

A RAPID SYSTEM OF CANE LEAF ANALYSIS USING X-RAY SPECTROMETRY AND INFRA-RED REFLECTANCE

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Abstract

An evaluation of a non-destructive technique for the determination of the elements P, K, Ca, Mg, S, Zn, Fe, Cu and Mn in cane leaves using a Philips PW 1410/20 X-ray fluorescence spectrometer is described. Regression analyses indicated that nutrient values determined by the X-ray method correlated well with values obtained by the chemical method (all *r* values greater than 0,90). Furthermore, reproducibility was acceptable with coefficients of variation ranging from 3 to 10%. Together with the infra-red reflectance analyser to determine nitrogen in cane leaves, the X-ray method provides a rapid integrated system of analysis which has replaced time consuming chemical methods. The new system will be capable of handling likely increases in the demand for leaf analysis in the foreseeable future.

Introduction

The Experiment Station of the South African Sugar Association has conducted a fertilizer advisory service (FAS) for more than 30 years. The demand for soil tests has always exceeded that for foliar diagnosis as shown in Table 1. During the 1973/74 season, more than 17 500 soils and only about 6 000 leaves were analysed. During the 1982/83 season, 22 500 soils were analysed while the number of leaves analysed had risen to more than 16 500. This large increase in the number of leaf samples which was analysed can be attributed partly to the wider acceptance by cane growers of whole cycle recommendations, in which fertilizer advice is given for a plant crop and four successive ratoons. Leaf analyses are used to check on the adequacy of these recommendations during the ratoon cycle.

TABLE 1
Soil and leaf samples analysed (1973-1983)

Year	Soil	Leaf
1973/74	17 546	6 246
1974/75	14 549	6 899
1975/76	14 224	8 470
1976/77	17 059	9 149
1977/78	22 032	9 893
1978/79	20 867	13 121
1979/80	23 597	11 899
1980/81	20 554	13 444
1981/82	21 621	12 699
1982/83	22 614	16 649

The analysis of more than 16 000 leaf samples by the traditional chemical digestion procedure often involved two digestions, one using sulphuric acid for the macro-elements and the other using nitric acid and perchloric acid for the micro-elements. Apart from being hazardous and unpleasant, these were time consuming procedures involving many laboratory operations to determine the various plant nutrients (see Figure 1). It became apparent that to cope with the increased number of samples, an alternative system of analysis was required. It would have to be quicker and at least as reliable as the old methods, preferably automated, labour-saving and suitable for processing any future increase in the number of samples.

With the above criteria, a comprehensive range of analytical equipment was evaluated. The most suitable combination of instruments was found to be an infra-red reflectance analyser for nitrogen determinations, in conjunction with an X-ray fluorescence spectrometer which would be used to determine non-destructively, the remaining macro-and micro-elements in the leaf. The rapid determination of nitrogen in sugarcane leaves using the Technicon infra-red reflectance analyser has already been reported in detail by Meyer,⁶ so that reference will be made here mainly to the elemental analysis of cane leaf material by X-ray spectrometry.

Interest in X-ray fluorescence spectrometry as a means of elemental analysis has grown rapidly in the past 30 years, as adequate electronic and counting equipment has become available to render the method suitable for routine laboratory determinations. The use of X-ray fluorescence for analysing plant tissue has been reported by various workers, among them Bowen;¹ Evans;³ Jenkins *et al.*;⁴ Livingstone;⁵ Norrish and Hutton;⁷ and Whittig.⁹ Norrish and Hutton⁷ pointed out that plant material is in many respects ideal for analysis by X-ray spectrometry for all elements except those with atomic numbers less than 10, e.g. carbon, nitrogen and oxygen, or those present in quantities of only a few parts per million. Generally the concentration of the elements required is less than 5% and simply pressing an adequately ground sample into a suitable pellet or disc is the only preparation that is required.

The main advantage of the X-ray method for plant analysis is the elimination of the time consuming acid digestion procedure. It was decided that its applicability to the determination of P, K, Ca, Mg, S, Zn, Fe, Cu and Mn in cane leaf samples on a routine basis should be investigated. The equipment, the principle of the method, the procedure used, and an evaluation of the accuracy and precision of the method under routine operating conditions, are described.

Method

Equipment

The Philips X-ray spectrometer system (see Figure 2) consists of a PW 1410/20 sequential semi-automatic vacuum X-ray spectrometer which is used in conjunction with a PW 1130/90 X-ray generator to provide both high voltage and current for the X-ray tube, a PW 1390 channel control unit for timing and counting output of pulses, a PW 1394 motor control and a PW 1395 programmer for automatic operation. There is also a pen recorder to chart pulse heights and peak angles, and an HP 9825A computer for calculating and printing results. Other accessories include a Herzog press for manufacturing leaf sample discs and a supply of gas consisting of 90% argon and 10% methane which is required for the flow counter of the spectrometer.

