A NEW SAMPLE PROCESSOR – ABSORPTIOMETER SYSTEM FOR RAPID SOIL AND LEAF ANALYSIS

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Abstract

The need for replacing the diluting/dispensing equipment and absorptiometer used for colorimetric determinations in the Fertiliser Advisory Service (FAS) laboratory is discussed. The new instrumentation is described. Modifications to existing methodology with specific reference to extractable soil phosphorus and aluminium in soils and total phosphorus in foliar tissue are explained while other relevant methods such as those for leaf nitrogen and sulphur have been identified. Investigations used to evaluate the equipment are detailed. The new system has enabled laboratory throughput and productivity to be improved with no loss in accuracy or precision. Other advantages related to the introduction of the system are indicated.

Introduction

The South African Sugar Association Experiment Station has operated a Fertiliser Advisory Service (FAS) for more than 36 years. Between March 1990 and February 1991 over 32 000 soil and leaf samples were analysed by the FAS laboratory. Based on a trend analysis conducted by Burrows (1977) and updated in 1990, it is estimated that sample numbers could increase to 44 000 per annum by the turn of the century (Schroeder *et al.*, 1992). Such an increase in sample throughput would result in the number of liquid transfer operations in the laboratory increasing from the current 170 000 per annum to about 240 000 annually. This projected figure is not unrealistic, particularly in view of expansion in sugarcane development in the Eastern Transvaal and KwaZulu. The large number of liquid handling operations includes the transfer of extracts and digests, and dilution and dispensing of reagents. The system previously used for these operations was introduced to FAS in 1973. It consisted of a semi-automatic LKB sample processor capable of trans-

ferring liquid volumes in the semi-micro range and a calculating absorptiometer used for various colorimetric soil and leaf determinations (Burrows and Meyer, 1976). After several possible replacement systems had been evaluated, the Kemtek Sample Processor and Anthos 2000 Plate Reader were chosen. This system allows for unlimited combinations of accurate liquid transfer operations within a defined space and the absorptiometer is designed specifically for colorimetric determinations using microtitre plates. Despite the fact that the microtitre plate technique is often used routinely in many medical laboratories, the transfer of liquids and use of optical density determinations on a micro scale is regarded as a new approach to leaf and soil testing.

This paper describes the instrumentation and methods used for its evaluation. Modifications to existing methodology for various colorimetric procedures are detailed and advantages of the new system are discussed.

Procedure

Equipment

The equipment comprises two separate units. The Kemtek Sample Processor (Figure 1) is made up of a defined work area in which liquid handling operations are executed by a double nozzle dual probe system. The liquid column in each nozzle is independently controlled, resulting in flexibility of sampling and dispensing of solutions and reagents. The probes, which are fitted with liquid level sensing devices, are connected to separate three port pumps each with a single syringe. No restriction on liquid vessels or tray layouts is imposed. The Kemtek is fully computer controlled. The Anthos 2000 Plate Reader (Figure 2) is a programmable absorptiometer for microplate applications using pre-selected filters. The statistics software package allows for data reduction and results may be printed or stored.
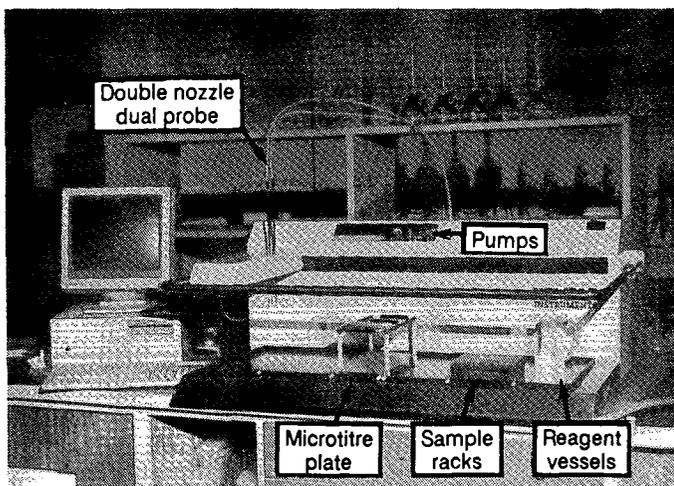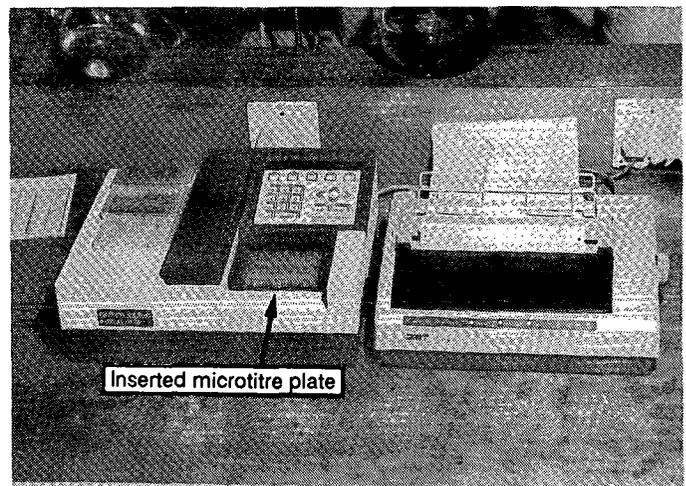

