Chapter (non-refereed)

Parkinson, J. A.. 1987 The determination of total sulphur in vegetation. In: Rowland, A. P., (ed.) *Chemical analysis in environmental research.* Abbotts Ripton, NERC/ITE, 12-15. (ITE Symposium, 18).

The determination of total sulphur in vegetation

J A PARKINSON
ITE, Merlewood Research Station, Grange-over-Sands

1 Introduction

The standard method traditionally used in ITE's Merlewood Research Station chemical laboratories for estimating total sulphur in vegetation has been a dry ashing/barium sulphate turbidimetric procedure based on Butters and Chenery (1959). Over the years, this method has proved to be reliable and reproducible. However, it is tedious and time-consuming for routine use when large numbers of samples are involved. Throughput can be increased by replacing the dry ashing stage with a nitric/perchloric acid digestion, but this technique has been found to have limitations with some types of sample. A better alternative is X-ray fluorescence (XRF), which is both rapid and convenient. The sample matrix is largely cellulose and the spectrometer can, therefore, be calibrated by using, as standards, samples which have been carefully analysed by a suitable chemical method, such as the standard one mentioned above.

During a calibration exercise of this kind, it was found that X-ray fluorescent intensity of the sulphur Kα line was highly correlated with the total sulphur content of the specimen used. However, for 2 species, dog's mercury *(Mercurialis perennis)* and bracken *(Pteridium aquilinum),* the relationship did not hold, and further tests revealed errors in the chemical analysis results for these 2 species. Therefore, various chemical procedures were investigated for the analysis of a wide range of vegetation types, with a view to finding a suitable method for XRF calibration.

2 Materials and methods

A selection of air-dry, ground (0.7 mm) samples of vegetation were obtained covering several different species, including dog's mercury and bracken (Table 1).

Four chemical methods for the determination of total sulphur were compared, involving 4 different oxidation procedures and 2 methods for determination of the sulphate-sulphur formed on oxidation.

i. The standard dry ashing/barium sulphate turbidimetric procedure based on Butters and Chenery (1959). The full procedure is described by Allen *et al.* (1974).

ii. Wet oxidation/barium sulphate turbidimetric, in which the sample is oxidized with a mixture of nitric and perchloric acids in the presence of vanadium (Mottershead 1971).

iii. Schoniger flask oxidation followed by ion chromatography (Busman *et al.* 1983). In this study, chemically supressed ion chromatography was carried out on a Dionex (model 2010i) chromatograph using an AS4A column operated under the manufacturer's standard conditions.

iv. Oxygen bomb oxidation, followed by ion chromatography. This procedure was a development from method 3, in which the oxidation was carried out under pressure (20 atmospheres of oxygen). The bomb used was part of a conventional adiabatic bomb calorimeter (Gallenkamp & Co, Model CB-100). The samples were pressed into pellets, 13 mm diameter, and placed in a silica crucible designed

Table 1. Samples used in comparison tests

Sample	Site	Species	Component
1	Merlewood	Dog's mercury	
2	Oxenthwaite	*(Mercurialis perennis)*	Above-ground growth
3	Merlewood		
4	Humphrey Head	Meadowsweet	
5	Oxenthwaite	*(Filipendula ulmaria)*	Leaves
6	Angerham		
7	Merlewood	Rosebay willow-herb	
8	Lindale	*(Chamaenerion augustifolium)*	Leaves
9	Hampsfell	Bracken	
10	Lowick	*(Pteridium aquilinum)*	Fronds
11	Kirkby Moor	Heather *(Calluna vulgaris)*	Above-ground growth

for use with the apparatus. The crucible was positioned in the bomb, pressurized with oxygen and fired according to the manufacturer's instructions, except that 25 ml water was added to the pressure vessel initially. After firing, 15–20 minutes were allowed before releasing the pressure. The walls and cap of the bomb were washed and the washings added to the 25 ml water already present. After dilution of the solution obtained to 50 ml, sulphate was determined by ion chromatography.

v. The samples were also analysed by XRF using the calibration mentioned earlier, but excluding the results for the samples which gave trouble in the chemical analysis.

