A Comparative Determination of Heavy Metals in Moss Tissue by Atomic Absorption Spectroscopy, Differential Pulse Anodic Stripping Voltammetry, Direct Current Plasma Spectroscopy and X-ray Fluorescence Spectroscopy

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Heavy metal contents of mosses are usually determined routinely for pollution studies, by atomic absorption spectroscopy. Results obtained from a such study are here compared with levels obtained from a comparative determination of the metals in the mosses by three other techniques: Differential pulse anodic stripping voltammetry (DPASV), Direct current plasma (atomic emission) spectroscopy (DCPS) and X-ray fluorescence (XRF) spectroscopy.

The levels of Pb, Cu and Cd determined by DPASV did not differ significantly from those obtained from AAS. Metal levels from DCPS analyses tended to be significantly higher than the AAS values. Although the XRF values were slightly higher than those of AAS for Pb and Zn, the differences were statistically insignificant. The implication of these trends in comparing the extents of pollution in different regions, is highlighted.

KEY WORDS: Heavy metals, mosses, AAS, DPASV, DCPS, XRF.

INTRODUCTION

Adequate monitoring programmes are essential for effective environ-
mental pollution control measures. Accurate data obtained from
such exercises are invaluable for determining trends in pollution
levels, developing abatement tactics, judging compliance with quality
standards, and guidance in land use and transportation planning. A
variety of techniques are available for monitoring the levels of the
numerous pollutants in the atmosphere. These range from the use of
expensive automatic samplers/analyzers, to the application of various
biological materials as sinks for semi-quantitatively mapping gradients
in the levels of the pollutants. The use of plants as bioindicators
has become particularly useful in view of the relative cheapness,
rapidity and effectiveness of the method. In this regard, mosses have
been extensively used in various parts of the world as sensitive indi-
cators of the burden of trace metals in the air.[1-5] These species of
lower plants characteristically accumulate trace metals and other
pollutants into their tissues by mechanisms which include ion-
exchange and chelation. Based on the levels of trace metals ac-
cumulated in indigenous live mosses, peat mosses or "moss bags",
zones of relative pollution levels have been discerned. In some cases
the levels of pollution in different regions of the world have been
compared. This is particularly so for parts of Scandinavia and
elsewhere.[2,3,6,7]

A variety of chemical analytical methods are available for the
determination of trace metals in plant tissues.[8] Several of these have
been applied to the analysis of mosses in the course of pollution
studies.[22] Apart from atomic absorption spectroscopy (AAS) which
is the most widely used, other techniques which have been reportedly
used include X-ray fluorescence (XRF) spectroscopy,[9,10] neutron
activation analysis,[11] differential pulse anodic stripping voltammetry
(DPASV)[6,12] and colorimetry.[13] The comparisons of pollution levels
of different regions based on results from different studies, usually
have not considered what significant variations may result from the
analyses of the moss samples by different methods. Results from
some inter-laboratory comparative determinations of trace metal
concentrations in plant tissues[14,15] show that the differences between
techniques may be larger than the differences between individual
laboratories in which the same techniques are used. If results of

metal analyses from different studies involving different analytical techniques are to be accurately compared for- the purposes of determining the extents of pollution, it should be understood, how the levels may vary from one technique to another.

Mosses have also been used for pollution studies in Nigeria by the author and others.[16,17] The AAS technique was employed for the analyses. In one of these studies, a comparative analysis of a selection of samples by three other methods was carried out in order to determine how results from AAS analyses compare with those from the other methods. These other techniques were: differential pulse anodic stripping voltammetry, direct current plasma (atomic emission) spectroscopy (DCPS) and X-ray fluorescence spectroscopy. The results are presented in this paper.

MATERIALS AND METHODS

Analysis by AAS

Cleaned, air-dried moss samples were ground in a laboratory mill and redried at 70–80°C. About 1.00 g of each sample was treated with 20.0 cm^3 of Analar grade 4:1 mixture HNO_3:$HClO_4$ acids and left to predigest overnight.[18] They were then heated, up to 180°C over a period of 24 hours on a temperature-programmed Tecator digestion block. A little quantity of siliceous matter, "opal",[19] was usually dispersed in the residual solution. The digests were quantitatively transferred to calibrated tubes and made up with double-distilled water to 25.0 cm^3. The solutions were left to stand for about 30 mins and then decanted to separate the settled siliceous matter. Analysis was subsequently carried out with a Varian-AA5 Atomic absorption spectrophotometer for the elements Pb, Cu, Cd, Ni, Mn, Zn, Fe, using working standards prepared in 11% $HClO_4$ acid.

