

SEMI-AUTOMATION WITH REFERENCE TO THE DETERMINATION OF ALUMINIUM AND PHOSPHORUS IN SOIL AND CANE LEAVES

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Abstract

The semi-automated equipment purchased to cope with the increasing numbers of samples analysed by the Fertilizer Advisory Service Laboratory is described. Experiments used to evaluate the equipment and modifications to methodology are detailed, with specific reference to the determination of "extractable" aluminium and phosphorus in soils and total phosphorus in cane leaves. It was concluded that there was no loss in precision due to automation and that work output was increased. With the exception of aluminium determinations, no bias was introduced into the results by changes in methodology.

Introduction

The Experiment Station has now had 22 years experience in running for the sugar industry a fertilizer advisory service based on soil and leaf analysis. The number of samples has increased steadily from 10 700 in 1955 to 24 000 in 1974. During this period the scope of analytical services has also increased considerably. For example, new soil tests to determine more accurately the phosphorus (Meyer⁶) and lime requirements (Moberly and Meyer⁷) of soils from the Natal Midlands have been introduced into the analytical programme. In order to minimise delays which could arise from the increased workload an improved semi-automated system of analysis has been introduced.

The previous system, in which macroquantities of reagents were dispensed with glass pipettes, and readings manually recorded from hand operated instruments, was first critically examined using a simple work study technique. Time-consuming steps which limited the output of the system were identified by drawing up an operations flow chart. Priorities were then assigned to these features in the design of an improved system. Various analytical systems available in South Africa were evaluated in the light of this information and the LKB Ultralab discrete sample system was chosen as being most suitable for our requirements.

This paper describes the equipment and the methods used for its evaluation, with specific reference to the colorimetric determination of "extractable" aluminium and phosphorus in soils and total phosphorus in cane leaves.

Materials

Oven-dried reference samples of top soil and top visible dewlap cane leaf material, collected from a range of soil types in the sugar belt, and soils submitted for routine analysis were used for evaluation.

Equipment

The equipment was purchased on a stepwise basis. Initially the *measurement step* was automated with an LKB Model 7400 calculating absorptiometer (see Figure 1). This instrument

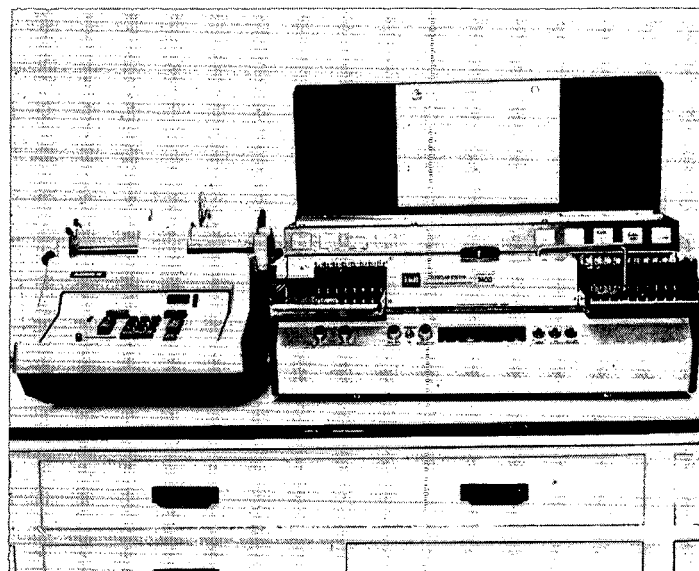


FIGURE 1 LKB calculating absorptiometer.

is a high speed, ten channel filter photometer capable of determining 100 samples in five minutes. Samples for analysis are contained in disposable cuvettes, held in aluminium racks, which are progressively fed through the instrument. The absorptiometer is linked to a printer so that results are recorded directly onto paper tape.

The colour complex *preparation step*, which involved up to four manual operations, was automated as the second phase using an LKB 2071 sample processor (see Figure 2). This instrument automatically transfers aliquots, and dispenses up to three reagents at previously selected volumes into disposable test tubes or cuvettes. As with the absorptiometer, aluminium