FIGURE 1 Kemtek sample processor

FIGURE 2 Anthos 2000 plate reader

Materials

Oven dried reference topsoil and top visible dewlap or third leaf sugarcane samples, together with miscellaneous plant and soil samples supplied by the Fertiliser Society of South Africa (FSSA) Quality Assurance Scheme, were used for evaluation purposes.

Methodology

Protocols and programmes for the Kemtek/Anthos system were developed for the determination of extractable soil phosphorus, exchangeable aluminium index (EAI) in soils and total leaf phosphorus based on existing methodology (Burrows and Meyer, 1976; Meyer, 1970). As a detailed description of the method for determination of extractable soil P using the new system is presented below, only essential details of the other two procedures are summarised in Tables 1 and 2. Irrespective of the method, suitable standard solutions and reagent blanks are included in designated wells on every microtitre plate to enable automatic calibration of the reader with each run. Other relevant methods, such as those for leaf nitrogen and sulphur and soil phosphorus desorption index determinations, are being modified to ensure compatibility with the new system and will shortly be incorporated as standard routine procedures.

Soil phosphorus

'Available' soil P was determined after extraction with 0,02N sulphuric acid (solution:soil ratio of 50:1) using the colorimetric procedure described by Murphy and Riley (1962) and developed by Burrows (1978).

Sample preparation – A volume of 3 ml of the extract is transferred to a preparation tube held within the sample rack using a 5 ml Oxford pipette. Sample racks, microtitre plates and a vessel containing mixed reagent (0,75 g ascorbic acid in 250 ml molybdate stock solution) are placed in defined areas on the Kemtek sample processor work space. The stock solution is prepared by adding 24 g ammonium hepta-molybdate, 1 g potassium antimony tartrate, 1 litre 1N H_2SO_4 and 7 g sodium lauryl sulphate and making up to 10 litres with deionised water.

Table 1

Summary of method for determination of exchangeable aluminium index (EAI) in soil extract (Meyer, 1970) using the micro technique

Volume μℓ	Solution
20	Soil extract
150	Pyrocatechol violet solution containing pyrocatechol indicator (0,144 g/500 mℓ deionised water) and ammonium acetate buffer (77 g/litre deionised water adjusted to pH 6,30) in a ratio of 1:2
35	1,10 Phenanthroline hydrate solution (0,385 g/500 mℓ deionised water)
135	Hydroxyl ammonium chloride solution (15,4 g/500 mℓ deionised water)

