, and
(v) applicable to a large variety of samples.

In addition, an ICP-AES method is described for the determination of trace elements in soil samples after extraction of those elements into DTPA/TEA reagent. Extraction is one of the most frequently used sample pre-treatment techniques applied to the determination of trace metals in environmental samples by ICP-AES. The extraction serves the dual purposes of concentrating the metals of interest and separating them from an interfering matrix. Isolation from the matrix significantly decreases any background signal caused by concomitants and the solids content of the solution. On other hand, the results obtained provide a general overview on air pollution patterns within the investigated area.

EXPERIMENTAL

Survey area. The sample sites are 200 -300m east and west of the factory chimneys, and along an east – west transect which runs from the town, along the Ampoi Valley, to a site 25km downstream. A map of the sites chosen was published in a previous paper [7]. The sites, listed in order of distance from the pollutant source are:

site 1 in Zlatna town (300m SSW of two smelter chimney)
site 2 in Zlatna town (opposite to site 1 at 500m NE from new factory)
site 3 in Patrangeni station (3.5km SW Zlatna)
site 4 opposite Patrangeni forest (4km WSW Zlatna, 300m below a calcareous stone with cross)
site 5 in Presaca Ampoiului (9km SW Zlatna)

site 6 Metes (16km WSW Zlatna)
site 7 Ampoita (25km WSW Zlatna).

Sampling. Soil and associated terricolous lichen, *Cladonia floerkeana* samples were collected from the sites. The soil samples (approximately 200g) were collected from the surface mineral horizon, after removing the organic layer, in locations free of growing plant material. These samples were air-dried, crushed to a fine consistency in agate mortar and sieved through an 80 mesh sieve. Only a small amount of lichen could be collected from some sites and it was not possible to collect any *Cladonia* in Zlatna town (sites 1 and 2) because this species was absent due to high levels of air pollution. *Cladonia floerkeana* was also absent from sites 6 and 7. All samples were collected during October 1998.

Sample preparation methods.

Reagents and standard solutions. Deionised water and, where possible, analytical grade or better reagents were used for all the procedures described.

Nitric acid (65% m/v), hydrochloric acid (37% m/v) and hydrogen peroxide (30% m/v) were used for the lichen digestions.

Soil extraction solution: 0.005M diethylenetriaminepentaacetic acid (DTPA), 0.01M calcium chloride and 0.1M triethanolamine (TEA) adjusted to pH 7.3 with HCl. To prepare 1 litre of this solution 14.92g of reagent grade TEA, 19.67g of DTPA and 1.47g of $CaCl_2 \cdot 2H_2O$ were dissolved in approximately 200mL of deionised water. The pH of this solution was adjusted to 7.3±0.05 with 1M HCl while stirring and it was then diluted to 1.0L with deionised water[8].

Stock multi-element standard solutions were prepared from 10,000µg.ml^{-1} single element, high purity standards. Working standard solutions were prepared daily by appropriate dilution of the intermediate solutions with deionised water.

Lichen dissolution procedure*.* Lichen thalli were cleaned carefully under a binocular microscope without washing. Samples were dried at 108^{0}C for 8 hours and ground. Samples of 50mg of dry weight were measured into borosilicate test tubes with reflux bulbs and digested in 3ml HNO_3 (concentrated) at 50^{0}C overnight, then for 3 hours at 120^{0}C, cooled and 1ml 30% H_2O_2 was added. The solutions were reheated to 50^{0}C for half hour, then to 120^{0}C until brown fumes were no longer evolved. The water-clear contents of the test tubes were filtered through Whatman No 42 filter paper and brought to a volume of 15ml with deionised water. The solutions were analysed for Pb, Cu, Zn, Fe, Cd, Ni, Ag, As and S.

Extraction procedure. 10g of the air-dried, sieved soil samples were weighed into 125ml conical flasks and 20ml of the DTPA extraction solution were added. Each flask was sealed with stretchable paraffin film and secured upright on a horizontal shaker with a speed of 120 cycles min^{-1}. After exactly 2 hours of shaking the suspensions were filtered by gravity through Whatman No 42 filter paper. The shaking time is very important because extraction is not complete after 2 hours, the labile and non-labile trace metals fractions will continue to dissolve.