The parameters for analysing the desired elements are selected and programmed on the computer and this information is then loaded into the programmer. Program 1 for the elements Zn, Ca, K, P, S and Mg and Program 2 for Mn, Fe and Cu.

Principle of X-ray spectrometry

The basic spectrometer arrangement is shown in Figure 3. The power from the generator causes the X-ray tube to emit

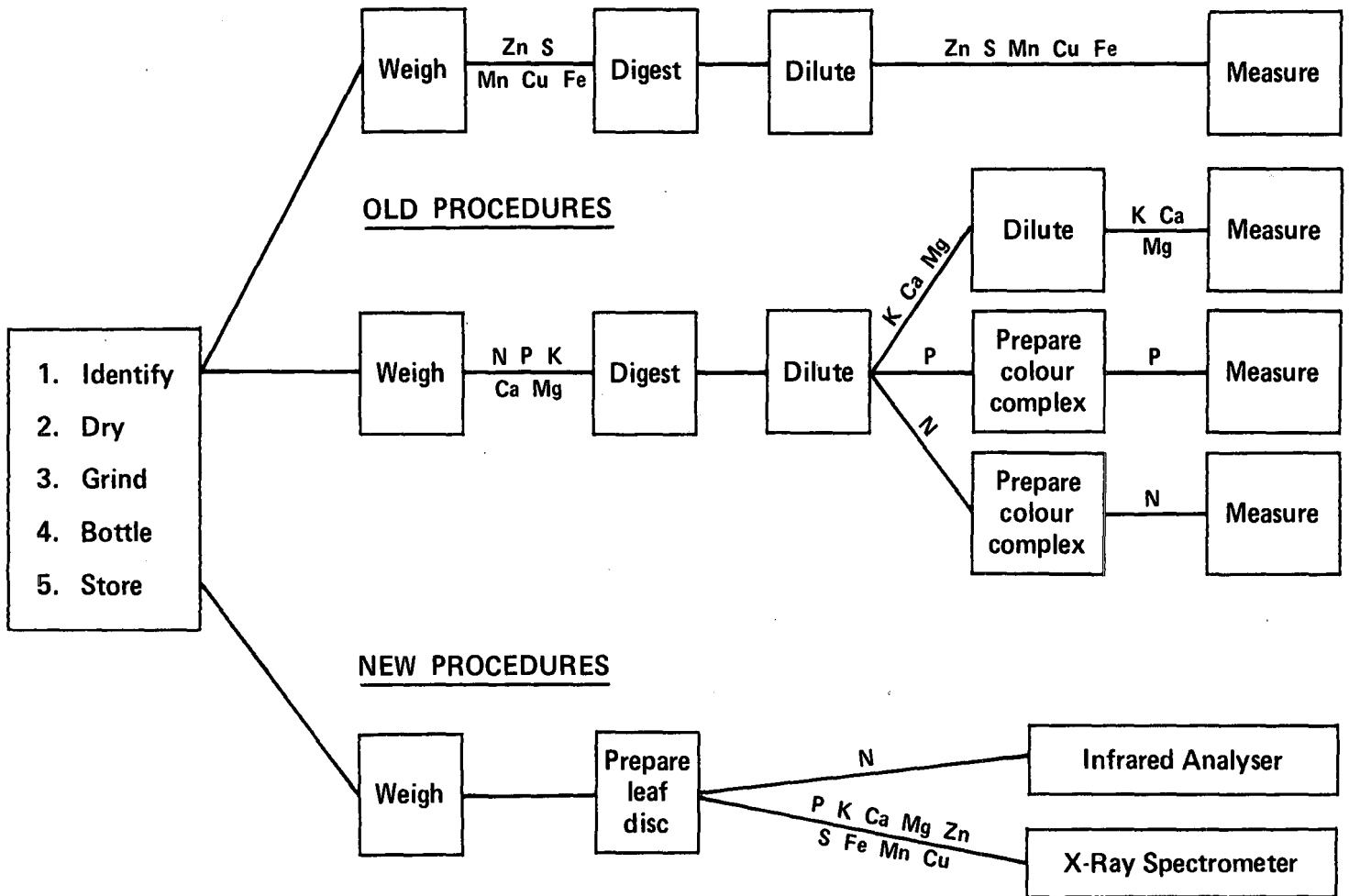


FIGURE 1 Procedures of old and new systems of leaf analysis.

