

# FLAME PHOTOMETRIC DETERMINATIONS OF CATIONS IN CANE LEAVES AND STALKS

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## Summary

Flame photometric determinations of potassium, calcium, magnesium and sodium in plant tissues are discussed. The difficulties due to phosphate suppression and potassium enhancement of calcium readings are covered at some length. A description of practical methods of overcoming these interferences is given.

In sugarcane tissue there are four metals which can be conveniently determined on the flame photometer. These are potassium, magnesium, sodium and calcium. Since the first three elements present little or no difficulty due to radiation interference, the bulk of this paper will be taken up with calcium determination.

Phosphorus in a solution has a marked suppressing action on calcium emission, when the solution is sprayed in a flame photometer. To illustrate this point, a set of 10 standards was made up. Each standard contained 18 p.p.m. CaO, which is equivalent to 0.3 per cent CaO on plant tissue when 150 mg. of dry material is dissolved in 25 ml. of extract. This is an average calcium value to be expected in the analysis of cane leaves. No phosphate was added to the first standard, but phosphate was added in successively greater amounts to the remaining standards. When sprayed in the Beckman D.U. flame photometer, using hydrogen at 5 p.s.i., and oxygen at 15 p.s.i., slit width 0.02 m.m., and wavelength 554 millimicrons, the standards gave scale readings as shown in graph 1. It can be seen that a calcium emission reading of 100 units on the scale can be reduced by roughly two-thirds to a minimum of about 34 units. Due to the varying amounts of phosphorus found in the plant material to be analysed, it is obvious that no reliance can be placed on any calcium results without taking phosphorus content into account.

One method of obviating the phosphate effect is to add a large amount of this element to each of the standards, and to each of the unknowns. All calcium readings on the flame photometer are then depressed to a uniform maximum amount. At ratios of  $P_2O_5$ :CaO greater than about 1:1, as graph 1 shows, the curve flattens out — i.e. variations in phosphate content will have no further effect on the calcium readings. Since calcium is such a strong emitter, the large depression of readings is no material set-back. The depression is easily countered by opening the slit further, or increasing the sensitivity of the machine.

A second method is to remove all the interfering phosphate by the use of an ion-exchange column. The unknowns are run through a column, which is made of resins designed to remove the anions present. The resulting solutions are then sprayed, and compared with suitable standards containing neither phosphate nor sulphate ions.

A third method operates by adding a "substitution" element (e.g. barium, strontium, or magnesium) to all samples and standards. By adding a relatively highly concentrated solution of one of these elements, it is assumed that the phosphate ions link with the added element rather than with the calcium. In theory, the unencumbered calcium ions then give their full emission in the flame. (See graph 2.)

A fourth method is the "internal standard" procedure. The theory of this method is that, when an equal proportion of the unknown sample is added to each of the standards, the interfering substance in the sample will affect both the calcium in the standard and that in the sample equally. In the case of phosphate, this method will not work satisfactorily, because the suppression caused is not independent of the varying P:Ca ratio normally found in cane samples.

A fifth method envisages the addition of glycerin to all samples and standards. Only a short reference to this method has been seen in the literature, and it has not yet been tried out here.

A further method tested here was the addition of E.D.T.A. solution to all samples and standards. The method seemed to work reasonably well, but rather a high flame background was encountered. This limited the use of the method in low calcium concentrations.

It should be mentioned here that any method of phosphate suppression should not impair the rapidity and ease with which flame photometer readings can be made. If a solution requires time-consuming treatment before spraying, then the advantages of the flame photometer are largely offset.

Initial testing of calcium determinations by flame methods was done both on cane-leaf extracts and on simulated leaf-extract solutions. In such solutions the substances present in sufficient quantities to affect calcium readings were considered to be potassium, phosphorus, magnesium and sulphuric acid. The effect of each of these substances was tried in turn. Potassium did tend to enhance calcium readings, but provided the calcium standards contained an average amount of potash found in cane-leaves, this effect was not serious. Thus a very low potash in an unknown would depress an average calcium figure by about 6 per cent, whereas a very high potash would cause a 4 per cent enhancement.