Filter	Wavelength nm
Measurement	570
Reference	414

Table 2

Summary of method for determination of phosphorus in cane leaf samples (Dickman and Bray, 1940) using the micro technique

Volume μℓ	Solution
14	Digest
78	Stannous chloride solution (5 mℓ concentrate (1,1 g stannous chloride dihydrate in 6 mℓ concentrated hydrochloric acid and made up to 100 mℓ with deionised water)
245	Sodium molybdate solution (250 mℓ mixed reagent, 0,5 g sodium lauryl sulphate and 1 litre deionised water) Mixed reagent is prepared by adding 125 g sodium molybdate to 2300 mℓ 32% HCl and made up to 5 litre with deionised water.

Filter	Wavelength nm
Measurement	650
Reference	414

Colour development – The required pre-programmed procedure for development of the colour complex is selected using prompt guides provided by the computer software. The number of samples to be analysed is entered.

Sequence description – According to the protocol developed for soil phosphorus, the dual probes (Figure 1) move to the reagent position where 200μl of mixed reagent (as above) is aspirated by nozzle 1 on each probe.

The probe then moves to the sample rack position to aspirate 150μl of extract from the sample preparation tubes. A volume of 330μl of the solution held within each nozzle is transferred to the appropriate wells in the microtitre plates. The probe finally moves to the washbowl position where the remaining 20μl of solution is dispensed and each nozzle is washed with 300μl of diluent. The dual probe then moves to the next sample position to repeat the above procedure until the counter reaches the number of samples specified. Samples are allowed to stand for 10 minutes to ensure full development of the colour complex before the microtitre plate is placed in position on the Anthos Reader. The required analysis is selected by the computer menu options. The measurement filter (690 nm for soil P) is automatically selected. An option exists also for selecting a reference filter for background correction. Sample numbers, optical density values and concentration values are printed on request.

Evaluation

The capabilities of the new system together with the effects of the procedural changes were evaluated in terms of performance, accuracy, precision and throughput. The methodology associated with the LKB Ultralab system was used as the reference (Burrows and Meyer, 1976).

Testing Beer's law

The system was evaluated in terms of conformation to Beer's law which states that absorbance is proportional to concentration at a given wavelength. Absorbance or optical density values were read for a series of standards prepared for each element under consideration. These values plotted as a function of concentration produced calibration curves (Figure 3) which indicated linear relationships and therefore adherence to Beer's law over the normal working concentration ranges. Correlation co-efficients (r) were in excess of 0,99.
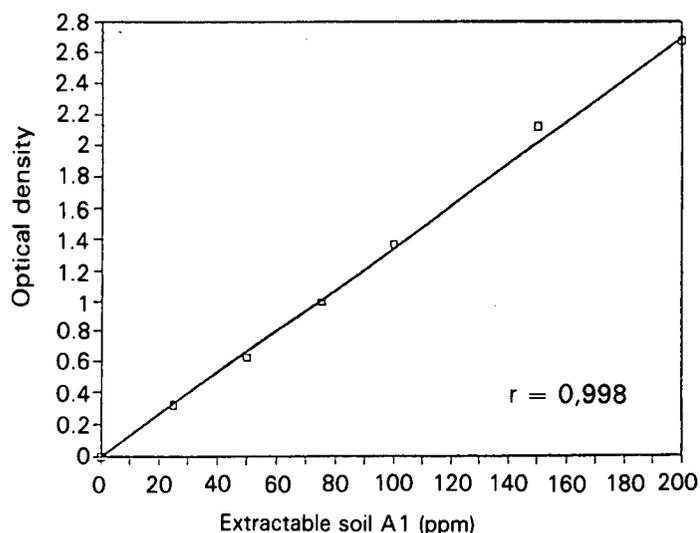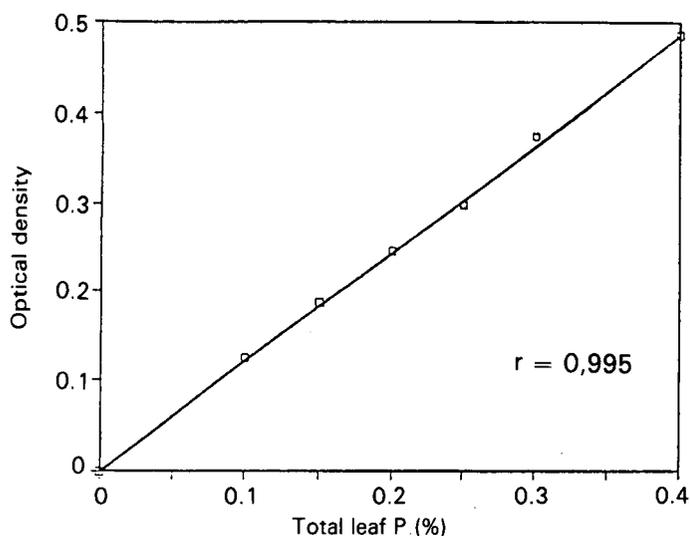
Table 4