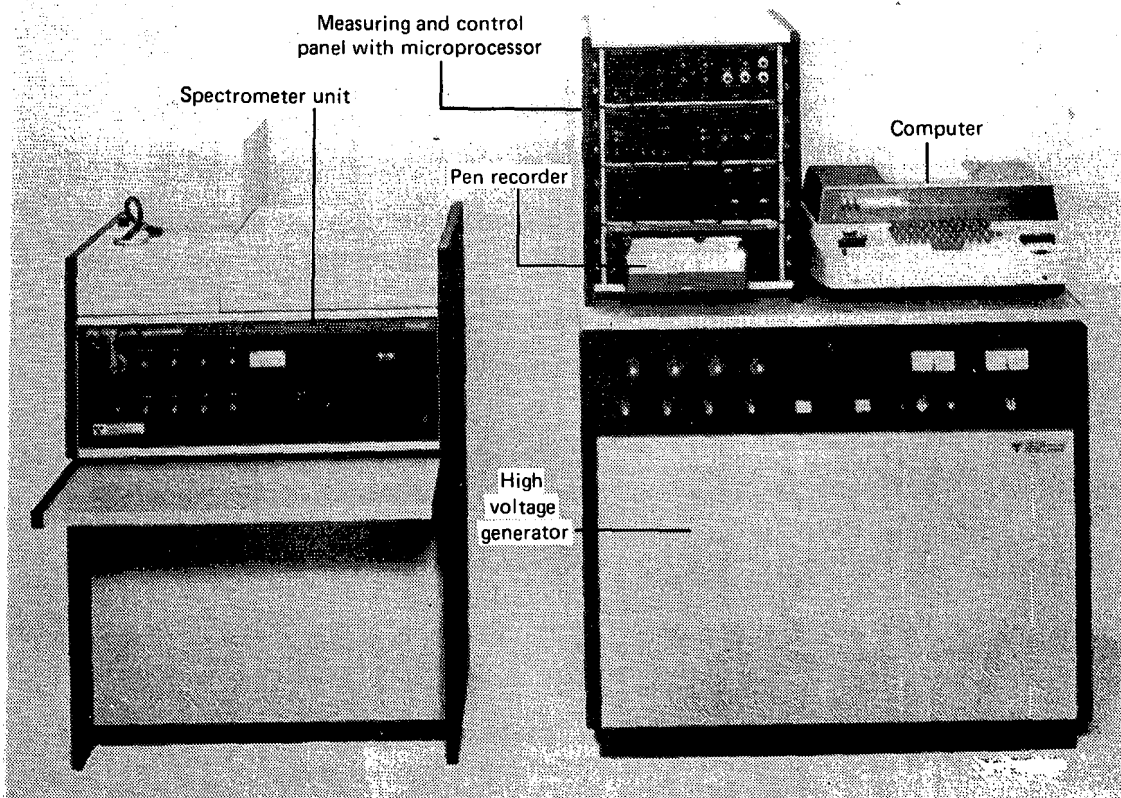


FIGURE 2 The PW 1410/20 X-ray spectrometer system.