Initially it was thought that magnesium tended to depress calcium readings, since, by adding magnesium sulphate to calcium solutions the readings were lowered. Later tests, however, proved that it was the sulphate ions that were causing the depression, and not the magnesium. In these test solutions the sulphuric acid normally present in leaf extracts had been omitted. The depression due to sulphuric acid or

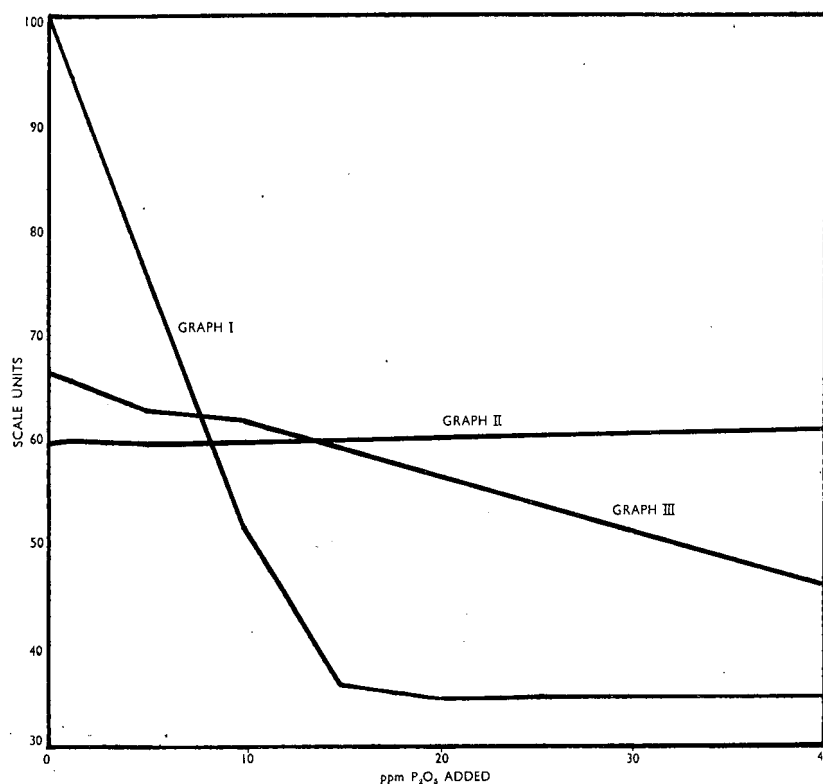
sulphate ions was found to be stable for all concentrations of sulphuric acid likely to be encountered in our digested extracts. Thus, provided the acid is present in all standards, no errors in calcium determination are detectable through differences of acid concentration in individual extracts. The presence of sulphuric acid has the effect of largely eliminating the fluctuations caused by various phosphate concentrations. At very low phosphate values, sulphate ions depress calcium readings, but at medium and high values, sulphate ions enhance calcium readings. (See graph 3.) Sulphuric acid on its own, however, is not sufficiently effective in overcoming phosphate interference.

In view of the previously mentioned suppression of calcium readings due to phosphorus, it was decided to "swamp" all future sample solutions and standards with this substance. Reasonably accurate calcium determinations on leaves were possible, provided that the standards also contained average quantities of potassium and sulphuric acid. The accuracy of results was checked both by the use of titration methods and by determining synthetic sample extracts of known, but varying, concentrations of calcium, potash and phosphorus.

