

# Analytical performance of some methods for the determination of trace elements in lichens used as air quality assessment

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**Abstract** A study was carried out in Havana City using lichen as monitor of air pollution. The concentration of several trace elements (Ca, Mn, Fe, Ni, Cu, Zn, Sr, Cd, Pb) in a total of 225 samples was determined by Total Reflection X Ray Fluorescence (TRXRF), Flame Atomic Absorption Spectroscopy (FAAS) and Anodic Stripping Voltammetry (ASV). In addition several Biological Certified Reference Materials (CRM's) were analyzed in order to assess the analytical performance of the results. In general a relatively good agreement was found among the techniques. No significant differences were found between the obtained results and the certified reference values.

**Keywords** Trace elements · Lichen ·  
Atomic absorption spectroscopy ·  
Total reflection X-ray fluorescence ·  
Anodic stripping voltammetry

## Introduction

Several biological materials have been used in air monitoring studies, such as lichens, mosses, tree bark, and recently *Thillandsia*. Lichens have been found to be sensitive to many types of pollution processes and are

considered as good biological monitors for air quality assessment [1–3]. The lichen's capability to accumulate some elements in its tissues aids the chemical analysis and facilitates the detection of elements which are usually present in very low concentrations in the environment. The use of biomonitors in atmospheric pollution studies has also the advantage of obtaining an extensive data set of element concentration at different sampling sites [4].

The relatively low concentration levels of metals in environmental samples impose the need to implement high sensitivity analytical techniques, such as ICP [5], ICP-MS, AAS (flame and graphite furnace), PIXE [6], INAA [7, 8], ASV [9] or TRXRF [10]. The assessment of atmospheric pollution studies using biomonitors requires of the implementation of reliable analytical techniques, able to ensure commensurate results.

The traceability of the results is often assessed through the measurement of certified reference materials and achieving quantification results that are consistent with the certified unit values (i.e. elemental weight fractions). However, certified reference materials are not always available as to ensure an appropriate matching of the range of elemental mass fraction, matrix and potential interferences, as well as to have relative uncertainty values smaller than those fitting the purposes of the intended interpretation of the analytical results. As an alternative, the traceability can be assessed by comparison with the results of reliable independent methods [11, 12].

This paper reports the results of a comparative study of the analytical performance of FAAS, TXRF and ASV for the determination of a mass fraction of a group of elements in lichens samples collected from the territory of Havana City, in an effort to strengthen the quality of the results provided for air-pollution assessment.

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## Materials and methods

### Sampling and sample treatment

A total of 225 lichen samples were collected from 181 spots in Havana city and its surroundings (see Fig. 1 in Annex) during a sampling campaign carried out in the 2002–2003 winter/dry season (November–February). The location of the spots is shown in the map provided in Fig. 1.

Following the results of a previous study [7] the lichen species selected for sampling was the epiphytic *Physcia alba* (Fée) Müll. Arg. The samples were taken from *Roystonea Regia* trees (*Roystonea regia* (HBK) O. F. Cook) at a height of 0.5 to 2 m above the ground. Once at the laboratory, the lichens were removed from any remains of tree bark, freeze-dried, grinded and homogenized.

Two grams of the lichens were subject to dry ashing at 550 °C during 3 h, followed by acid digestion in Pt vessel using a mixture of concentrated HF, HNO<sub>3</sub> and HClO<sub>4</sub> acids (2:2:1). The solution was spiked with 50 mg/ml of Ga solution as internal standard for further TXRF determinations.

Three Certified Reference Materials of biological origin [IAEA-336 (Lichen), CRM 482 (lichen), NRCC-Tort-2 (Lobster Hepatopancreas)] were selected to assess the analytical performance as part of the validation of the methods.