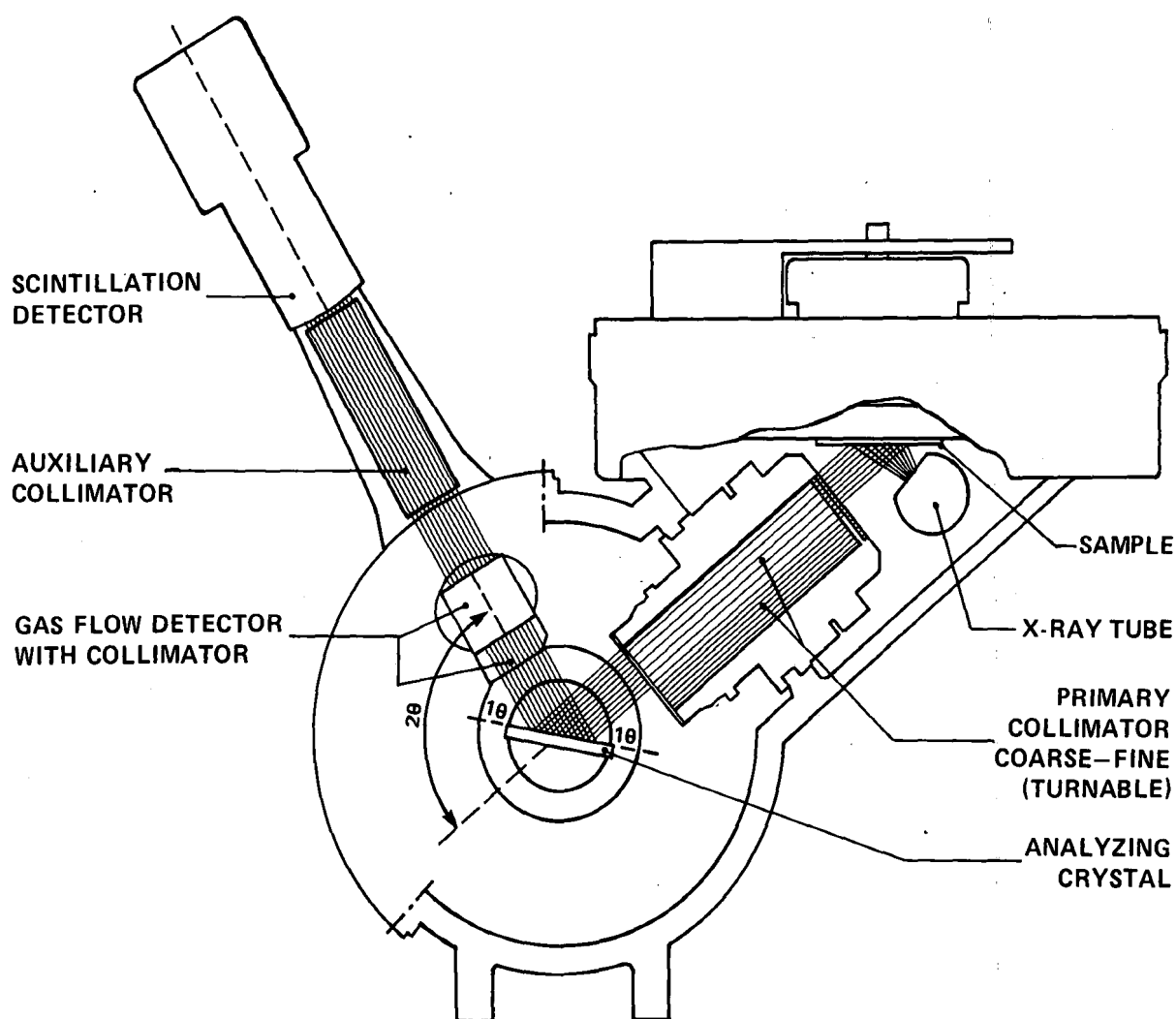


FIGURE 3 The basic spectrometry arrangement.

high energy primary X-rays which bombard the sample, causing it to emit secondary fluorescent X-rays. Peaks of different radiation intensity are obtained and are characteristic for each element in the sample. The secondary X-rays are channelled into parallel beams by a primary collimator and directed onto an analysing crystal, from where they pass through a second collimator and are directed towards the gas flow detector and the scintillation detector. A goniometer aligns the two detectors in the direction of the beams reflected by the analysing crystal. The pulses received by the detectors are further amplified and then relayed to the channel control where they are displayed in kilo counts per second (kc^{-s}). The channel control relays the kc^{-s} to the computer, which converts them into the desired units and prints the result.

The main operating parameters for the elements that were tested are listed in Table 2. The flow proportional detector (counter) plus pulse height selection were used for detecting all elements. In addition, the scintillation counter was required for detecting Zn and Cu. The lithium flouride crystal (LiF 200) was the one most widely used (for Zn, Ca, Fe, Mn, K and Cu), while the germanium (Ge III) crystal was used for P and S and the ADP crystal for Mg. The cps^{-ppm} and detection limits in Table 2 indicate that X-ray fluorescence is very sensitive to Zn, Cu, Fe, Mn and Ca (all less than 5 ppm detection limit), moderately sensitive to P, K and S (less than 50 ppm), and insensitive to Mg (more than 200 ppm). Fortunately, the concentration range of Mg in third leaf samples is above the limit of detection (0,10 to 0,40% Mg). Some problems are encountered with the trace

element Cu because it occurs in the range 4 to 10 ppm which is just above the limit of detection.

TABLE 2

Instrumental conditions for the elements tested

General conditions: Philips PW 1410/20 spectrometer fitted with a Cr target 3 kW tube, spinner on and using vacuum

Element	Detector	Collimator	Crystal	Angle 2θ	Counting time, secs	cps^{-ppm}	Detection limits, ppm
P	F	C	Ge(111)	140,98	4	0,84	31
K	F	F	LiF(200)	136,78	4	3,60	20
Ca	F	F	LiF(200)	113,19	4	3,32	4
Mg	F	C	ADP(101)	136,80	10	0,08	260
S	F	C	Ge(111)	110,65	4	1,36	24
Zn	F+S	F	LiF(200)	41,80	20	6,25	3
Cu	F+S	F	LiF(200)	45,06	10	4,20	5
Fe	F	F	LiF(200)	57,52	4	2,94	1
Mn	F	F	LiF(200)	63,00	20	1,40	2

Collimator: Fine (F) or Coarse (C)
 Detector : Flow (F) or Scintillation (S) counter or both

Figure 4 represents a scan showing wavelength maxima corresponding with $K\alpha$ transitions for selected elements when using the LiF 200 crystal. In routine analysis, the spectrometer is programmed to measure the intensity of the X-ray fluorescence at the element's particular wavelength reading and to convert this to a concentration reading.