Later on, when larger numbers of cane stalk analyses were being done, it was decided to explore the possibility of using ion-exchange columns to remove the phosphates from sample extracts. Ten columns were

set up and filled with the correct amounts of activated anion-retaining resin. Ten "sample" solutions were made up (series "A"), using varying quantities of calcium, magnesium, potassium and phosphorus to correspond with the ranges of these elements to be found in cane stalks. Enough sulphuric acid was added to each solution to correspond with that used in the wet-digestion process whereby 2 gms. of dry material ends up in solution together with 1 ml. of concentrated sulphuric acid in 50 ml. of water. Five ml. from each of the ten sample solutions was put through an ion-exchange column. This was then washed through and made up to 50 ml. (Series B). The series B solutions were then sprayed, and compared with suitable calcium standards, having an average potash content. It was noted that where the potash content of the sample was higher than that in the standards, the resulting figure for calcium was higher than the known amount, and vice versa. Thus in material such as cane stalks, where the  $K_2O$  figures are commonly 10 or 15 times the  $CaO$  figures, potash has a definite enhancing effect on calcium readings. Before obtaining further sets of figures to prove the last statement, the magnesium chloride addition method was tried as a means of overcoming the phosphate interference.

A 5 N. solution of magnesium chloride was added to each of the "A" series in the proportion of 1:4. Results were similar to the previous ones — namely,

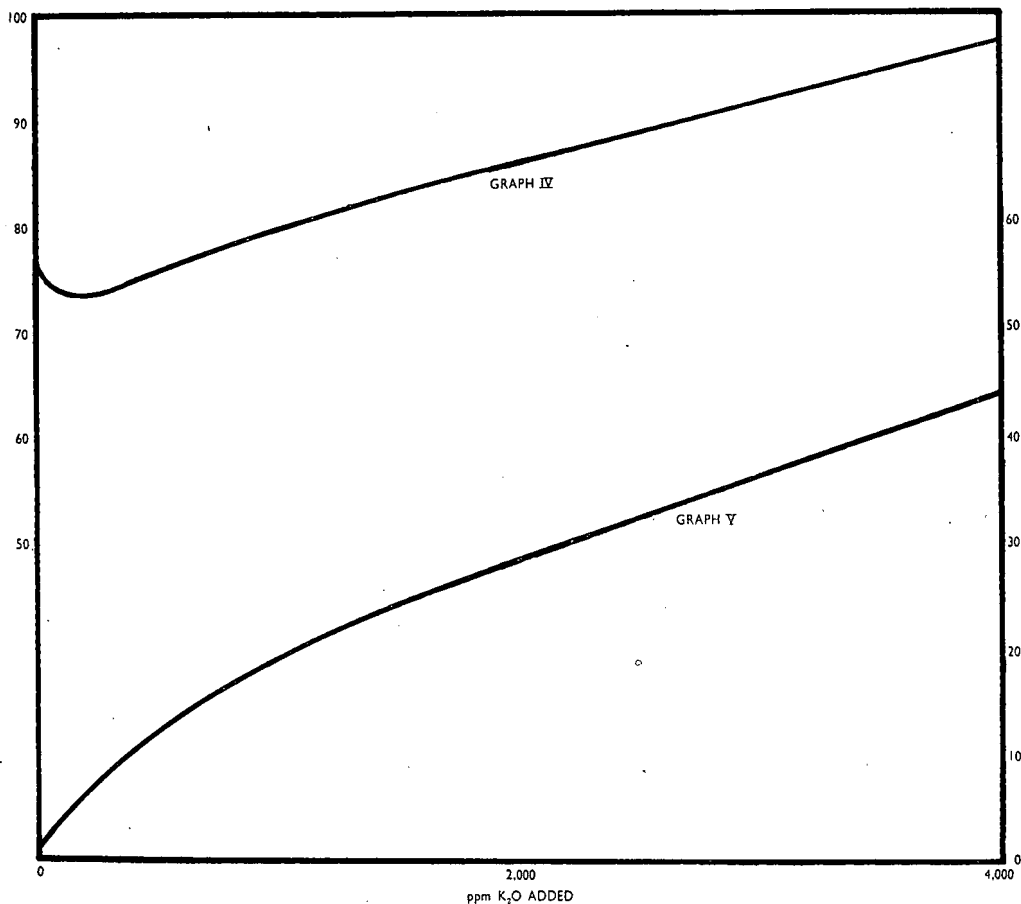


**Graph 1.**—18 p.p.m.  $CaO$ , with increasing quantities of  $P_2O_5$ . No sulphuric acid or magnesium chloride present.