3 Results and discussion

The species used in the comparison tests were chosen because low recoveries of total sulphur had been obtained with the dry ashing procedure. With the exception of dog's mercury, all species proved difficult to ash. Nevertheless, the presence of unburnt carbon at the filtration stage did not necessarily result in significantly low recoveries of sulphur. In fact, attempts to ensure complete combustion by extending the time in the furnace often caused more serious losses of sulphur. Possible recovery problems other than incomplete ashing is borne out by the dog's mercury sample, which, although invariably completely ashed, often gave inconsistent values of sulphur content. Reproducible results could only be obtained by controlling closely all stages of the ashing procedure for each individual determination. Such control is tedious but necessary.

The results of the comparison tests are given in Table 2. It was noticeable that the samples which were difficult to ash by dry ashing were also difficult to oxidize by wet digestion. The latter method is known to give low recoveries when used to oxidize methionine alone (Randall & Spencer 1980; Bethge 1956) and this has been confirmed for the Mottershead procedure (H M Grimshaw, pers. comm.). Two species, meadowsweet *(Filipendula ulmaria)* and rosebay willowherb *(Chamaenerion augustifolium),* always gave low and variable results with the wet digestion procedure, despite several attempts and careful attention to the digestion procedure. No attempt was made to determine whether these samples were particularly high in methionine, but the wet digestion procedure is unsuitable in these cases.

The Schoniger oxidation resulted in low recovery of S in most cases. Incomplete combustion was a contributory factor, as there was almost always a residue of unburnt carbon in the platinum basket used to hold the filter paper containing the sample. In addition, the procedure requires some manual skill and practice to obtain good results. The figures given are, therefore, not a true reflection of the potential of the method, and further work would probably have overcome the problems. During the course of the study, however, the Schoniger oxidation was superseded by the oxygen bomb combustion, which was immediately more successful, and work on oxygen flask combustion ceased at that point.

The products of combustion in oxygen tend to remain associated with the water formed during the burning process (Mott & Parker 1958) and, as a result, a large part of the sulphur is condensed on to the walls and lid of the vessel as sulphuric acid, rather than being absorbed by the added water. It is, therefore, important to wash down the walls and lid of the container to ensure that all the sulphate produced is determined by ion chromatography. This was found to be the main precaution necessary in the bomb oxidation procedure. Otherwise, the combustion of the sample pellet proceeded smoothly and the addition of a firing aid was not necessary, as no car-

Table 2. Comparison of results for total S (%) in vegetation by various methods

Sample number	Dry ashing	Wet oxidation	Schoniger oxidation	Bomb oxidation	XRF
1	0.348	0.335	0.312	0.328	0.331
2	0.419	0.407	0.382	0.397	0.400
3	0.298	0.310	0.194	0.309	0.313
4	0.132	0.114	0.122	0.123	0.122
5	0.136	0.088	0.113	0.122	0.119
6	0.157	0.107	0.165	0.145	0.143
7	0.138	0.119	0.164	0.153	0.154
8	0.158	0.106	—	0.145	0.137
9	0.366	0.343	0.211	0.344	0.347
10	0.225	0.220	0.190	0.239	0.217
11	0.130	0.134	0.112	0.129	0.132
Mean	0.228	0.207	0.197	0.221	0.220

Results are means of duplicates, except for sample 11 which are day-to-day running means of heather laboratory reference sample (see text and Table 3) bonaceous residue remained in the crucible.

The sample means for each method indicate that lower results were obtained for wet digestion and Schoniger oxidation flask than for the other procedures, for the respective reasons given earlier. Statistical examination of the data from the other 3 procedures showed no significant differences between them ($P<0.05$). However, the bomb oxidation figures agree more closely with those of XRF than do the data from dry ashing, which suggests either better precision for the bomb oxidation method, or a slight positive bias in this set of dry ashing results.