Assessment of the micro technique for determining total leaf P

Composite leaf samples	No. of dets.	Leaf P %				
		KEMTEK/ANTHOS			LKB	Diff.
		Mean	SD	CV %	Mean	
1	20	0,117	0,006	5,13	0,120	−0,003
2	20	0,202	0,007	3,47	0,192	0,010
3	20	0,205	0,004	1,95	0,210	−0,005
4	20	0,208	0,004	1,92	0,207	0,001
5	20	0,245	0,008	3,27	0,244	0,001
6	20	0,270	0,008	2,96	0,265	0,005
7	20	0,231	0,010	4,33	0,232	−0,001
8	20	0,292	0,009	3,08	0,292	0,000
9	20	0,196	0,008	4,08	0,195	0,001
10	20	0,350	0,006	1,71	0,346	0,004
Overall mean			0,007	3,19	...	0,001
Calculated 't'						0,97

Assessment of the system

An assessment of accuracy, precision and reproducibility of the new methodology was made by repeatedly analysing a number of samples for extractable soil phosphorus, aluminium and total leaf P according to the modified and existing procedures. The results are summarised in Tables 3 and 4.

Calculated 't' values (0,75, 1,21 and 0,97) based on paired data indicated that no significant differences existed between results obtained from the new and LKB systems for the three different determinations. Low co-efficients of variation over the whole range of samples (less than 9% in all cases) as well as those calculated as mean values for each of the methods (3,0 to 3,6%) indicated that the Kemtek/Anthos procedure achieved a high level of precision and reproducibility. Close agreement between values (mean differences of 0,3 ppm, 1,12 ppm and 0,001% for soil P, soil Al and leaf P respectively) showed that bias was not introduced as a result of converting to the new system. Accuracy of the micro technique was further tested by determining P in soil and leaf samples supplied by the FSSA quality assurance scheme. No significant differences were apparent when results of analyses

FIGURE 3 Calibration curves for total leaf P and extractable soil A1 based on Kemtek/Anthos procedure

Table 3

Assessment of the micro technique for determining extractable soil P and Al

Composite soil samples	No. of dets.	Soil P (ppm)					EAI (ppm)				
		KEMTEK/ANTHOS			LKB	Diff.	KEMTEK/ANTHOS			LKB	Diff.
		Mean	SD	CV %	Mean		Mean	SD	CV %	Mean	
A	20	9,1	0,5	5,5	9,7	−0,6	12,9	1,1	8,5	12,5	0,4
B	20	17,7	0,8	4,5	15,9	1,8	13,9	0,8	5,8	13,4	0,5
C	20	19,7	0,7	3,6	18,9	0,8	28,5	0,5	1,8	28,6	−0,1
D	20	24,4	0,6	2,5	24,4	0,0	24,3	1,2	4,9	24,8	−0,5
E	20	57,4	1,0	1,7	55,9	1,5	42,4	1,7	4,0	44,3	−1,9
F	20	26,0	0,8	3,1	28,1	−2,1	60,4	0,8	1,3	60,3	−0,1
G	20	29,9	1,0	3,3	29,3	0,6	91,0	1,0	1,1	99,8	−8,8
H	20	46,7	1,0	2,1	44,8	1,9	118,9	2,7	2,3	118,5	0,4
I	20	56,3	1,1	2,0	56,5	−0,2	142,6	3,9	2,7	143,4	−0,8
J	20	65,7	1,1	1,7	66,4	−0,7					0,0
Overall mean			0,9	3,0		0,3		1,5	3,6		−1,2
Calculated 't'						0,75					1,21