Analysis by DPASV

The measurements were made with a Princeton Applied Research 174A Polarographic Analyzer, charted with a Hewlett Packard 7044A X–Y recorder. The working electrode was a Hanging Mercury Drop Electrode. The samples were aliquots of digests earlier analysed by

AAS. To prevent saturation of the working electrode, the digests were further diluted (60 × for Cu and Pb; 15 × for Cd) with distilled water. The $HClO_4$ acid matrix served as background electrolyte. $30.0 \, cm^3$ of the diluted digest to which $30 \, \mu l$ perhydrol was added, was UV-irradiated at 700 W for 2 hours. On cooling, the solution was quantitatively transferred to the electrolytic cell. It was stirred, deaerated (10 mins), and electrodeposition carried out for 3 mins. A quiescence period of 20 secs was allowed, followed by electrolytic anodic stripping from $-0.8 \, V$ (vs Ag/AgCl) at 10 mV/sec. For most samples, the current ranges were Cd—$0.05 \, \mu A$, Pb and Cu—$0.5 \, \mu A$. Sequential standard additions were made with appropriate microlitre volumes of $0.05 \, \mu g/ml$ Cd, $1.0 \, \mu g/ml$ Pb and $1.0 \, \mu g/ml$ Cu standards.

Analysis by DCPS

The instrument used is the SpectraSpan IIIB System (SpectraMetrics Inc.) incorporating an argon plasma which reaches a temperature of $6000°C$. To suppress ionization interference, the matrices of the samples and standards were made into $4,000 \, \mu g/ml$ Li, by spiking with appropriate volumes of a stock solution $(20,000 \, \mu g/ml)$ of Li. The analysis was carried out as detailed in the instrument manual. The DCPS method suffers from frequent instability of the argon plasma, as a result of which drifts in emission intensities may occur. No significant drift occurred during this analysis. The relative standard deviation of the intensities of the higher standards during the analysis, were: Zn—4.0%, Fe—3.0%, Mn—3.7%, Cu—5.9%, Pb—3.1% and Ni—3.0%.

Analysis by XRF technique

The analyses were carried out with an automated Phillips PW1410 X-ray Spectrometer coupled to a PW1710 Diffractometer control microprocessor. The moss samples were ground to a fine powder in a mikrodismembrator (Braun model). No significant contamination resulted from this mode of grinding. Sample pellets (32 mm diameter) were prepared in stainless steel holders, using about 1.0 g sample and 1.0 g cellulose as backing. A pressure of 100 kN was applied for one minute with a mechanical press, and a thin sheeth of cellophane was wrapped over the exposed surface of the sample. The fluorescence

intensities of the metals of interest were then determined. For each metal, the background intensities on both sides of the analytical angle 2θ were measured, and the corrected intensities of the metal thereby evaluated.

For a set of eight samples, the fluorescence intensities of each metal in the samples were correlated with the concentrations of the metal (μg/g dry moss) determined from AAS analysis. The following coefficients were obtained: Cu–0.999 (AAS conc. range, 17.9—94.2 μg/g); Fe—0.996 (4,210—10,502 μg/g); Mn—0.994 (221–395 μg/g); Pb–0.984 (23.4–80.5 μg/g) and Zn—0.999 (75.8–1,533 μg/g). The absolute concentrations of Pb and Fe in a few samples were subsequently determined by the XRF technique. Because no matrix-correction programme or suitable plant standards for XRF analysis was readily available, the analysis was carried out by standard addition. The addition of Fe was made with milligram amounts of fine analar grade Fe metal powder. Use of a standard solution of Fe would have required spiking with more than a tolerable volume of solution, to make the required additions (10,000–20,000 μg/g) to 1.0 g moss. Pb was added to the same moss samples as microlitre volumes of a 1,000 μg/ml Pb standard. After drying and carefully mixing with clean glass rods, pellets of the spiked samples were prepared and the fluorescence intensities determined.