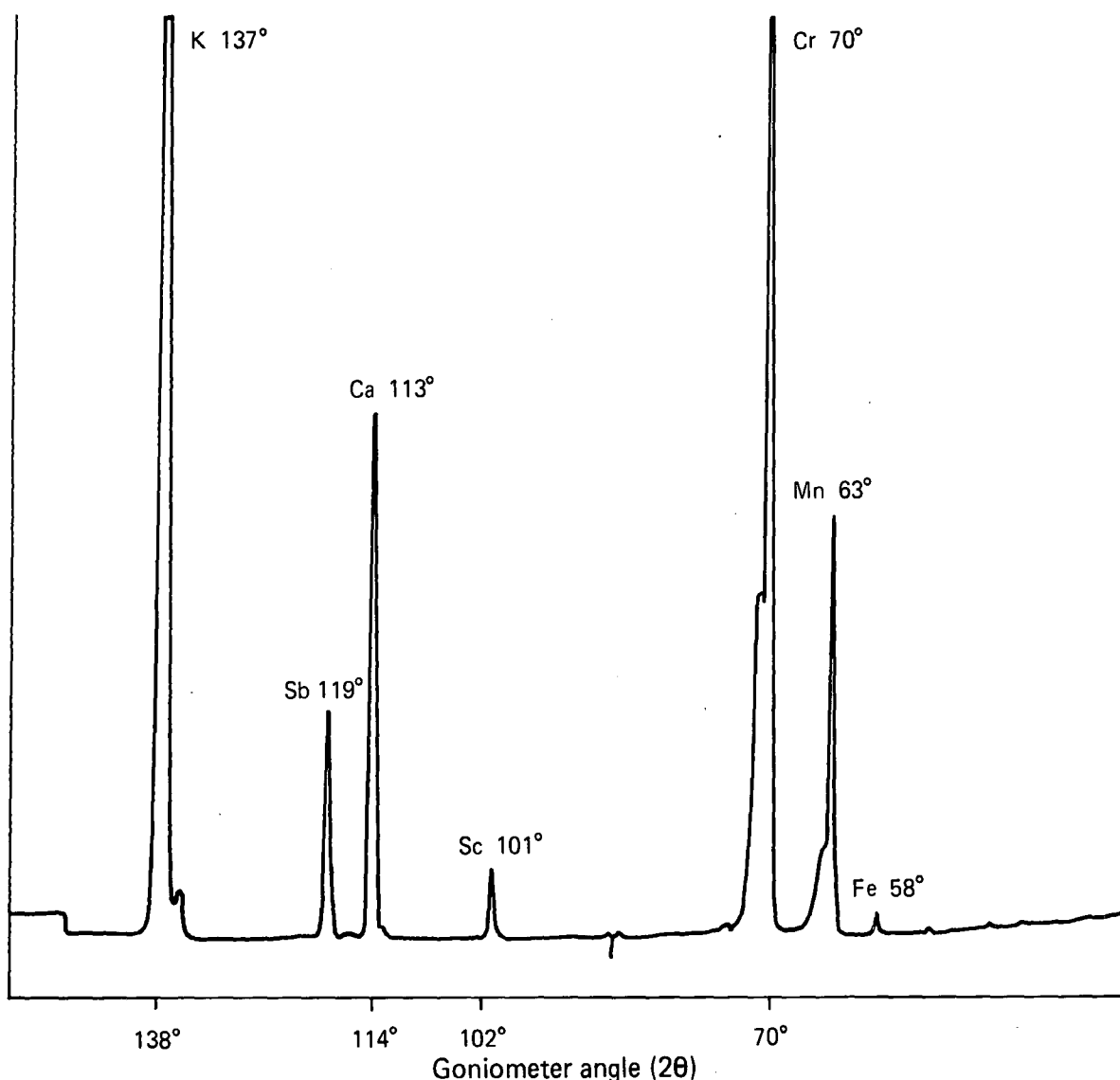


FIGURE 4 X-ray fluorescence wavelength scan for selected elements using a LiF 200 crystal.

Procedure

Sample preparation

After third leaf samples are dried in a forced draught oven at 65°C for 48 hours, they are finely ground in a mill by passing them twice through a 0,5 mm perforated screen. Five grams of the ground sample are thoroughly mixed with three grams of a binder (EMU powder, which is a proprietary brand of styrene copolymer as used by van Zyl⁸). A Herzog hydraulic press is used to press the mixture into a disc. The discs are held at a pressure of 153 megapascals for 20 seconds and then stored in a desiccator until required for analysis.

Calibration of the spectrometer

The spectrometer is calibrated for the elements to be analysed by choosing a range of leaf samples to cover the lowest and highest expected values for each element. Calibrations are checked daily by using reference samples before analysing unknown samples. The instrument, does not have to be calibrated daily, but has to be re-calibrated either when the high voltage is altered, or when the flow counter window or gas cylinder is replaced.