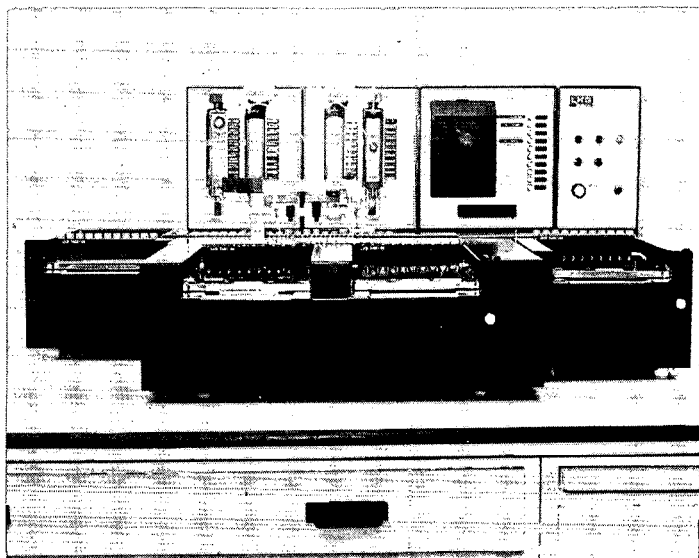


FIGURE 2 LKB sample processor.

* Postgraduate material by the senior author for submission to the University of Natal Applied Chemistry Department.

racks are used to hold the sample tubes. The rated throughput is 100 samples per 15 minutes when sampling, and 100 samples per 7½ minutes when dispensing only.

A third phase involving the replacement of the *filtration step* by centrifugation is envisaged for the rapid separation of soil from extractant. A special centrifuge head will be used to accommodate 10 LKB aluminium racks.

Methods

Phosphorus

“Available” phosphorus in soils was extracted using a modified Truog method (Beater¹) with 0,02N sulphuric acid as the extractant in a ratio of 50 : 1 with soil. Total phosphorus in leaves was obtained by digesting portions of leaf sample material in a Kjeldahl flask with 98% sulphuric acid, using selenium as a catalyst.

The phosphorus contents of both extracts were determined colorimetrically using a phosphomolybdate complex reduced with stannous chloride (Dickman and Bray³).

Aluminium

The exchangeable aluminium index (EAI) of soil samples was determined by using an extraction procedure of Reeve and Sumner,⁸ while the aluminium contents of the extracts were determined colorimetrically with a pyrocatechol violet complex. (Meyer⁵.)

Experimental

To evaluate the equipment and the effects of procedural changes on the analytical results, the following experiments were conducted.

Testing of Beer's law

Confirmation of the colour complexes to Beer's law was determined by preparing synthetic standards covering the normal working range of concentrations. To compensate for the effects of interfering ions, these standards were prepared in the same matrix as the unknowns. The calibration curves obtained by plotting absorbance values against concentration are shown in Figures 3 and 4 for phosphorus and aluminium respectively. In the case of phosphorus, a linear relationship was obtained in the range 0-2,3 ppm P in the colour complex.

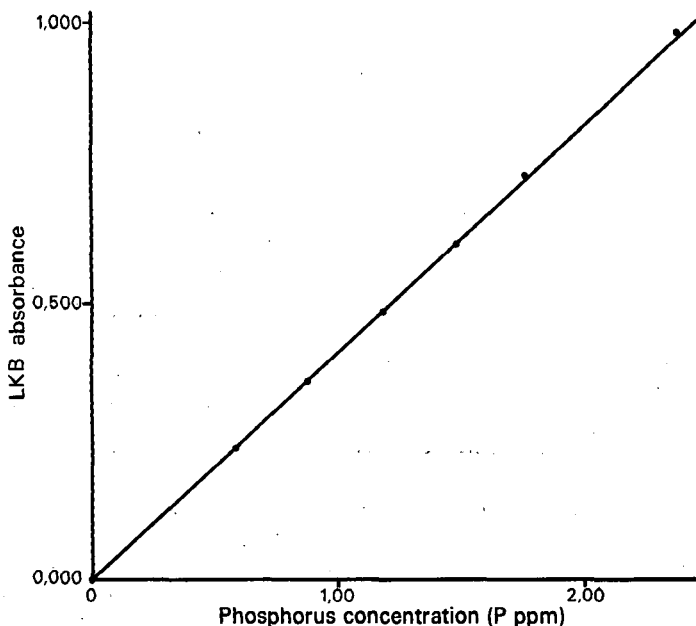


FIGURE 3 Phosphorus calibration curve.

