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Determination of Total Sulfur in Lichens By Combustion-Infrared Analysis

by

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Abstract

Sulfur was determined in Parmelia sulcata and P. chlorochroa by combustion of the sample and infrared detection of evolved sulfur dioxide using an automated sulfur analyzer. Vanadium pentoxide was used as a combustion accelerator. Pelletization of the sample prior to combustion was not found advantageous. Washing studies showed that leaching of sulfur was not a major factor in the sample preparation. The combustion-IR analysis usually gave higher sulfur content than the turbidimetric analysis as well as shorter analysis time. Relative standard deviations of less than 7% were obtained by the combustion-IR technique when sulfur levels in lichens ranged from 0.05-0.20%. Determination of sulfur in National Bureau of Standards orchard leaves standard reference material showed good agreement between the combustion-IR technique and other instrumental procedures.

Introduction

Lichens have proven to be viable air quality indicators for pollutants such as the heavy metals and sulfur. Numerous studies have shown correlations between the elemental content of lichens and anthropogenic sources (Tomassini and others, 1976; Gough and Erdman, 1977; Goyal and Seaward, 1981; Nygard and Harju, 1983; Taylor and Bell, 1983). In environmental studies such as these the methodology used varies greatly both in the preparation and in the analysis of the lichens. Frequently the methodologic biases are not clearly understood which makes broad interpretation of the data difficult, if not impossible.

The determination of sulfur in plants has generally been restricted to turbidimetric procedures (Tabatabai and Bremner, 1970; Basson and Bohmer, 1972; Chan, 1975) and X-ray fluorescence spectroscopy (Alexander, 1965; Tomassini and others, 1976; Norrish and Hutton, 1977). Combustion techniques with either photometric or titrimetric quantitation of evolved sulfur have also been used to a limited extent (Tiedemann and Anderson, 1971; Jones and Isaac, 1972; Bartels and Pham, 1982).

We have applied a combustion technique with infrared (IR) detection to the determination of sulfur in lichens and other plants and have examined the viability of using this procedure in long-range studies such as the detection of contaminants from emission sources over several years. The analytical methodology used in studies of this nature must be both accurate and precise to detect small changes with time. Therefore, the effects on precision of sample size, pelletization, and use of accelerator were examined. We also examined sample preparation techniques, washing and grinding, which may affect the determination of sulfur in lichens through leaching or inhomogeneity effects. Through detailed study of sample preparation and the analytical technique we have attempted to define the methodologic biases and their effects on data interpretation.

Methods

Determination of Sulfur. A Leco* combustion-IR sulfur analyzer, Model SC-132 (Leco Corp., St. Joseph, MI), was used in this work. The instrument is composed of a microprocessor control unit, an electronic balance, resistance furnace, and a solid state infrared detector. The sample (0.25 g) was

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*The use of trade names is for descriptive purposes only and does not constitute endorsement by the U.S. Geological Survey.

combusted in a stream of pure oxygen at 1370°C for approximately two minutes. Thirty seconds after combustion was initiated oxygen was blown directly into the combustion crucible. The evolved SO_2 was detected and measured in an IR cell after the removal of water.

The instrument was calibrated daily with National Bureau of Standards reference material 1572, citrus leaves (0.407% sulfur). A coal sample was combusted after every fourth sample to flush condensed organic matter through the combustion train. Also several coal samples were combusted as conditioners after the changing of the $Mg(ClO_4)_2$ moisture trap which was replaced after 20-30 samples.