Analysis

Four prepared discs are placed in sample holders and are analysed simultaneously. The sample holders are inserted one at a time into the spectrometer airlock which is then evacuated (Figure 5). Thereafter the sample holders are placed automatically on the four position turret in the sample chamber. Anal-

ysis is initiated by depressing the appropriate key on the computer. The first sample to be analysed then moves into the measuring position.

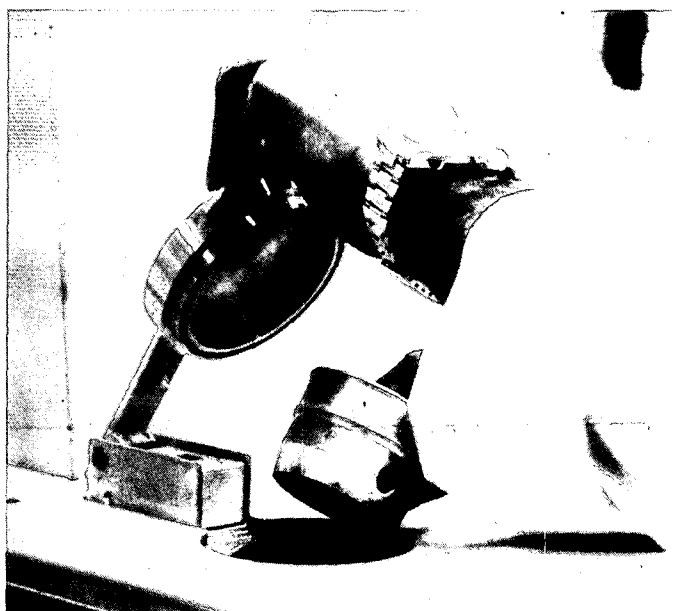


FIGURE 5 The leaf sample holder being inserted into the spectrometer airlock.

As a standard procedure, analysis is only initiated when the vacuum in the sample chamber is less than 0,3 torr. A complete analysis for Zn, Ca, K, P, S and Mg in four samples takes approximately six minutes. The analysed samples are then replaced by a further set of four samples. Accuracy of the analyses is monitored by including a reference sample after each batch of 20 samples has been analysed. Every twentieth sample is analysed in duplicate, and reference samples are also run at the beginning and end of each day's analysis.

Evaluation

Assessing the suitability of the X-ray spectrometer for routine analyses consisted of:

- calibration intensity measurements
- preliminary evaluation of the accuracy of the method and reproducibility of the results in relation to the standard chemical digestion procedure incorporating the colorimetric determination of phosphorous and determination of the remaining nutrients (apart from sulphur) by atomic absorption spectrophotometry
- evaluating the performance of the spectrometer under routine operating conditions over a wider range of nutrient values.

Calibration

Calibration curves were constructed for the major and minor elements by plotting count rates against element concentration for 13 reference samples (Figures 6a and 6b). The slope of the calibration curves represents the sensitivity for a particular element expressed in terms of counts per second percent or ppm. Sensitivity for the macro-nutrients decreased in the order K, Ca, S, P, Mg, while for the micro-nutrients the order was Zn, Cu, Fe, Mn. Overall, the sensitivity was directly related to the atomic number of an element. Mg has the lowest atomic number (12) and had the lowest sensitivity (0,08 cps-ppm) compared with Zn which has the highest atomic number (30) and had the greatest sensitivity (6,25 cps-ppm).

Comparison of the X-ray and the chemical digestion methods

An initial assessment of the accuracy and precision of the X-ray method was made by repeatedly analysing seven reference samples over a period of 30 days. The results are sum-

marised in Table 3. The small difference between the mean results, and the small variation in values for each element (CV range 3 to 10%) suggest that the method is reasonably accurate and precise. The values for P compared favourably with those reported by Burrows and Meyer² using a colorimetric procedure, and for the remaining nutrients with values obtained by atomic absorption.

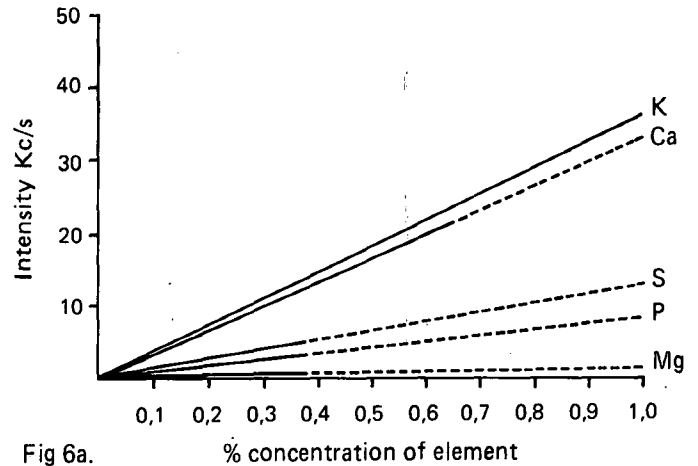


Fig 6a.