The filtrates were analysed for the trace elements Cu, Ni, Zn, Pb, Fe, Cd and S by ICP- AES.

Instrumentation.

Table 1.

Operating conditions for the ICP.

Forward power	650 W
Reflected power	3-5W
Integration time	1 second
Plasma argon	0.8 $L.min^{-1}$
Auxiliary argon	7.5 $L.min^{-1}$
Carrier (nebuliser) argon	0.7 $L.min^{-1}$
Sample uptake rate	2.9 $mL.min^{-1}$, free aspirating

Analytical lines, nm

As: 189.042; Ag: 328.068; Cd: 228.802; Cu: 324.754;
Fe: 259.940; Ni: 231.604; Pb: 220.353; Zn: 213.856;
S: 182.037.

The determination of trace elements by ICP-AES in all of the samples investigated was performed using a model 3410 ARL "Minitorch" sequential spectrometer with pneumatic nebuliser (Meinhard concentric, type TR-30 – K3). The instrument was optimised for routine multi-element analysis by aspirating a solution containing 2$\mu g.ml^{-1}$ of each of the elements of interest. All measurements were background corrected. Linear working curves generated from five multi-element external standards were used for quantification. Lichen standard solutions were prepared in the same acid concentration as the samples, whilst blank solutions were prepared by following the steps of the sample dissolution procedure. Also, multi-element soil extract standards were prepared for quantification, every solution containing 2%HNO_3 and 400$\mu g \cdot mL^{-1}$ Ca. All measurements were made in duplicate for the samples and standard solutions. The instrument operating parameters are listed in Table 1.

RESULTS AND DISCUSSION

The results obtained for the BCR CRM 482 lichen sample [9,10] are listed in Table 2. The values measured for most elements are higher than the published certified concentrations, except for Zn. The high results for the elements measured were attributed to memory effects from preceding samples rich in those elements. Thus, the data presented in Table 3 show the high levels of contamination in lichens. However, as can be seen the metal concentrations determined by the proposed method are in satisfactory agreement with the certified values.

Table 2.

Measured and certified concentrations of elements ($\mu g \cdot g^{-1}$) in BCR CRM 482 lichen sample.

Element	Certified concentration ($\mu g \cdot g^{-1}$)	Measured concentration ($\mu g \cdot g^{-1}$)
Pb	41.0±0.2	49.7±0.9
Cu	7.0±0.2	7.8±0.3
Zn	101.0±3	64.0±3
Cd	0.56±0.02	0.60±0.03
Ni	2.5±0.07	3.2±0.05
As	0.85±0.07	1.28±0.2

Tables 3 and 4 show the results of our analysis of the lichen and soil samples collected from the area studied. Mean and extreme values of the data are listed. The concentrations of contaminant elements found in lichens and soils show remarkable differences according to the different exposures to air pollution in the Zlatna region. Relative standard deviations given in Tables 3 and 4 indicate spatial or biological variation of the mean values. The variation of the concentrations found within sites differ considerably. In all our results, we are confronted by high statistical variability that indicates regional differences in airborne heavy metal pollution. Besides the pollutant element content of soil, other ecological parameters of the habitats (pH, organic matter content, etc.) governing the uptake of metals have to be taken into account.

Conclusions

The analysis of soils for the evaluation of the contaminants level of an area is very important, as are also assessment of the bioavailabilty and uptake by organisms. Therefore the analysis of total metal contents in soils alone is not sufficient. Lichens, due to their morphological characteristics, yield reliable data on the distribution and concentration of contaminant elements. In fact, from these data it is possible to assess deposition rates and map variations in concentration across either small or large areas. All can be done at a very low cost when compared to the traditional physicochemical techniques. Certainly, to obtain comparable results (lichen – soil) more emphasis has to be put on standardising sampling and analysis procedures.