All working solutions were prepared using analytical reagents grade chemicals and distilled and double de-ionized water (grade 2) [13]. Each calibration in FAAS analysis (Mn, Fe, Ni, Cu, Zn, Sr, Cd and Sr) and ASV techniques (Cu, Zn, Cd and Pb) was made by measuring suitable dilutions from 1,000 µg/ml stock standard

solutions (MERCK, Darmstadt, Germany). Double distilled and deionized water was used in blank sample preparation.

### Implemented methods

The determinations by FAAS and ASV were made using calibration curves prepared following the requirements of the Guide ISO 8466 [14]. The detection limits (DL) for FAAS and ASV were calculated according to Vogelgesang [15].

#### FAAS analysis

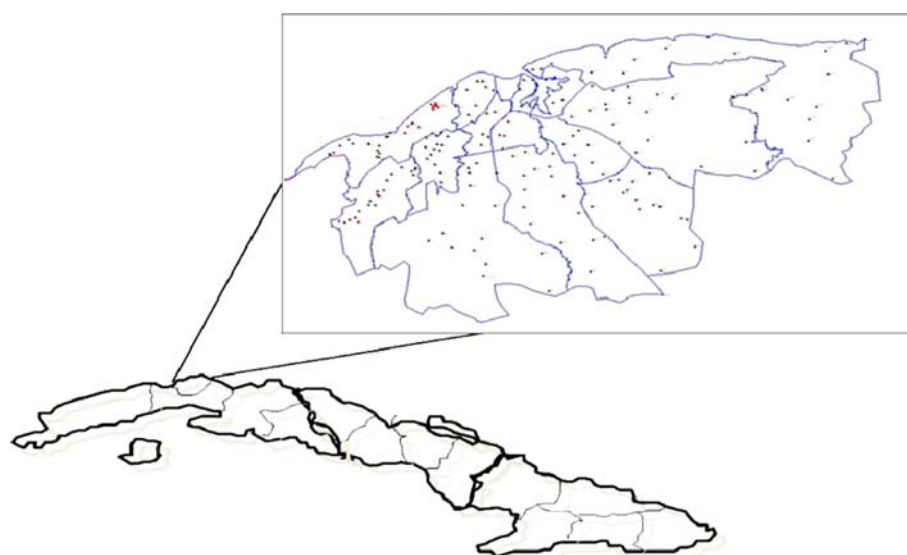
A Pye Unicam Atomic Absorption Spectrophotometer Model SP-9 was used. The elements Mn, Fe, Ni, Cu, Zn, Cd and Pb were measured using an air-acetylene flame. For Ca and Sr determination, a buffer solution (0.2% of K) was previously added, and measured with nitrous oxide-acetylene flame. The instruments conditions for each element are given in Table 1.

#### ASV analysis

A PA4 Polarographic Analyzer with mercury drop electrode (Differential Pulse Polarographic and Anodic Stripping Voltammetry) was used to determine Cu, Zn, Cd and Pb concentrations.

Ten milliliters of sample solution were placed into a fused quartz-measuring vessel of the Polarographic Analyzer. Samples were previously flushed with N<sub>2</sub> for 15 min in order to eliminate the oxygen interferences, anodic stripping was performed in the differential pulse mode with the following parameters:

**Fig. 1** Sampling Sites



**Table 1** Operational parameters for the FAAS determination of Ca, Mn, Fe, Ni, Cu, Zn, Cd, Sr and Pb

	Ca	Mn	Fe	Ni	Cu	Zn	Cd	Sr	Pb
Wavelength (nm)	422.7	279.5	248.3	232.0	324.8	213.9	228.8	460.7	217.0
Slit (nm)	0.5	0.5	0.2	0.2	0.5	0.5	0.5	0.5	0.5
Background correction	No	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Deuterium lamp									

- Working electrode—HMDE
- Auxiliary electrode—Pt
- Reference electrode—Saturated calomel
- Pulse height—50 mV
- Clock time—0.2 s
- Scan rate—5 mV/s.