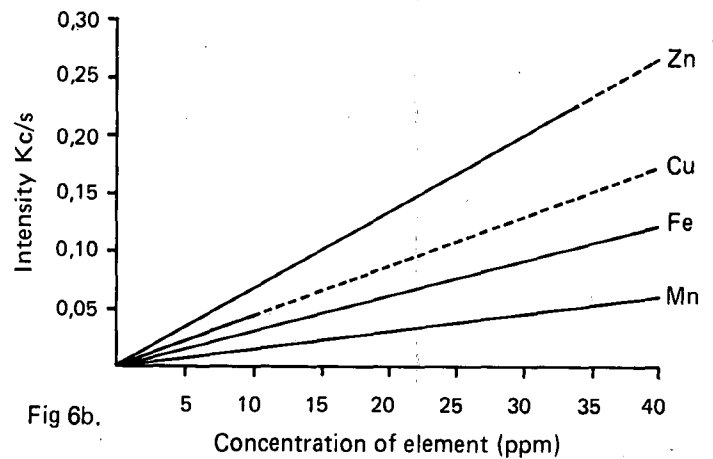


Fig 6b.

FIGURES 6a & 6b Relative sensitivity of macro- and micro-elements to X-ray fluorescence.

TABLE 3
Accuracy and precision of the X-ray method

Composite sample	No. of determinations	Mean value: X-ray method						Difference between X-ray and chemical methods					
		P%	K%	Ca%	Mg%	S%	Zn ppm	P%	K%	Ca%	Mg%	S%	Zn ppm
B	30	0,15	0,83	0,23	0,20	0,13	18,3	0,00	-0,05	0,00	0,00	-0,01	+1,6
C	30	0,17	0,95	0,23	0,22	0,15	18,6	0,00	-0,05	-0,02	-0,04	0,00	-0,7
D	30	0,18	1,05	0,24	0,20	0,15	18,4	-0,01	-0,02	-0,01	-0,04	0,01	+0,6
F	24	0,21	1,30	0,25	0,19	0,14	18,2	-0,01	-0,03	-0,05	-0,04	0,00	-0,9
G	25	0,23	1,34	0,26	0,17	0,14	17,7	0,00	0,04	-0,05	-0,03	-0,01	-1,5
H	24	0,26	1,47	0,25	0,16	0,14	16,9	0,01	0,02	-0,05	-0,01	-0,01	-1,8
I	24	0,28	1,57	0,25	0,17	0,15	17,3	0,00	-0,03	-0,05	-0,01	-0,02	-1,1
Overall mean		0,21	1,22	0,24	0,19	0,14	17,9	0,00	-0,02	-0,03	-0,02	-0,01	-0,5
Std deviation		0,16	0,04	0,009	0,019	0,014	1,16						
CV %		7,77	3,20	3,87	10,02	10,01	6,50						

The reproducibility of the method using the results of a single reference sample that was analysed on six separate occasions, is shown in Table 4. The CV data suggest that the method is extremely precise for K and Ca (CV less than 5%), while for Mg, Zn and S, the method is less precise (CV 7 to 9%), but nonetheless acceptable.

TABLE 4
Reproducibility of the X-ray method for various elements

Nutrient	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Mean	Standard deviation	Mean CV %
P	0,16	0,15	0,16	0,16	0,15	0,14	0,15	0,012	7,9
K	0,85	0,85	0,87	0,87	0,88	0,82	0,83	0,033	4,0
Ca	0,25	0,24	0,25	0,25	0,24	0,23	0,23	0,010	4,5
Mg	0,21	0,22	0,22	0,25	0,22	0,21	0,20	0,016	8,0
S	0,13	0,13	0,15	0,14	0,13	0,12	0,13	0,011	8,4
Zn	16,4	23,1	22,1	19,3	19,6	17,9	18,3	1,590	8,7

Performance of the X-ray spectrometer under routine conditions

The performance of the spectrometer under routine conditions in the FAS laboratory was assessed by analysing a batch of 72 leaf samples and comparing the results with those obtained by the chemical procedures. The level of accuracy that was obtained for P, K, Ca and Mg over a wide range of element values is shown in Table 5. The respective r values of 0,94, 0,99, 0,94 and 0,97 from regression analyses confirm that the X-ray method correlated closely with the chemical method for all four elements.