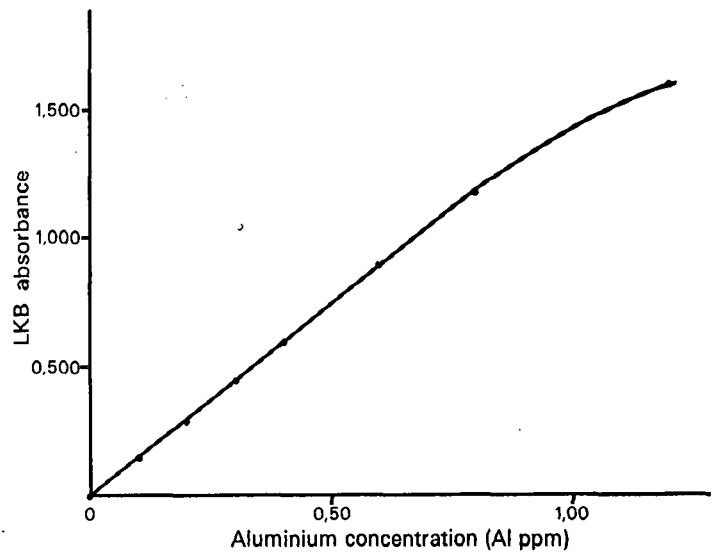


FIGURE 4 Aluminium calibration curve.

This covered the working ranges of 0-60 ppm soil and 0-0,40% P in leaf. For aluminium the relationship was linear in the range 0-0,8 ppm Al in the colour complex. Above this range the complex showed a marked deviation from Beer's law, but this was outside the normal working range of 0-200 ppm Al in soil.

Routine testing of the proposed changes in procedure

The existing method and the proposed changes were compared by analysing routine samples on a parallel stream basis, to assess in a simple manner the relative effects of changing the system. It was intended that differences in values obtained would reflect the sum of the errors involved in both systems from the point at which the parallel streaming begins, to the measurement step. This is also a period when operators are taught any new techniques which may be necessary, and the modification is tested under routine conditions with a moderate work load. Table 1 illustrates how this method was used to

TABLE 1
Comparison of foliar P (%) results given by the LKB calculating absorptiometer and the Klett colorimeter*.

No.	LKB	Klett	Difference
L8936	0,22	0,23	- 0,01
37	0,20	0,21	- 0,01
38	0,20	0,21	- 0,01
39	0,19	0,19	0
40	0,18	0,20	- 0,02
41	0,20	0,21	- 0,01
42	0,24	0,26	- 0,02
43	0,25	0,27	- 0,02
44	0,24	0,26	- 0,02
45	0,20	0,23	- 0,03
46	0,21	0,22	- 0,01
47	0,24	0,26	- 0,02
48	0,14	0,13	+ 0,01
49	0,20	0,23	- 0,03
50	0,22	0,22	0
51	0,21	0,25	- 0,04
52	0,21	0,23	- 0,02
53	0,23	0,25	- 0,02
54	0,21	0,23	- 0,02
55	0,22	0,24	- 0,02
56	0,25	0,25	0
57	0,21	0,23	- 0,02
58	0,21	0,24	- 0,03
59	0,22	0,26	- 0,04
60	0,26	0,25	+ 0,01
61	0,23	0,25	- 0,02
Means	0,21	0,22	- 0,01

* Used for existing method.

compare Klett colorimeter readings with those of the calculating absorptiometer when assessing the phosphorus contents of cane leaf digests. The close agreement between values indicated that both methods were reasonably precise and that the use of the absorptiometer would not introduce a significant bias into the results. A comparison of allowing soil plus extractant to stand for 30 minutes after shaking before filtration, with immediate centrifugation after shaking, when determining the EAI of soils is shown in Table 2. Here a significant systematic bias was revealed, filtered samples tending to have lower values. Since a rapid extraction technique was used it is possible that the aluminium contents of the extract and soil had not reached equilibrium and that the soil reabsorbed aluminium during the settling phase. Bias effects appear to be independent of the EAI value obtained, and would seem to be a function of the particular soil under investigation.

TABLE 2
Filtration compared with centrifugation in the determination of Exchangeable Aluminium Index

Sample	No. of observations	Mean filtered ppm	Mean centrifuged ppm	Mean difference ppm	Standard error of the mean difference
A*	7	12,9	15,1	2,2	± 0,38
C*	6	28,1	34,6	6,5	± 0,35
D*	6	64,0	69,2	5,2	± 1,05
E	6	0,5	0,7	0,2	± 0,18
F†	7	88,8	90,7	1,9	± 0,67
G	4	76,5	77,4	0,9	± 0,48
I	5	37,5	39,4	1,9	± 0,70
J	6	159,1	159,6	0,5	± 2,63

* denotes significance at 99% confidence limit

† denotes significance at 95% confidence limit

TABLE 3
Assessment of instrument errors arising from the colour-determining step for soil phosphorus

Sample	No. of observations	Mean soil P ppm	Standard error	CV%	95% confidence limits
A	22	10,50	± 0,52	4,95	± 1,08
B	24	23,21	± 0,82	3,53	± 1,69
D	12	3,53	± 0,41	11,61	± 0,89
E	20	4,21	± 0,54	12,83	± 1,13
F	10	19,18	± 0,76	3,96	± 1,69
G	22	7,13	± 0,57	7,99	± 1,18
H	18	22,02	± 0,98	4,45	± 2,06
J	26	6,21	± 0,69	11,11	± 1,42
K	12	116,70	± 2,41	2,07	± 5,25
L	24	7,20	± 0,51	7,08	± 1,05

Satisfactory completion of the trial period occurred when either good agreement of data on a routine basis, or a satisfactory explanation of any bias effects, was obtained. Bias problems can easily be resolved by using external reference samples but in many cases, where empirical soil extraction techniques are used, these samples are not available. In these cases rationalisation of any bias problems before proceeding further is extremely important.