### TRXRF

A modular spectrometer supplied by the International Atomic Energy Agency (IAEA) was used in this study. Sample excitation is achieved using a total reflection module designed in the Atominstitut, Vienna. Effective excitation is achieved by successive modifications made to the beam emitted by a fine focus Mo-anode X-ray tube: an initial collimation produces a fine parallel beam that impinges into a polished silicon reflector under an angle smaller than that required to achieve the total reflection only of x-rays having energy less than that of the anode characteristic lines. That way the continuum background in XRF spectra resulting from scattering of the excitation radiation at the sample is drastically reduced. Only the radiation reflected at the cut-off reflector impinges into the sample (a thin film resulting from the evaporation of a 10  $\mu$ L drop of aqueous solution in the polished surface of a fused Silicon disc), again under the critical angle required for total reflection.

The X-ray fluorescence emerging from the sample is measured with a Si(Li) detector positioned at a small distance from the sample surface. Spectrum evaluation and quantification is made using the AXIL-QXAS software package, freely distributed by the IAEA [16].

The quantification procedure is based in instrumental sensitivity calibrations made by measuring standard solutions spiked with known amounts of gallium (internal standard). The use of internal standardization enhances the method robustness, since some effects affecting the measurement results (differences in sample deposition, fluctuations in x-ray tube flux, among others) are cancelled out. Quantification of unknown samples is made according to Eq. (1)

$$W_i = \frac{I_i S_{st} W_{st}}{I_{st} S_i} = \frac{N_i S_{st} W_{st}}{N_{st} S_i} \quad (1)$$

where  $W_i$  is the concentration of element of interest  $i$ ,  $N_i$  and  $N_{st}$  are the net peak area counts for the element  $i$  and

the internal standard, respectively. The ratio  $S_i/S_{st}$  is the sensitivity calibration factor (ratio of the response of the instrument to the unit of concentration of the element  $i$  to that of the element used as internal standard).

Following the general recommendations provided in [17] the detection limits can be calculated as

$$DL = 3 \frac{(\sqrt{N_{bck,i}} + \sigma_{N_{blank}}) W_{ST}}{N_{ST} S_i/S_{st}} \quad (2)$$

where  $N_{bckg,i}$  is the counting corresponding to the spectrum continuum under the peak of the element  $i$  and;  $\sigma_{N_{blank}}$  is the standard deviation of the peak area of the element  $i$  (if found) in the measurement of a blank sample. As blank sample it is understood a sample of grade 2 water subject to complete sample treatment.

## Results and discussion

As mentioned before in the introduction, the traceability of the results can be assessed using suitable CRMs. Two reference materials corresponding to dried lichen samples were available for this study: the IAEA-336, prepared by the IAEA Nuclear Applications Analytical Laboratories at Seibersdorf and the CRM-482 supplied by the Community Bureau of Reference (BCR) of the Commission of the European Communities since 1995. A third CRM (TORT-2, Lobster hepatopancreas, prepared by the Marine analytical Chemistry Standards Program of the National Research Council Canada in 1987) was selected for this study, in order to enlarge the interval of certified values. Although this material does not correspond to a lichen matrix, the capability of achieving a full digestion of the sample using the same preparative procedure was assumed as sufficient condition to fit for the intended purpose.

The results obtained for Mn, Fe, Ni, Cu, Zn, Sr, Cd and Pb in the CRM's by using the different methods are summarized in Table 2. The results of the analysis of blank samples by the three techniques served to calculate the detection limits for each method (see Table 3). The concentration values are expressed as mean  $\pm$  one standard deviation obtained in the analysis of four replicates ( $n = 4$ ). The value of a  $t$ -Test (shown in the column next to each result) reveals no significant difference ( $t\text{-exp} < t = 3.18$  for  $\alpha = 0.05$ ) between the obtained