There were significant differences between the results obtained from the AAS analyses and those from the XRF method involving this form of standard addition. The Pb concentrations from XRF were about 25–60% higher, while Fe concentrations were 70–100% higher. It was later found that up to 14% depression of the fluorescence intensity of Pb was caused by the addition of 20,000 μg/g Fe powder, due perhaps to differences in grain size between powders of the moss and Fe. Particle size of samples is known to be a critical factor in XRF measurements.[20] The analyses were subsequently repeated for only Pb and Zn which did not require addition of metal powders. Better comparisons between the two techniques were then obtained.

RESULTS AND DISCUSSION

The statistical parameters used in comparing the techniques are given in Table I. An average relative deviation of about 10% is

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TABLE I

Statistical comparison of AAS results with those of DPASV, DCPS and XRF techniques

Technique	Element	No. of samples	Correlation with AAS (r)	Student t-test[a]		Average relative deviation from AAS (%)
				t-tabulated	t-calculated	
DPASV	Pb	15	0.982	2.14	2.072 (NS)[b]	7.6 ± 10.0
DPASV	Cu	12	0.647	2.22	0.320 (NS)	1.6 ± 15.6
DPASV	Cd	4	0.995	3.18	0.317 (NS)	3.5 ± 3.5
DCPS	Pb	16	0.988	2.13	4.56 (S)[b]	7.5 ± 4.8
DCPS	Fe	16	0.995	2.13	4.78 (S)	6.5 ± 6.7
DCPS	Zn	16	0.970	2.13	2.32 (S)	6.6 ± 6.0
DCPS	Mn	16	0.985	2.13	9.54 (S)	13.8 ± 5.1
DCPS	Ni	16	0.887	2.13	9.68 (S)	32.2 ± 14.7
DCPS	Cu	16	0.998	2.13	11.45 (S)	33.6 ± 10.9
XRF	Pb	3	0.973	4.30	2.46 (NS)	13.1 ± 10.3
XRF	Zn	3	0.858	4.30	1.90 (NS)	22.3 ± 18.8

[a] $t = \dfrac{D}{Sd}\sqrt{N}; \nu = N-2.$

[b] (NS) = not significant; (S) = significant.

regarded as normal and insignificant for this purpose. The duplicate analysis of some moss samples show that the precision of about 1–5% was comparable for the AAS, DPASV and DCPS techniques (Tables II, III, IV).

There was no significant difference between the results from DPASV and AAS (Table II). This is statistically established from the t-test, and the low average relative deviation values. The correlation with AAS values was comparatively poorer for Cu. Despite the close comparison between the two techniques, DPASV is not suitable for routine analysis of a large number of samples as is required in large-scale pollution studies, due to its relatively poor speed.[22] It is therefore in this regard, best applied for crosschecking few samples with a view to validating the results obtained from AAS analysis.

The DCPS values showed a bias in comparison with AAS (Tables III and IV). The former tended to have higher values. Though the t-test indicates that the differences between the two techniques are

TABLE II

Concentrations of metals in moss digests, from analyses by AAS and DPASV techniques

Sample	Pb (μg/ml)		Cu (μg/ml)		Cd (ng/ml)	
	AAS	DPASV	AAS	DPASV	AAS	DPASV
S-1A	0.88	0.70		$-^a$	10.0	10.1
S-1B	0.88	1.01	0.60	0.75		—
S-2B	0.12	0.14		–	<2.0	0.2
S-3A	0.85	0.90	0.54	0.51		—
S-3B	0.85	0.96	0.59	0.54		—
S-4A	1.40	1.43	0.77	0.71		—
S-6A		—		—	5.0	5.3
S-6B	1.20	1.20		—	2.0	1.2
S-8A	1.35	1.44	0.46	0.45		—
S-8B	1.35	1.54	0.46	0.42		—
S-11A	0.95	0.87	0.54	0.45		—
S-11B	0.95	1.02	0.54	0.54		—
S-16A	0.30	0.35	0.53	0.66	—	
S-16B	0.30	0.34	0.53	0.49		—
S-19A	0.35	0.41	0.57	0.61		—
S-19B	0.38	0.44	0.54	0.70		—

^{a}Not determined by DPASV.