The concentrations of pollutant elements measured in the samples studied indicate high levels of pollution in the area of the smelter. Of the pollutants investigated, Pb was the most abundant in both soils and lichens. The smelting process to recover Cu from its ores results in much particulate matter escaping to atmosphere via the chimney of the plant. Significant amount of Pb plus small amount of Cd, Ni and As are released into the environment. Lichen samples exhibited element concentrations higher than that for soil samples. It is normal, because soils are leaching and lichens are leaching too and bioaccumulators same time are.

The proposed method is efficient for determination of elements in soil samples. The main advantage offered by the fast solvent extraction of elements prior to determination are the suppression of interferences and an increase in the sensitivity by pre-concentration of the analytes. The ability to analyse such contaminated soil samples, with little sample preparation will clearly benefit environmental waste remediation efforts. ICP-AES can be used for multielement analysis of contaminate lichen samples (in this study: Pb, Cu, Zn, Cd, Ni, Fe, Ag, As and S). The accuracy of ICP-AES has been shown to be acceptable for routine environmental soils and lichens work.

Table 3.

Minimum, maximum, mean and relative standard deviation of element contents (μg·g^{-1}) found in *Cladonia floerkeana* at selected sites.

Element	Statistic	Site		
		3	4	5
Pb	minimum	1377.0	317.2	832.1
	maximum	5399.0	354.0	2571.7
	mean	3254.0	331.1	1609.5
	RSD %	62	5	55
Cu	minimum	424.7	169.4	257.7
	maximum	575.1	328.4	387.6
	mean	483.0	245.1	306.3
	RSD %	17	75	23
Zn	minimum	192.8	159.4	195
	maximum	267.4	247.1	191.6
	mean	226.5	198.1	117.2
	RSD %	16	21	76
Cd	minimum	2.6	2.9	2.0
	maximum	4.0	6.4	4.6
	mean	3.3	4.1	3.2
	RSD %	21	39	42
Fe	minimum	934.0	1016.0	1031.6
	maximum	4440.3	1825.2	5948.8
	mean	2908.0	1489.0	3575.0
	RSD %	60	23	69
Ni	minimum	4.0	3.7	7.0
	maximum	6.0	5.6	9.3
	mean	4.8	4.7	8.0
	RSD %	22	18	14
Ag	minimum	5.2	4.1	3.7
	maximum	73.0	5.4	8.3
	mean	35.5	5.0	5.7
	RSD %	96	10	41
As	minimum	16.1	0	10.3
	maximum	46.3	9.3	12.6
	mean	28.0	5.7	14.2
	RSD %	57	88	35
S	minimum	1290.8	1158.5	1192.8
	maximum	2893.1	1640.4	1989.7
	mean	2046.0	1405.0	1575.0
	RSD %	39	74	25

Table 4.

Minimum, maximum, mean and relative standard deviation of element contents of soils ($\mu g \cdot g^{-1}$) from selected sites. (not determined: n.d.)