**Table 2** Results of the analysis of biological origin CRM's (values expressed in  $\mu\text{g/g}$ )

Element	CRM	Certified	FAAS	<i>t</i> -Test <sup>a</sup>	ASV	<i>t</i> -Test <sup>a</sup>	TRXRF	<i>t</i> -Test <sup>a</sup>
Mn	NRC TORT-2	13.6 ± 1.2	12.0 ± 1.5	2.13	ND <sup>c</sup>		12.8 ± 1.1	1.45
	BCR CRM-482	33.0 <sup>b</sup>	31.5 ± 1.4	2.14			31.6 ± 1.2	2.33
	IAEA-336	63 ± 7	59 ± 3	2.67			64.5 ± 4.2	0.71
Fe	NRC TORT-2	105 ± 13	101 ± 14	0.57	ND <sup>c</sup>		106 ± 4	0.50
	IAEA-336	430 ± 50	405 ± 25	2.00			414 ± 26	1.23
	BCR CRM-482	804 <sup>b</sup>	815 ± 36	0.61			780 ± 39	1.23
Ni	BCR CRM-482	2.47 ± 0.07	2.58 ± 0.89	0.25	ND <sup>c</sup>		DL <sup>b</sup>	
	NRC TORT-2	2.50 ± 0.19	2.9 ± 0.7	1.14			DL <sup>b</sup>	
Cu	IAEA-336	3.6 ± 0.5	3.25 ± 0.53	1.32	3.58 ± 0.09	0.44	4.0 ± 0.6	1.33
	BCR CRM-482	7.03 ± 0.19	7.24 ± 0.98	0.43	6.93 ± 0.62	0.32	6.90 ± 0.60	0.43
	NRC TORT-2	106 ± 10	102 ± 4	2.00	101 ± 5	2.00	101 ± 4	2.56
Zn	IAEA-336	30.4 ± 3.4	29.0 ± 2.0	2.50	29.8 ± 1.8	1.89	29.8 ± 2.5	1.36
	BCR CRM-482	100.6 ± 2.2	98.7 ± 3.9	0.97	96 ± 3.5	2.63	95.5 ± 3.6	2.83
	NRC TORT-2	180 ± 6	178 ± 7	0.57	185 ± 8	1.25	175 ± 5	2.00
Sr	IAEA-336	9.3 <sup>b</sup>	8.9 ± 0.8	1.00	ND <sup>c</sup>		9.3 ± 0.4	0.25
	BCR CRM-482	10.5 <sup>b</sup>	10.3 ± 1.6	0.31			10.13 ± 0.35	2.11
	NRC TORT-2	45.2 ± 1.9	46.3 ± 2.4	0.92			48.3 ± 2.9	2.14
Cd	IAEA-336	0.117 ± 0.017	DL <sup>d</sup>		0.124 ± 0.010	1.40	ND <sup>c</sup>	
	BCR CRM-482	0.56 ± 0.02	0.67 ± 0.10	2.20	0.55 ± 0.05	0.40		
	NRC TORT-2	26.7 ± 0.6	26.0 ± 1.0	1.40	27.2 ± 0.6	1.67		
Pb	NRC TORT-2	0.35 ± 0.13	DL <sup>d</sup>		0.39 ± 0.03	7.69	ND <sup>c</sup>	
	IAEA-336	4.9 ± 0.6	4.6 ± 0.5	1.20	4.7 ± 0.4	1.00		
	BCR CRM-482	40.9 ± 1.4	38.2 ± 2.1	2.57	39.9 ± 0.8	2.50		

Notes

<sup>a</sup> *t*-test calculated for  $\alpha = 0.05$  ( $t = 3.18$ )<sup>b</sup> Informative values<sup>c</sup> Not determined<sup>d</sup> Values found less than detection limits (see Table 3)**Table 3** Detection limits obtained ( $\mu\text{g/g}$ )

	Ca	Mn	Fe	Ni	Cu	Zn	Sr	Cd	Pb
FAAS	2.0	0.5	2.5	1.6	2.1	1.0	0.5	0.3	3.1
TXRF	50	3.0	4.0	3.5	2.0	1.0	0.5	ND	ND
ASV	ND	ND	ND	ND	0.4	0.1	ND	0.05	0.09

Note: ND–Not determined

results and corresponding certified value for all the elements and techniques. The repeatability expressed as standard deviation in % was in general less than 10%. As expected, for concentration values close to the detection limit (see Table 3 for reference) the trueness and the precision are consequently worse.