TABLE III

Concentrations of Pb, Fe and Zn in moss digests, from analyses by AAS and DCPS techniques

Sample	Pb (μg/ml)		Fe (μg/ml)		Zn (μg/ml)	
	AAS	DCPS	AAS	DCPS	AAS	DCPS
S-1A	0.88	0.85	155	194	2.83	3.12
S-1B	0.88	0.87	160	178	2.85	2.82
S-3A	0.85	0.90	165	187	2.10	1.91
S-3B	0.85	0.98	170	190	3.95	3.02
S-4A	1.40	1.56	295	321	5.00	5.12
S-4B	1.43	1.37	295	289	5.05	4.76
S-5A	1.50	1.63	313	320	2.10	1.91
S-5B	1.45	1.52	305	305	2.05	1.92
S-6A	1.20	1.40	395	415	2.20	2.05
S-6B	1.20	1.37	397	406	2.20	2.08
S-8A	1.35	1.50	300	324	4.45	4.33
S-8B	1.35	1.43	305	332	4.50	4.47
S-14A	2.35	2.50	495	506	3.20	2.73
S-14B	2.35	2.44	515	523	3.05	2.88
S-15A	1.78	1.83	285	286	3.75	3.77
S-15B	1.75	1.80	280	284	3.95	3.87

TABLE IV

Concentrations of Mn, Ni and Cu in moss digests, from analyses by AAS and DCPS techniques

Sample	Mn (μg/ml)		Ni (μg/ml)		Cu (μg/ml)	
	AAS	DCPS	AAS	DCPS	AAS	DCPS
S-1A	15.0	18.7	0.20	0.27	0.60	0.91
S-1B	14.5	17.2	0.18	0.26	0.60	0.87
S-3A	13.5	15.0	0.17	0.23	0.54	0.75
S-3B	13.0	15.4	0.17	0.23	0.59	0.83
S-4A	12.0	13.6	0.23	0.34	0.77	1.11
S-4B	11.8	12.4	0.24	0.30	0.80	1.06
S-5A	10.1	11.0	0.24	0.39	0.69	0.87
S-5B	9.5	10.5	0.21	0.25	0.64	0.85
S-6A	16.4	18.4	0.22	0.30	3.55	4.08
S-6B	16.1	17.8	0.22	0.29	3.50	3.89
S-8A	8.4	9.7	0.21	0.29	0.46	0.63
S-8B	8.4	10.3	0.21	0.31	0.46	0.65
S-14A	18.6	20.7	0.34	0.38	0.72	0.94
S-14B	19.7	21.9	0.37	0.40	0.76	0.95
S-15A	11.5	13.1	0.32	0.37	0.89	1.16
S-15B	11.5	13.0	0.31	0.38	0.90	1.20

significant for Pb, Fe and Zn, the relative deviations of the DCPS values from AAS values are low and within tolerable limits ($7.5 \pm 4.8\%$, $6.5 \pm 6.7\%$ and $6.6 \pm 6.0\%$ respectively). Visual inspection of the data (Table III) indicates that there is a reasonable degree of agreement between the two techniques, for these metals. For Cu, Ni and Mn (Table IV) however, all parameters show that the differences between the techniques are significant. The reason for the higher levels with DCPS for these metals is not readily known. The AAS technique is however generally more liable to chemical interferences than the DCPS in which the hot plasma effects a more efficient dissociation of the samples. The pattern observed in this study is in agreement with the observations of some routine users of the DCPS technique.[21] This offsets the advantage of the multielement capacity of the DCPS.

The XRF values for Pb and Zn were slightly higher than AAS values (Table V), though the differences are statistically (t-test) insignificant. A higher deviation between the two techniques was obtained for Zn levels. The generally higher XRF values are possibly due to the absence in the technique of sample dissolution steps which may be incomplete, or lead to loss of analytes.

In summary, the average trend in metal concentrations from analysis by the four methods is typified by the trend for Pb, which is; $AAS \simeq DPASV \leqq DCPS \leqq XRF$. It is clear that for some of the metals such as Mn, Cu, and Ni, significant differences could occur between the commonly used AAS and other techniques such as the DCPS. This is of importance for comparative pollution studies of different

TABLE V

Concentrations of metals in moss tissues, from AAS and XRF analyses

Sample	Element	AAS conc.	XRF conc. (μg/g)
S-13	Pb	34.0	35.9
S-14	Pb	62.7	69.4
S-15	Pb	48.4	59.6
S-16	Zn	48.9	70.2
S-18	Zn	33.2	37.0
S-19	Zn	54.7	60.9

regions. It is vital that analyses be made by techniques of comparable accuracies, if the concentrations so determined are to be used for establishing zones of relative pollution levels.

Acknowledgement

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