Three factors which might influence the analysis were examined in a balanced 2^3 factorial design: coarse or fine ground lichen material as the two levels of factor one; powdered or pelletized lichen material as the two levels of factor two; and the absence or presence of an accelerator during combustion as the two levels of factor three (cf. table 1). *Parmelia sulcata* collected in Boulder County, Colorado was washed with distilled water as described in the next section. The cleaned material was air-dried for 48 hrs. at 40°C and then ground in a Waring blender. This coarse-ground material was divided into two groups: the first group received no further grinding and the second was ground to a finer and more uniformly sized powder in a Spex Industry mixer/mill.® The finer material was obtained by grinding for 20 minutes in polystyrene vials with methacrylate balls. The coarse and fine ground groups were each divided into two parts: the first was analyzed as the powder and the other was pelletized prior to analysis. Pellets were obtained by pressing 0.25 g of lichen in a 0.5 in. die under 10,000 psi. The powdered and pelletized groups were each further subdivided into two groups which were analyzed either with or without accelerator during combustion. One gram of vanadium pentoxide (Hagerman and Faust, 1955) was spread evenly over the powdered or pelletized lichen sample. The eight treatment groups were analyzed ten times each, randomizing both within and between groups. No instrumental memory effects were detected between analyses.

The turbidimetric determination of sulfur was performed by the method of Tabatabai and Bremner (1970). The plant material (0.25 g) was digested with nitric and perchloric acid. A barium chloride-gelatin mixture was added to the digest. The sulfur content was quantified by comparison of the barium sulfate turbidity in the sample solution to a calibration curve prepared from the analysis of standard solutions.

Preparation and Cleaning of Lichens. *Parmelia sulcata* was collected from trunks, stumps, and downed logs of red juniper in Dunn County, North Dakota. Thalli were broken into uniformly sized pieces, with diameters of about 2.5 cm. The pieces were floated in tap water, and adhering bark, moss, and other lichen species were removed with forceps. Pieces of thalli were randomly separated into three treatment groups, each to receive progressively more intense washings. Group 1 was set aside and labeled "grossly washed." Groups 2 and 3 were recombined and washed eight times with distilled water. For each wash the material was floated in distilled water, stirred for about 15 seconds, and the floating lichen layer was removed. The thalli were randomly separated into two groups. Group 2 received no further washing and was labeled "distilled-water washed." Group 3 was "ultrasonically washed" four times. For each wash the material was cleaned in distilled water using an ultrasonic probe for 15 seconds, and the floating lichen layer was removed.

Treatment groups 1, 2, and 3 were dried overnight at 40°C in a forced-air oven, ground in a Waring blender, and then shaken for 10 minutes using a ceramic container and bead on a mixer/mill. Following thorough homogenization, each group was separated into 16 analytical splits of about 1.5 g each.

Results and Discussion

The sample combustion characteristics are the most important parameters with this analysis technique since the rate of sulfur evolution as well as incomplete conversion to SO_2 affects both the accuracy and the precision of the measurement. As described in the experimental section, three factors, sample particle size, pelletizing, and the use of accelerator, were examined for their effects upon the sulfur determination in lichens. Particle size was chosen as a factor to test for inhomogeneity effects and possible size-related combustion effects. Pelletizing and the use of accelerator were also chosen as factors which might influence the rate of sulfur evolution or the formation of SO_2.

The mean apparent sulfur content for each treatment group and the F values for the main effects and interaction effects are shown in tables 1 and 2, respectively. The results show an interaction between the pelletizing and use of accelerator treatments. When the lichen sample was both pelletized and combusted with accelerator the apparent sulfur content decreased by 20%. Due to this interaction, interpretation of the effects of pelletizing and accelerator alone must be based on the examination of subsets of the data.

Table 1.--Mean apparent sulfur content (%S) found in 2^3 factorial experiment with replication (n=10) using samples of Parmelia sulcata

	Blender Ground		Blender + Mixer Ground	
	Powdered Sample	Pelletized Sample	Powdered Sample	Pelletized Sample
Combusted without Accelerator.....	0.139 (1)	0.141 (b)	0.139 (a)	0.142 (ab)
Combusted with Accelerator.....	0.143 (c)	0.113 (bc)	0.142 (ac)	0.115 (abc)

Table 2.--Analysis of variance of factorial experiment examining combustion effects using samples of *Parmelia sulcata*