A reference material of heather *(Calluna vulgaris)* (Rowland 1987) was used to assess the long-term reproducibility of the different procedures and also to check whether there were significant differences between them. The data are summarized in Table 3. The dry ashing and wet digestion data were obtained over a period of several years, the Schoniger and bomb oxidation results over a few weeks, and the XRF values over one year. As might be expected, the XRF procedure is the most precise (coefficient of variation (CV) 1.7%) followed by the bomb oxidation method (CV 4.1%). The other methods all had coefficients between 5% and 10%. Excluding the Schoniger oxidation, the means from the other procedures were not significantly different from each other ($P<0.05$). The Schoniger mean was significantly different from all the rest, for the reasons given earlier. If a wider range of species were analysed, then it is probable that the wet digestion would also show significant differences, but this procedure appears to be satisfactory for heather.

Table 3. Results of multiple analyses of heather reference sample for total S

	Dry ashing	Wet oxidation	Schoniger oxidation	Bomb oxidation	XRF
n	30	35	7	7	12
Mean (%)	0.130	0.134	0.112	0.129	0.132
SD	0.0096	0.0131	0.0111	0.0053	0.0023
(CV)	(7.4)	(9.8)	(9.9)	(4.1)	(1.7)
SE	0.0018	0.0022	0.0042	0.0020	0.0007
95% confidence limit	0.0037	0.0044	0.0103	0.0049	0.0015

The only standard reference material available in the laboratory at the time of writing was National Bureau of Standards (NBS) SRM-1575 pine needles. This sample is not certified for sulphur content but a recommended value of 0.117% has been published by Alvarez and Uriano (1985). This material was analysed by the 2 most precise procedures as a check on accuracy (Table 4). The figures show that both the bomb oxidation and XRF methods are accurate, but this fact needs to be confirmed using other certified reference vegetation samples.

Table 4. Analysis of NBS-SRM-1575 pine needles

	% S
Quoted value (Alvarez & Uriano 1985)	0.117
Value obtained by XRF	0.113
Value obtained by bomb oxidation	0.114

4 *Conclusions*

Both dry ashing/barium sulphate turbidimetry and bomb oxidation/ion chromatography appear to be suitable methods for calibration in X-ray fluorescence, which is rapid and reliable for the determination of total sulphur in vegetation. The bomb oxidation procedure is more precise and more convenient, and is therefore to be evaluated further with a wider range of species. The wet digestion/barium sulphate turbidimetric procedure is not recommended because it is unreliable for some species. Schoniger flask oxidation/ion chromatography requires some manual dexterity, but would probably be satisfactory if the necessary skill was acquired.

5 *Acknowledgements*

The author would like to thank Liverpool Polytechnic students, A G Geraghty and A O'Shea, for help with this project, during their period of industrial training at ITE's Merlewood Research Station.

6 *References*

Allen, S.E., Grimshaw, H.M., Parkinson, J.A. & Quarmby, C. 1974. *Chemical analysis of ecological materials.*Oxford: Blackwell Scientific.

Alvarez, R. & Uriano, G.A. 1985. New developments in NBS biological and clinical standard reference materials. In: *Biological reference materials,* edited by W. R. Wolfe, 19—44. Chichester: Wiley.

Bethge, P.O. 1956. Determination of sulphur by wet combustion with perchloric acid. *Analyt. Chem.*, **28**, 119—122.

Busman, L.M., Dick, R.P. & Tabatabai, M.A. 1983. Determination of total sulphur and chlorine in plant materials by ion chromatography. *Soil Sci. Soc. Am. J.*, **47**, 1167–1170.

Butters, B. & Chenery, E. M. 1959. A rapid method for the determination of total sulphur in soils and plants. *Analyst,* Lond., **84**, 239–245.

Mott, R.A. & Parker, C. 1958. Studies in bomb calorimetry IX: formation of sulphuric acid. *Fuel,* **37**, 371–381.

Mottershead, B.E. 1971. Determination of sulphur in biological materials using the Technicon AutoAnalyzer. *Lab. Pract.*, **20**, 483–484.

Randall, P.J. & Spencer, K. 1980. Sulphur content of plant material: a comparison of methods of oxidation prior to determination. *Commun. Soil Sci. Plant Anal.*, **11**, 257–266.

Rowland, A.P. 1987. Reference materials for ecological analysis. In: *Chemical analysis in environmental research,* edited by A. P. Rowland, 88–91. (ITE symposium no. 18.) Abbots Ripton: Institute of Terrestrial Ecology.