**Graph 2.**—18 p.p.m.  $CaO$ , with increasing quantities of  $P_2O_5$ . All samples had sulphuric acid. 1 ml. of 0.5N magnesium chloride solution was added to 4 ml. of test solution in each case.

**Graph 3.**—This graph again shows 18 p.p.m.  $CaO$  solutions with increasing  $P_2O_5$  increments. Here sulphuric acid was present, but not magnesium chloride.

Note that instrument settings for graphs 1 and 3 were the same, but for graph 2 the slit width was increased, since the addition of the magnesium chloride solution had in effect diluted the other ingredients. Wavelength was 554 millimicrons,



**Graph 4.**—This represents 30 p.p.m. CaO with increasing amounts of K<sub>2</sub>O added.

**Graph 5.**—This is K<sub>2</sub>O (0 to 4,000 p.p.m.) without any CaO. In both cases the wavelength was 554 millimicrons.

high potash additions gave enhanced calcium figures and vice versa. The phosphate depression was as effectively countered as it had been in the previous tests. However, since the calcium readings are not depressed as much as they are when phosphate swamping is employed, smaller slit-widths could be used. This in turn reduced the amount of potassium radiation allowed to fall on the photocell, and thereby diminished the potash interference.

The effect of potash interference was tested by adding from 0 to 4,000 p.p.m. K<sub>2</sub>O to 30 p.p.m. solutions of CaO. When these solutions were sprayed, it was found that as more potash was added, so the readings were enhanced almost linearly. All settings on the photometer were then maintained, and solutions of potash only (0 to 4,000 p.p.m.) were sprayed. The two sets of results were plotted in the form of graphs. The second graph showed a considerable response to potash on the calcium wavelength used (554 millimicrons). This graph was roughly parallel to the previous one, showing that most, if not all, the enhancement due to the presence of potassium was due to the radiation of this element on the calcium wavelength. The experiment was repeated at 422.7 millimicrons with very similar results. At this wavelength the sensitivity to calcium emission was not as

great, and the slit had to be opened further, giving the potash an even greater effect on readings.

The last stage was merely a check on previous stages. Sets of calcium standards were made up containing extremes of calcium, magnesium, phosphorus, sulphuric acid, and potassium in the various permutations corresponding to whole-cane, or stalk digestions. Magnesium chloride swamping was done on all standards prior to spraying. The different levels of magnesium, phosphate, and sulphuric acid had no effect on either the high or the low calcium figures, but potash affected the readings. Potash introduced errors of up to 20 per cent in the low calcium range, and up to 10 per cent in the high calcium range. Since the potash effect is not overcome by conventional means, a correction table must be drawn up from multiple graphs. Potash analyses are normally done on all cane tissue extracts, and where the potash figure is found to differ from the average K<sub>2</sub>O content in the standards, the appropriate correction is then made.

The following analytical procedure has now been adopted by the research chemistry section of the Mount Edgecombe Experiment Station: For routine N P K analyses of leaf laminae samples, 50 mg. of the dry, powdered material is wet-digested with sulphuric acid and hydrogen peroxide. When clear, the extract

is made up to 25 ml. with de-ionised water. A filtered aliquot is sprayed in the flame photometer at 766.5 millimicrons, and compared with potassium standards corresponding to 0.5 per cent, 1.0 per cent, 1.5 per cent and 2 per cent  $K_2O$  on the original material.