The minimal and maximal values obtained in the analysis of 225 lichen samples are presented in the Table 4, showing that the three biological CRM's used for this study do not ensure an appropriate matching of the range of

**Table 4** Interval of elemental mass fraction found in lichens samples ( $\mu\text{g/g}$ )

Element	Range (Min–Max)
Mn	9–170
Fe	19–8270
Ni	1.9–54
Cu	5.2–55
Zn	17.2–283
Sr	8.3–48
Cd	0.07–1.5
Pb	6.9–95

elemental mass fraction found in the natural lichens samples. Besides that, Ca contents are not certified in the CRM's. A comparison of the results obtained by the different techniques in the analysis of the 225 lichen samples collected in this survey was made to complement the methods validation. The results are schematically presented in Figs. 2–10. The slopes of the curves show the mean concentration ratios between the methods for

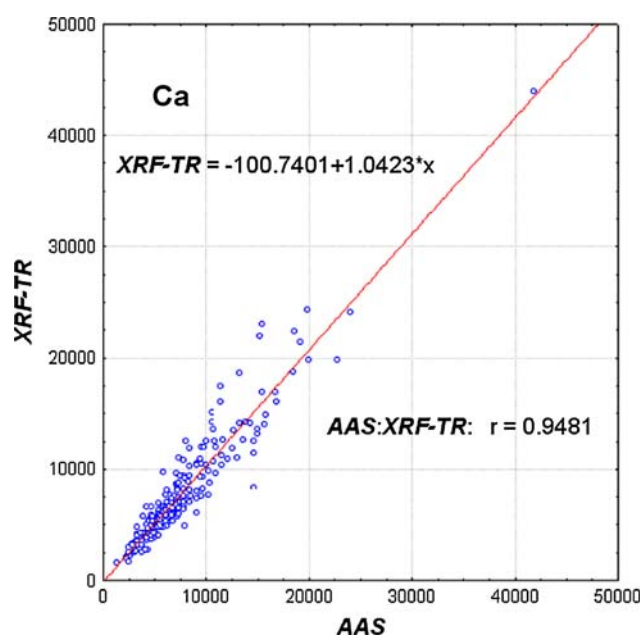


Fig. 2 Values obtained for Ca

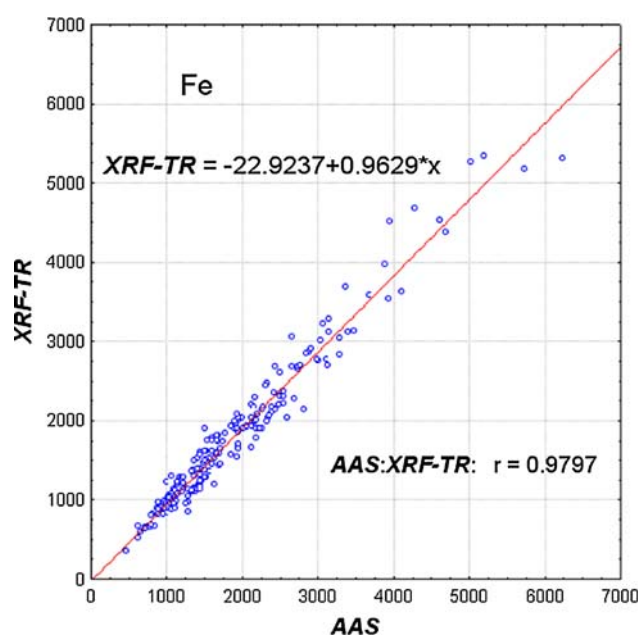


Fig. 4 Values obtained for Fe

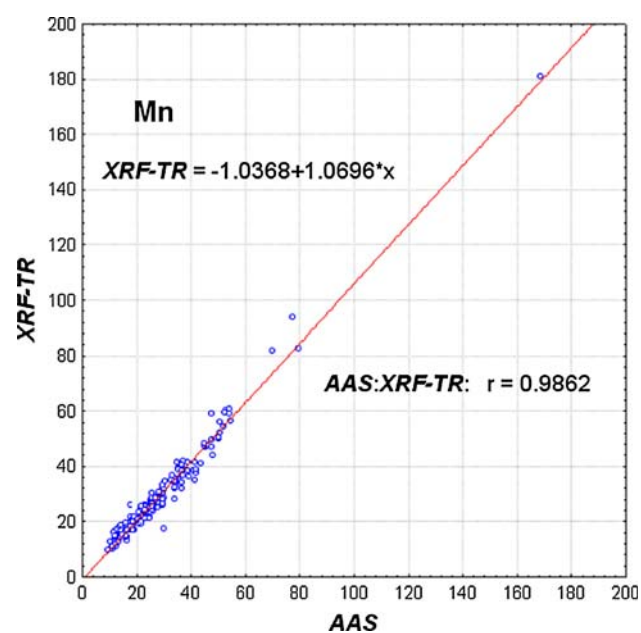


Fig. 3 Values obtained for Mn

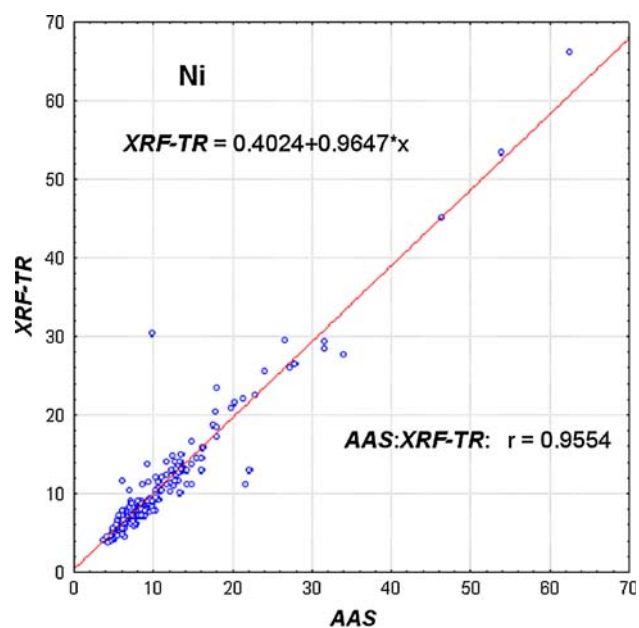


Fig. 5 Values obtained for Ni

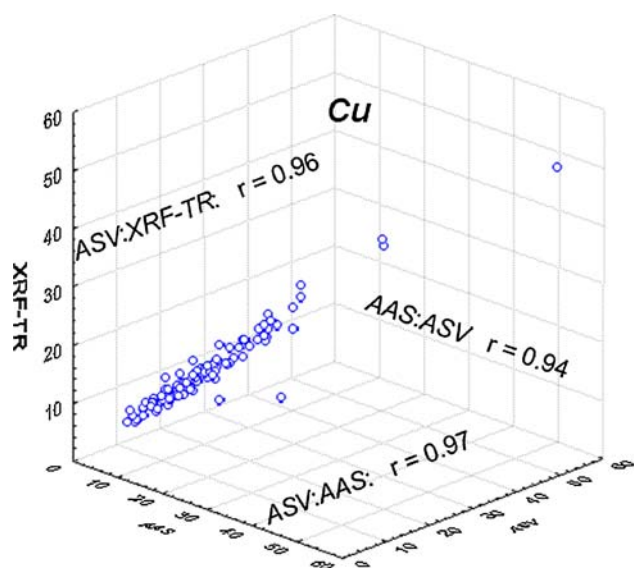
each element which are near to the unit, shown a good agreement between the methods.