Source of Variation	Degrees of Freedom	Mean Square $X10^4$	Calculated F Value
Grinding.................	1	0.101	0.2
Pelletizing.............	1	33.07	61.6 **
Accelerator.............	1	28.10	52.4 **
Grinding x Pelletizing...	1	0.149	0.3
Grinding x Accelerator...	1	0.004	0.007
Pelletizing x Accelerator	1	48.60	90.6 **
Grinding x Pelletizing x Accelerator..........	1	0.026	0.04
Error....................	72	0.537	

**Significant at the 99% confidence level.

The subsets in which these two factors were not confounders (e. g., treatments (1) vs. b, (1) vs. c, and b vs. c, cf. table 1) were examined. Comparison of the treatment means using the t test at the 95% confidence level indicated no apparent effect with pelletizing or the presence of accelerator when they were not used simultaneously. This was further examined by analyzing 31 lichen samples of *Parmelia sulcata* and *P. chlorochroa*. The samples were analyzed as powdered material covered with accelerator and as pelletized material without accelerator. Paired t tests indicated that the means of the two treatments were significantly different at the 95% confidence level for the lichens. The sulfur content averaged 10% higher without pelletizing. Since no detectable blank was found when using V_2O_5 the small bias was attributed to minor differences during combustion; and pelletizing of the sample was not pursued further. Although the use of accelerator was not indicated as being necessary we continued to use V_2O_5 for routine analysis since it may suppress the formation of sulfur trioxide (Ricke, 1964).

The factorial experiment revealed no sample inhomogeneity and combustion effects due to particle size, despite sieving experiments which showed that the additional grinding gave smaller and more uniform particle size. It can be seen in table 3 that simple grinding in a blender produced a particle size distribution that was not dramatically different from that of NBS citrus leaves. Thus blender grinding only was used for routine work.

Table 3.--Particle size distribution. Percentage of lichen sample passing through the sieve

U.S. Standard Mesh Size	P. sulcata Blender Ground	P. sulcata Blender + Mixer Ground	NBS 1572 Citrus leaves (as received)
35 (0.50 mm)..........	90%	95%	100%
60 (0.25 mm)..........	66%	80%	78%
100 (0.15 mm)..........	40%	56%	40%

Analysis of a lichen sample in replicate (n=5) using 0.25 g and 0.5 g sample weights indicated no difference in mean value (0.071 vs. 0.073 % sulfur, respectively). However, the relative standard deviation (RSD) did improve from about 6% to 2% with the increase in sample size. Since the larger sample size caused more frequent changing of the water trap and a decrease in the number of samples analyzed per day, the improvement in precision did not warrant using the larger size on a routine basis.

The excellent precision of the sulfur analyzer enhanced our ability to detect deleterious effects due to different grinding procedures, although none were found in the factorial experiment. It also allowed us to examine closely other factors in the sample preparation regimen, in particular the washing of the sample. For environmental studies that are concerned with contaminant levels in lichens, some washing procedure may be required. However, determining when the washing procedure has removed the extraneous particulates but has not leached analyte from the lichen itself is difficult. Goyal and Seaworth (1981) detected leaching of metals during their washing procedure, but did not find it to be statistically significant. Lawrey and Hale (1981) found washing effects when examining lichens for lead accumulation. Little (1973) used deionized water and 1% HNO_3 washes on aerially contaminated elm, oak, hawthorn, and willow leaves. He found lead and zinc present as surficial water-soluble and insoluble deposits which were distributed differently both within and between species. Water washing removed the majority of the surficial deposits, but the total amount removed was metal dependent. Thus, the sample preparation must be critically examined before environmental data can be interpreted.