Element	Statistic	Sites						
		1	2	3	4	5	6	7
Pb	minimum	245.5	154.1	114.7	171.1	49.0	32.7	10.6
	maximum	491.2	270.1	399.6	264.1	317.1	113.6	43.1
	mean	411.3	202.0	256.8	232.7	178.9	73.2	26.9
	RSD %	21	28	37	17	87	78	85
Cu	minimum	171.4	74.5	83.0	105.7	23.0	20.6	3.2
	maximum	339.7	282.8	252.2	233.9	118.3	45.8	10.1
	mean	223.3	162.9	152.2	157.1	68.9	32.2	6.7
	RSD %	30	55	39	39	70	56	75
Zn	minimum	9.1	10.8	11.8	47.9	8.3	5.8	1.3
	maximum	39.3	136.2	31.5	73.4	27.3	9.6	19.6
	mean	19.9	64.8	21.9	65.5	15.8	6.7	10.5
	RSD %	50	80	36	18	63	45	123
Cd	minimum	0.18	0.09	0.12	0.61	0.11	0.08	0.12
	maximum	0.50	2.89	0.28	1.33	0.40	0.20	0.59
	mean	0.27	1.33	0.19	1.12	0.22	0.14	0.36
	RSD %	44	96	42	29	77	71	83
Fe	minimum	284.8	30.7	281.2	211.5	53.4	75.7	116.2
	maximum	481.9	113.7	469.5	231.1	522.6	254.0	214.2
	mean	354.5	78.8	374.8	226.7	325.0	164.9	165.6
	RSD %	20	51	19	5	75	76	42
Ni	minimum	0.11	0.14	0.11	0.74	0.13	0.18	1.02
	maximum	0.24	0.65	0.24	1.73	1.02	0.53	1.05
	mean	0.14	0.39	0.16	1.33	0.44	0.36	1.04
	RSD %	36	67	31	32	111	69	2
S	minimum	108.9	50.0	100.8	53.9	38.4	n.d.	n.d.
	maximum	239.3	182.9	131.0	64.4	101.8	n.d.	n.d.
	mean	149.4	116.8	115.5	59.4	89.1	n.d.	n.d.
	RSD %	35	62	9	7	52	n.d.	n.d.

The high concentration of metals in the locality is damaging to soils, plants and human health. Other studies have indicated that high lead concentrations are deleterious to human health: particularly there is concern about the negative effects of lead on children – their mental abilities and growth. Both zinc and copper are bio-toxic in high enough concentrations, weakening the biological activity of vegetation and possibly damaging human health. In order to reduce emissions, the factory needs to improve its production technologies and install fume reduction equipment such as scrubbers and filters. Of course, such action is dictated by economics. Another option to reduce further damage to the environment would be to utilise bio-remediation methods: through widespread use of vegetation with a high affinity for the contaminant metals soil concentrations could be greatly reduced. In order to reduce human exposure to the pollutants, it is necessary to educate the local population about the potential hazards and to suggest ways in which they could minimise their personal exposure.

Acknowledgements

The authors gratefully acknowledge the financial support provided by grants from the Royal Society Joint Projects with Central/Eastern Europe and the former Soviet Union grant Ref: rc/jp/nov.

REFERENCES

1. M.S. Cresser, L. Ebdon, J.R. Dean, *J. Anal. Atom. Spectr.,* 1989, **4,** 1R.
2. K.J. Puckett, *Lichens, bryophytes and air quality*, Nash III, T.H., Wirth, V.(eds.)Bibliotheca Lichenologica Berlin and Stuttgart: Cramer, 1988, Vol. 30, 231-267.
3. P.W. James, *Air pollution and lichens*, B.W. Ferry, M.S. Baddeley, D.L. Hawksworth, (eds.), London, The Athlone Press, 1973, 143-175.
4. B.J. Williamson, O.W. Purvis, K.Bartok, N. Har, E. Manolache, D. Jones, C. Stanley, N. Vlad, *Studia Univ. Babes-Bolyai, Geologia,*1996, **XLI, 1,** 87-93.
5. K. Bartók, A. Nicoara, V. Bercea, T. Osvath, *Rev. Roum. Biol. Biol. Veget.,* 1992, **37**(**2)**, 135-143.
6. O.W. Purvis, B. Williamson, K. Bartok, N. Zoltani, *New Phytologist* 2000, **147**: 591-599.
7. A.M. Rusu, K. Bartok, B. Dubbin, W. O. Purvis, *Studia Univ. Babes-Bolyai, Chimia,* 2000, in press.
8. D.E. Baker, M.C. Amaker, Nikel, Copper, Zinc and Cadmium. In A.L.Page *et al.* (eds). *Methods of soil analysis.* Part 2, 2nd edition. American Society of Agronomy, Madison, WI, USA, 1982 p.331 – 333.
9. M. Bettinelli, S. Spezia, G. Bizzarri, 1996, **17**: 133-141
10. P. Quevauvillier, R.Herzig, H. Muntau, *Sci. Total Environ,* 1996, **187**: 143-152.