TABLE 5

Comparison of various nutrient values obtained by the X-ray and chemical method (72 samples)

Element	Means			Range		r
	Chemical	X-ray	Diff.	Low	High	
P	0,22	0,23	0,01	0,14	0,33	0,939
K	1,21	1,17	-0,04	0,75	2,06	0,992
Ca	0,24	0,27	0,03	0,19	0,39	0,935
Mg	0,19	0,20	0,01	0,12	0,37	0,974

The relationship between the potassium contents of the 72 samples determined by the chemical method based on atomic absorption spectrophotometry and those obtained by X-ray is shown in Figure 7. Regression analysis confirmed that the two methods were well correlated over the range 0,7 to 2,1% K ($r^2 = 0,98$). Furthermore, an examination of the results for individual samples showed that the differences were less than 0,10 units in 80% of the samples analysed. This close agreement is illustrated by the frequency distribution of differences given in Figure 8. Similar relationships were obtained for P, Ca and Mg.

Discussion and conclusions

The results obtained from this investigation indicated the suitability of X-ray fluorescence spectrometry for the routine analysis of all (except nitrogen) important plant nutrients in sugarcane leaves. The accuracy and precision of the method were found to

be comparable with those previously used by the Fertilizer Advisory Service. The use of the X-ray spectrometer for routine leaf analysis had no major disadvantages except that the equipment was very expensive. It is, however, extremely important that standardised procedures are established, especially with regard to all aspects of sample preparation, and regular calibration and instrument checks are required.

Together with the infra-red reflectance analyser for determining nitrogen in cane leaves, the X-ray method provides a rapid integrated system of analysis which has now replaced the hazardous and time consuming chemical methods. These methods required six full-time laboratory staff, whereas the new system requires only a technician and two other laboratory staff. The number of laboratory operations required to analyse each leaf sample has been greatly reduced (see Figure 1), while the number of leaf samples analysed per day has increased from 160 to 220. This increased capacity will considerably reduce delays during the peak leaf sampling period (October to March) and the new system will cater for any increases in demand for leaf analysis in the foreseeable future. Labour-saving resulting from the introduction of the new system has enabled the simultaneous analysis of soil and leaf samples during peak leaf sampling periods which previously was not possible, and this has led to improved sample throughput. As far as is known this is the first time that a non-destructive integrated system of plant analysis has been used for agricultural purposes in South Africa.

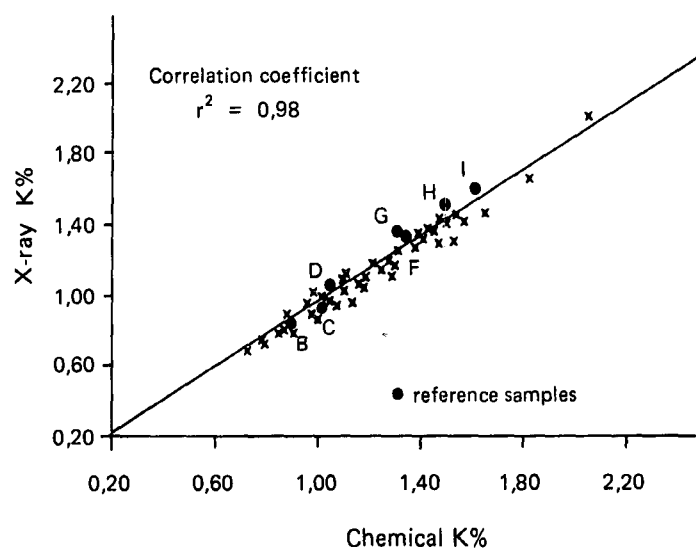


FIGURE 7 Comparison of the X-ray and chemical method for analysing potassium content of cane leaf.

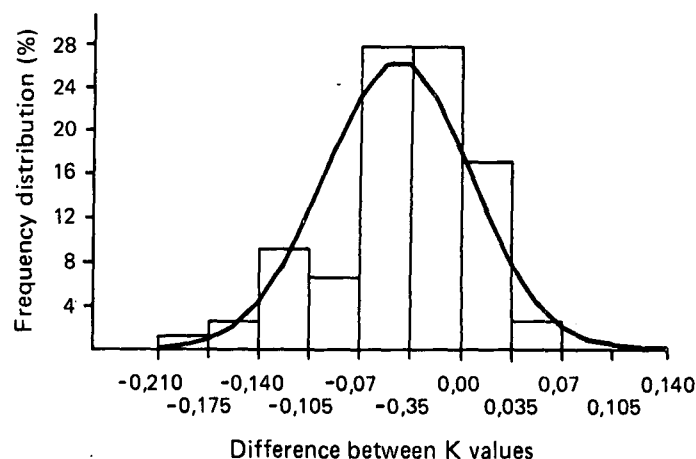


FIGURE 8 Agreement between the X-ray and standard atomic absorption method for K.

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