Where magnesium and calcium are to be determined, or when cane-stalk material is being analysed, 150 mg. of dry powder is digested. The magnesium determination is made on a filtered aliquot of this more concentrated extract, using a photometer wavelength of 285.2 millimicrons. The high background illumination coupled with a rather weak magnesium radiation results in a shallow, slightly curved graph at this wavelength. Nevertheless, fairly accurate and reproducible results can be obtained if care is exercised. For calcium determinations, 4 ml. of filtered extract is thoroughly mixed with 1 ml. of 0.5N magnesium chloride solution and sprayed at 554 millimicrons. Calcium standards, which have been similarly treated, are used for the comparison graph. The calcium figure read off from this graph is now corrected for potash effect.

The analytical techniques used in the Fertilizer Advisory Laboratory employ a larger quantity of plant tissue for digestion. Two and a half grams of powdered leaf material is wet-digested with nitric and sulphuric acids. When digestion is complete, the extract is made up to 100 ml. with water. Suitably diluted aliquots are taken for normal flame photometric determinations of potassium and sodium. A portion of the extract is diluted five times for subsequent calcium determination. Eight ml. of this solution is then stirred with 1½ ml. of 2N magnesium chloride solution, and sprayed in the flame photometer. At present magnesium is determined colorimetrically, but future analyses of this element will probably be done on a suitable flame photometer. Magnesium is more highly concentrated in the macro-method extracts obtained in the Fertilizer Advisory Laboratory. This higher concentration results in a steeper magnesium graph, favouring accuracy. At the same time, the photometric method is very much more rapid, and less costly in chemicals, than the colorimetric method.

Before you open this paper for discussion, Mr. Chairman, there is one fact I should like to mention. Since writing the paper, further evidence has shown a tendency for increasing quantities of potassium in the presence of all the other ingredients, including the magnesium chloride swamping solution, to approach a limiting enhancement for calcium readings. Thus, by adding 2.0 gm. of potassium chloride per litre of magnesium chloride swamping solution, it has been possible to obtain accurate calcium determinations in the presence of various amounts of potassium. The resultant graph is steep, and flame background is not increased much. Table 1 shows results obtained on the first trial run.

TABLE 1

$K_2O\%$	$P_2O_5\%$	CaO% Actual	CaO% Found
0.50	0.50	0.03125	0.031
0.125	0.125	0.125	0.12
1.25	0.50	1.25	1.23
1.25	0.50	0.25	0.24
0.25	0.25	0.25	0.24
0.25	0.375	0.875	0.85

TABLE 1 illustrates some calcium figures obtained on the flame photometer with various permutations of potassium and phosphorus. Swamping with magnesium and potassium was employed on all test solutions and standards. Percentages quoted would correspond to dry plant material.

**Mr. Alexander** after reading the paper said it was only in recent years had suitable apparatus been available and he then went on to describe the construction of the modern flame photometer.

**Mr. du Toit** (in the Chair) said that those people who did the type of work described in the paper would find it of inestimable value. The paper dealt to a large extent with the determination of calcium and the exposition was one of the most practical he had seen. Calcium reading was usually depressed by phosphate and the author had obtained excellent calcium reading results by swamping the effect of both phosphate and potash.

Test solutions made up without the author's knowledge and containing various interfering elements had been submitted to Mr. Alexander who had obtained the remarkable results shown in the addendum to the paper. The time saved by the use of the flame photometer was enormous as compared with the chemical methods previously used.

**Mr. Bishop** said that with the simpler type of flame photometer used by the Fertiliser Advisory Service it was found necessary in the determination of calcium to keep on increasing the amount of this element present in solution and he asked why this should be so.

**Mr. Alexander** thought that this was due to some kind of decay in the instrument itself, probably fatigue in the photo-electric cell or the capillary tube being coated with chemicals such as silica, but the latter was unlikely.

**Mr. Stewart** asked if high concentrations of calcium could affect the determination of phosphate in the colorimetric analysis.

**Mr. Alexander** replied that no such effect had as yet been found but no specific experiments had been done to investigate this aspect.