were compared with mean values calculated for all participating laboratories (Table 5).

Table 5

Comparison of leaf and soil P results using FSSA quality assurance scheme samples

Sample	Soil P (ppm)		Diff	Sample	Leaf P (%)		Diff
	SASA	FSSA			SASA	FSSA	
91/GS19	4,2	4,1	0,1	91/BL25	0,26	0,25	0,01
91/GS20	21,2	22,4	−0,2	91/BL26	0,07	0,08	−0,01
				91/BL27	0,57	0,55	0,02
				91/BL28	0,19	0,18	0,01

Efficiency evaluation

Kemtek Sample Processor – The time taken for completion of the colour complex development stage as described for soil P was about 20 minutes/100 samples compared with 15 minutes/100 samples for the LKB system. The time difference may be attributed almost entirely to the probe washing step after each sample/reagent transfer which eliminates cross contamination between samples. Probe washing was not possible with the LKB sample processor, so results had to be screened to establish where possible carry over had occurred.

Anthos Reader – Irrespective of the preceding colour complex development stage, standard 96-well microtitre plates can be read in less than six seconds at the selected and reference wavelengths. The LKB absorptiometer would take about 20 minutes to complete a similar number of determinations.

Discussion

In the general evaluation it was found that the system was quite robust and user-friendly. Advanced operator skills are not necessary once the system has been programmed, so it is highly suitable for routine analyses of many samples. As unlimited combinations of liquid transfer operations are possible on the Kemtek, the system is highly flexible in its application and protocols may be easily adapted to suit specific requirements. The efficiency of operation, particularly of the Anthos reader, should not only enable increased sample throughput in the laboratory, but also allow for more productive use of laboratory personnel as direct supervision is minimised once the instruments are in operation. This is primarily due to the liquid sensing and memory/counter mode options which are built into the system. Automatic data transfer also eliminates the tedious manual entry of data together with any associated human error.

The use of 96-well microtitre plates should result in substantial savings on the purchase of sample tubes and certain expensive chemicals required for colour complex development. Versatility of vessels used and their layout within the work area has allowed use of existing tubes and racks. The facility for washing probes has obviated the use of disposable nozzles which characterises many other sample processors. As the system is modular, upgrading is possible by the addition of further accessories such as a 96-tube carousel unit or a barcode reader. The multi-tasking disc operating computer software package enables the computer to be used simultaneously for controlling the sample processor and two additional systems.

Conclusions

- The selection of the Kemtek/Anthos sample processor-absorptiometer system has allowed colorimetric determinations on a micro scale to be satisfactorily introduced into the routine analysis function of FAS.

- The combination of the two instruments has resulted in more rapid soil and leaf analysis procedures, not only of potential sample throughput but also as a result of more productive use of laboratory staff due to increased automation.

- No loss of precision or accuracy has occurred despite substantial reductions in reagent and extract volumes associated with microtitre plate applications.

- Evaluation of the instrumentation indicated that results were reproducible, bias was not introduced by the changes and conformation to Beer's law was apparent over the normal working concentration ranges.

- Apart from the fact that the new system is able to link into existing laboratory routines with minimum disruption, it has assured that the liquid handling operations in FAS will be satisfied for at least the next ten years, particularly as it will be fully compatible with future laboratory computerisation, and despite the large increase in sample numbers expected.

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