We examined three increasingly rigorous washing procedures and found a statistically significant difference between the washes. However, the difference could not be clearly attributed to improved removal of extraneous matter as opposed to leaching of sulfur. Table 4 shows the mean sulfur content of sixteen analyses of each treatment category. The analysis of variance of these data gave a statistically significant (99% confidence level, CL) F value of 22.1 (degrees of freedom 2,45) when comparing the treatment variance with the error variance. Further examination using Duncan's multiple range test (Duncan, 1955) showed no difference between the grossly washed material and the distilled water washed material. It also showed no difference between the distilled water washed material and the ultrasonically washed material. There was a significant difference (99% CL) between the least rigorous and the most rigorous washes, that is, between the grossly washed and ultrasonically washed material. Although the decrease in sulfur content with rigor of washing may be due to leaching, it is possibly due to more effective removal of particulate matter since the ash content decreased with increased sample washing. The ash content was 14.9%, 11.6%, and 9.8% for the grossly washed, distilled water washed, and ultrasonically washed material, respectively. This decrease in ash content, however, could also be due to the leaching of calcium oxalate from the lichen sample (Erdman and others, 1977). Regardless of the cause, the overall relative decrease in sulfur content of less than 10% was not considered important. In order to minimize sample preparation time yet provide an effective wash, the distilled water washing regimen was chosen for routine analysis and will be applied in all subsequent studies.

Table 4.--Sulfur content of Parmelia sulcata in each wash treatment

	Treatments		
	Grossly washed	Distilled water washed	Ultrasonically washed
Range, %S...........	0.153 - 0.163	0.136 - 0.169	0.141 - 0.149
Relative Standard Deviation...........	2.4	4.8	1.7
Mean[1], %S........... (n = 16)............	0.156	0.150	0.145

[1] Means underscored by the same lines are not significantly different (95% confidence level) (Duncan 1955).

The NBS citrus leaves SRM and the lichen sample used in the factorial experiment were analyzed by a turbidimetric procedure(Tabatabai and Bremner, 1970), six times each. The turbidimetrically determined sulfur contents were 0.36% and 0.14% for the citrus leaves and lichen, respectively. The sulfur content of the citrus leaves was 13% lower than the certified value, whereas for the lichen it compared well with the combustion-IR result of 0.143%. When 17 higher order plants were analyzed by both procedures the combustion-IR technique gave sulfur contents which averaged more than 20% higher than those obtained by the turbidimetric analysis. The RSD for the turbidimetric analyses was 8% for the citrus leaves and lichen samples. The combustion-IR technique yielded slightly better precision, 2% and 4% RSD for the citrus leaves and lichen, respectively. Also an important improvement was obtained in the analysis time required; 60-80 samples per day by the combustion-IR technique compared to the 35 samples per day by the turbidimetric procedure.

TABLE 5.--Analysis of NBS botanical reference materials. Comparison of citrus leaves and coal as calibrating standards.

NBS Reference Material	Calibrating Standard	Range, %S	Mean, %S (n = 5)	Relative Standard Deviation
1567 Wheat flour	leaves	0.168 - 0.190	0.179	5.4
	coal	0.172 - 0.190	0.181	4.0
1568 Rice flour	leaves	0.136 - 0.143	0.140	1.9
	coal	0.132 - 0.147	0.138	4.3
1570 Spinach leaves	leaves	0.440 - 0.451	0.444 (n=3)	1.4
	coal	0.470 - 0.513	0.486	3.3
1571 Orchard leaves	leaves	0.200 - 0.214	0.204	2.7
	coal	0.194 - 0.204	0.196	2.2
1572 Citrus leaves	leaves	0.400 - 0.426	0.414	2.5
	coal	0.404 - 0.420	0.416	1.6
1573 Tomato leaves	leaves	0.610 - 0.634	0.626	1.6
	coal	0.643 - 0.666	0.655	1.4
1575 Pine needles	leaves	0.123 - 0.138	0.129	4.2
	coal	0.120 - 0.129	0.124	2.6

Table 5 demonstrates satisfactory agreement between the combustion-IR procedure and other instrumental methods for the analysis of NBS orchard leaves, except for the results of Jones and Isaac (1972), obtained using a Leco sulfur analyzer with an induction furnace and iodometric titrator, which were much lower than those found by the other investigators.

The combustion-IR sulfur determination technique proved suitable for the analysis of lichens. The excellent precision and the short sample analysis time required in large environmental studies were obtained. The results also agreed well with other analysis techniques.

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