In all cases correlation factors higher than 0.94 were obtained, except for Cd (ASV/FAAS ratio = 0.66 and  $r = 0.81$ ). The FAAS results for Cd were systematically higher than those obtained by ASV. This tendency is also corroborated by a 19.6% bias in the result obtained by FAAS in the analysis of the BCR CRM-482 (Table 2). For a larger concentration value (CRM TORT-2) the bias is not

significant. This difference seems to be conditioned by an inadequate compensation of the background in the used spectrometer (Deuterium Background Corrector) at very low concentration of cadmium.

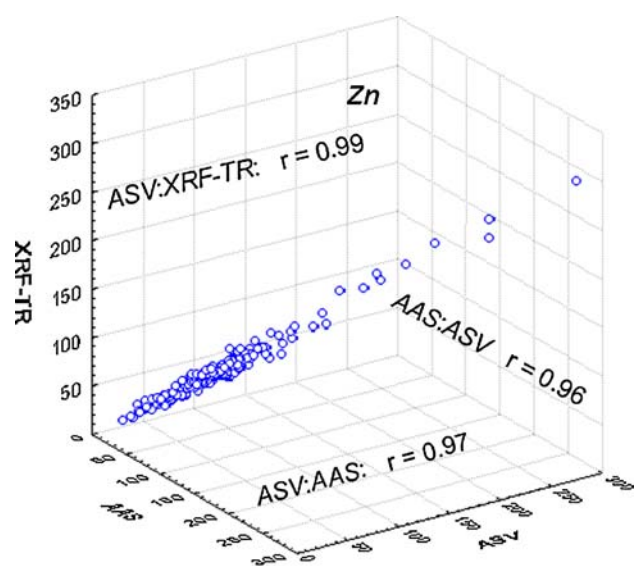
The mean relative uncertainty values (calculated according to internal procedure [18]) are presented in Table 5. These values are in most cases lower than 10%.

The uncertainty values in the determination of Cd in the lichen samples using FAAS were significantly higher