Assessment of reproducibility

An objective assessment of the precision of the equipment was achieved by making repetitive observations of the same operation over a period of weeks.

(a) Calculating absorptiometer

The precision of this instrument when determining soil phosphorus was assessed by taking duplicate measurements of

colour complexes prepared from extracts of ten reference soil samples. Since colour complexes were unstable, fresh extracts and complexes were prepared daily. The difference between duplicates analysed at random within an instrument batch was used as a measure of precision. The results are shown in Table 3. Coefficients of variation (CV%) ranged from 2,07% to 12,83% being higher in the lower phosphorus concentration range.

(b) Sample processor

The precision of the sample processor was assessed by weighing liquid dispensed from the calibrated pumps over a period of weeks. Leaf phosphorus calibration standards were dispensed with ammonium molybdate reagent into eleven cuvettes held in an aluminium rack. Table 4 shows the average weight obtained for tube contents of 44 racks. The precision assessed at the 99% confidence limit compared well with the rated precision. This test undoubtedly overestimated the true precision of the pumps, weights used being the sum of eleven operations. However, the absorbance readings for each standard varied little from rack to rack, discounting the possibility of gross compensation in weights from tube to tube.

TABLE 4
Comparison of rated precision of LKB pumps with that found by weighing

Mean weight of 11 operations	Standard error	99% confidence limits	Rated precision ± 0,5%
18,442 g (44 observations)	± 0,020g	± 0,054g	± 0,092g

The possibility of sample interaction during sampling (Broughton *et al*²) was also tested for the sample to diluent volumes used for leaf phosphorus measurements. Carry over of sample from a high phosphorus containing digest into a blank could not be detected.

Assessment of accuracy

An external assessment of accuracy of the leaf phosphorus method was obtained by the exchange of four leaf reference samples with the Rhodesia Sugar Association Laboratory at Chiredzi. Duplicate determinations were carried out at Chiredzi using a standard dry ashing technique. A comparison of the mean results obtained is given in Table 5. Reference to standard errors indicated no significant differences between results from the two laboratories.

TABLE 5
Comparison of mean foliar P (%) results of four samples analysed by two laboratories

Composite	RSA	SASEX	Difference
I	0,169	0,176	+ 0,007
C	0,222	0,227	+ 0,005
P	0,199	0,208	+ 0,009
R	0,221	0,236	+ 0,005

Discussion

Over the last three years approximately 75 000 analyses have been carried out using the calculating absorptiometer and 10 000 of these have been prepared during the current year using the sample processor. In the main, both items of equipment have functioned satisfactorily. The discrete sample

automated system has a number of advantages over a continuous flow system (Grigg⁴). Each step in the analytical pathway is operated independently so that specific time-consuming steps can be automated and the overall efficiency progressively improved to meet the increased demand. Loss of output due to breakdowns is minimised by a temporary return to manual operations for the stage affected. Throughput is very much faster than with a continuous flow system which is controlled by the slowest step in a long chain of interlinking operations. The rate determining step in the LKB system is the sample processor, with an output of 400 samples per hour compared with 40-60 samples per hour from a continuous flow system.

Conclusion

Improvements in the analytical system used by the Fertilizer Advisory Service have resulted in a number of benefits. The savings effected in man-hours have made additional analyses on a routine basis possible as and when required. During 1975 approximately 7 000 supplementary determinations of phosphorus desorption index and EAI were performed using the new equipment. There has been no loss in precision due to automation and the direct recording of instrument results by the printer has eliminated the human error in this step. With the exception of aluminium determinations, no bias was introduced into the results by changes in methodology. Work is in progress to develop other colorimetric methods of determining plant nutrients, in particular foliar nitrogen in order to utilise the spare capacity of the instruments.

Acknowledgements

Thanks are due to Mr. M. Murdoch for his help with the statistical design of experiments, to Mr. A. C. Long for the analysis of reference leaf samples and to Dr. R. A. Wood and Professor D. E. A. Williams-Wynn for their careful reading and constructive criticism of the manuscript.

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