**AAS:ASV:XRF-TR: Multiple R(z/xy) = 0.96**

**Fig. 6** Values obtained for Cu

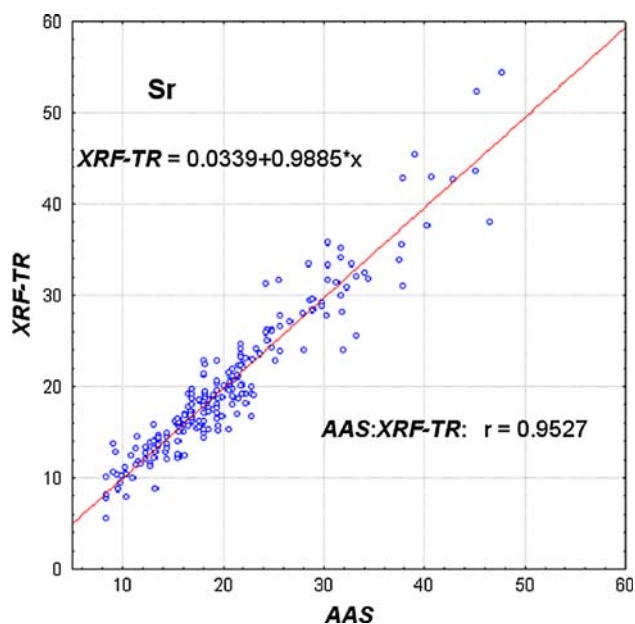


**AAS:ASV:XRF-TR: Multiple R(z/xy) = 0.99**

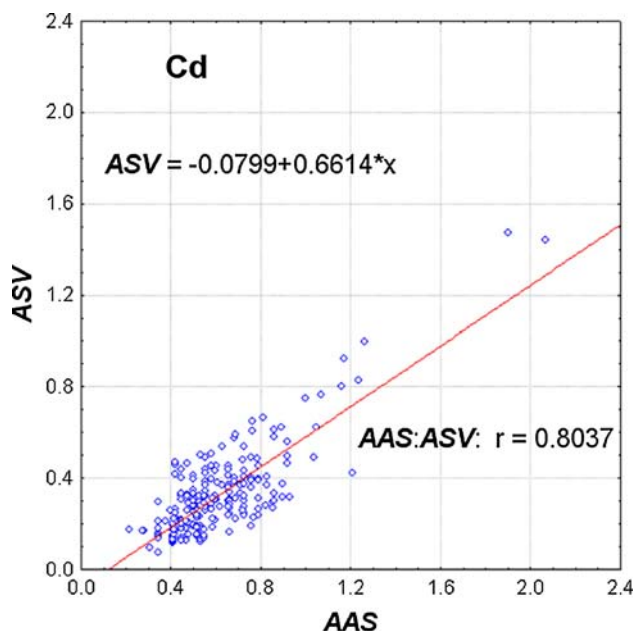
**Fig. 7** Values obtained for Zn

compared to ASV method. These results are in total agreement with those obtained previously in the MRC's analysis. On the other hand, the relative uncertainties for XRF-TR technique were slightly higher.

The ASV method is recommended for determination of very low concentration of cadmium, since its detection limits are approximately ten times lower than those obtained by FAAS.



**Fig. 8** Values obtained for Sr



**Fig. 9** Values obtained for Cd

**Conclusions**

The trueness, precision and detection limits of the methods used in this work for the determination of the concentration of Mn, Fe, Ni, Cu, Zn, Sr, Cd and Pb in three CRM's of biological origin were evaluated. As expected, for concentration values close to the detection limit the trueness and the precision are consequently worse.

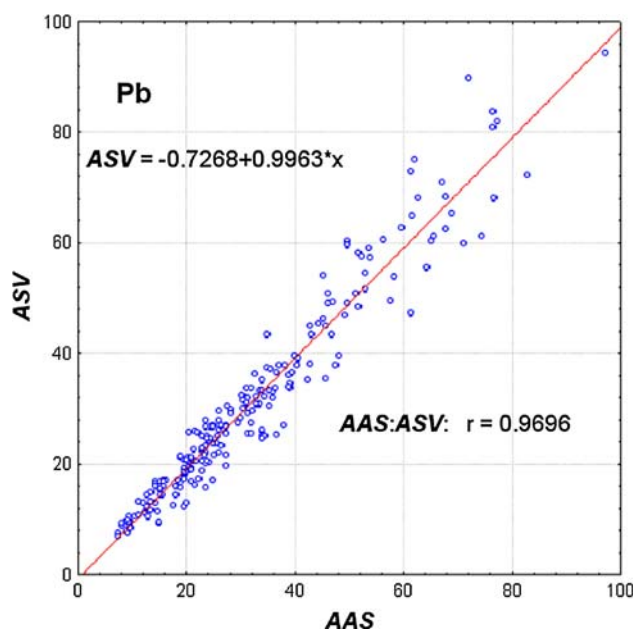


Fig. 10 Values obtained for Pb

Table 5 Relative uncertainties in the analysis of lichen samples

Element	Relative uncertainty (%)		
	FAAS	ASV	TRXRF
Ca	5.05		5.19
Mn	4.29		9.91
Fe	3.27		4.5
Ni	5.02		12.04
Cu	6.37	5.19	7.59
Zn	3.03	2.20	4.15
Sr	6.19		7.44
Cd	25.27	5.76	
Pb	3.52	5.41	

The comparison of the results obtained by application of FAAS, ASV and TRXRF methods to the determination of Mn, Fe, Ni, Cu, Zn, Sr, Cd and Pb in lichens collected from Havana City reveals a good agreement between the analytical results of the methods. The quality of the results obtained by these methods is adequate for the purposes of evaluating the level of atmospheric pollution using biomonitors.

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