

# *Flame Photometric Determination of Potassium and Calcium and the Chemical Estimation of Phosphorus, Magnesium and Manganese in Leaves of Hevea*

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A BRIEF NOTE ON THE NATURE and accuracy of the methods of analyses used in this laboratory was given previously (BOLLE-JONES 1954). Since that publication requests have been received for a more detailed account of our methods of leaf analysis. The object of this paper is to provide a fuller account of some of these methods and to describe others which are now being used by us for routine leaf analysis. The observations reported below cannot be regarded as the results of original research yet they comprise an essential pre-requisite for the implementation of research and advisory programmes within the Soils Division. Our main aim has been to achieve an analysis, by three laboratory assistants, of about four dozen samples of leaf material for potassium, calcium, phosphorus, magnesium and manganese, in four days, instead of the usual six. To achieve this objective it was necessary to abandon the slower chemical methods for potassium and calcium determination in favour of the more rapid flame photometric procedures. This paper briefly describes and reports our experiences with the flame photometric methods of potassium and calcium determination, and the chemical methods of phosphorus, magnesium and manganese estimation, as used by us.

## PREPARATION AND DIGESTION OF HEVEA LEAF SAMPLES

The petioles as well as midribs are discarded, and the remainder of the laminae cut into small pieces and dried rapidly at 80°C in the presence of a powerful current of air. When perfectly crisp and dry, the samples are ground in a Wiley Mill until the leaf powder will pass through a 40 mesh sieve.

One gram of this powder is weighed into a 200 ml pyrex conical beaker and 10 to 15 ml of concentrated nitric acid (Analytical Reagent Grade, sp. gr. 1.42) added. The percentage moisture in the leaf powder is assayed by oven drying a separate sample. The conical beaker is covered with a watch glass and is gently heated on a steam bath until all the powder passes into solution, which generally appears straw coloured. Three millilitres of perchloric acid (Analytical Reagent Grade, sp. gr. 1.54) are added and the acid mixture heated on a hot plate; brown fumes are at first evolved to be followed later by copious white fumes which leave behind a colourless solution, measuring about 1 to 3 ml. After cooling, 25 ml of distilled water are added, the solution boiled and passed through a Whatman No 40 filter paper to be collected and made up to a 100 ml in a measuring flask. This solution, referred to hereafter as the 'wet digest', is analysed for potassium, calcium, phosphorus, magnesium and manganese. Under routine circumstances two dozen leaf samples can be wet digested every one and a quarter working days in this laboratory by one assistant.

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## ESTIMATION OF POTASSIUM

Potassium is determined by a flame photometric procedure which is carried out directly on the wet digest, without difficulty. Such direct determinations have also been carried out by other workers (TOTH, PRINCE, WALLACE & MIKKELSEN 1948). Potassium can be determined at a wavelength of 404 millimicrons (BLACKWELL, YEAGER & KRAUS 1953) or 767 millimicrons (BAUSERMAN & CERNEY 1953). The region of the latter wavelength is preferred as manganese, often present in large amounts in wet digests, interferes to a greater extent at 404 millimicrons (ROY 1956). All our determinations are carried out at 766.5 millimicrons. It is not found necessary to use an internal standard in order to reach the standard of accuracy given below. The Beckman D.U. spectrophotometer with flame attachment and oxyacetylene atomizer burner, but without photomultiplier, are used. The instrument controls are set as follows:

Slit width .. .. .	0.05mm
Panel oxygen pressure ..	10 p.s.i.
Panel acetylene pressure ..	5 p.s.i.
Phototube knob .. .. .	'all in' (red phototube)
Load resistor switch at No 3	(10,000 megohm)
Selector switch at .. .. .	0.1

The transmittance scale is set to 100% using the luminosity of a 300 p.p.m. potassium standard. The instrument setting is checked after every three determinations of 'unknown' digests by using a potassium standard whose strength is of the same order as that of the digests; it is reset, if necessary, using a 300 p.p.m. potassium standard. Usually the values obtained for the potassium concentration in leaves are within 10% of the values obtained by chemical means; with an experienced operator this deviation may be considerably reduced. Table 1 provides a list of flame photometric and chemical values, each pair estimated on the same wet digest, recently obtained from a series of determination carried out in a routine manner.

Very little difficulty is experienced in the flame photometric determination of potassium. Each single determination takes, under routine unhurried circumstances, not longer than two minutes (this average allows for the resetting of the instrument and periodic checking of standards during a 'run' of determinations). The chemical method of potassium estimation, formerly employed by us, involved a cobaltinitrite precipitation and subsequent permanganate titration and requires the full attention of one laboratory assistant for one and a half working days to complete about two dozen determinations, from the wet digest stage. The chemical method also requires a minimum aliquot, of the wet digest, of 25 ml whereas the flame photometric method requires no more than 7 ml.

## THE ESTIMATION OF CALCIUM

It proved much more difficult to evolve a routine flame photometric method of estimation for calcium than it did for potassium. Calcium estimation was rendered more difficult because of the well known interferences of phosphate, iron and aluminium ions (HEMINGWAY 1956), apart from the evidence of our own trials which showed that cobalt enhanced, and chloride, nitrate and magnesium depressed, the luminosity of the calcium flame. The use of different wavelengths (422.7, 554 and 622 millimicrons) failed to overcome these interferences when the flame photometric estimation was carried out directly on the wet digest.

TABLE 1. COMPARISON OF POTASSIUM CONCENTRATION VALUES OBTAINED BY CHEMICAL AND FLAME PHOTOMETRIC METHODS OF ANALYSES FOR LEAF SAMPLES TAKEN FROM RUBBER PLANTS GROWN IN THE FIELD AND IN SAND

The terms high and low are used only in relation to concentrations usually found in healthy laminae All values expressed as percentage of oven dried laminae

Description of Sample	Potassium %		K % Difference	% Error
	Chemical	Flame		
FIELD GROWN				
Normal	0.54	0.49	0.05	9.3
Normal	0.62	0.63	0.01	1.6
Normal	0.72	0.79	0.07	9.7
Normal	0.80	0.79	0.01	1.2
Normal	0.96	1.02	0.06	6.2
Normal	1.22	1.27	0.05	4.1
Normal	1.81	1.79	0.02	1.1
Normal	1.80	1.86	0.06	3.3
High in N (4.41%)	1.31	1.39	0.08	6.1
High in P (0.58%*)	0.80	0.81	0.01	1.2
High in Mg (0.51%)	1.40	1.38	0.02	1.4
Low in Mg (0.10%)	0.50	0.52	0.02	4.0
High in K (2.13%)	2.13	2.15	0.02	0.9
High in Ca (1.79%)	0.49	0.47	0.02	4.1
High in Fe (21339 p p m*)	0.85	0.82	0.03	3.5
High in Mn (309 p p m)	1.18	1.27	0.09	7.6
High in N (4.41%) and also in P (0.68%*)	1.31	1.37	0.06	4.6
High in N (4.19%) and also in K (2.19%)	2.19	2.24	0.05	2.3
High in P (0.56%*) and also in Ca (1.79%)	0.49	0.48	0.01	2.0
High in P (0.67%*) and also in Mg (0.51%)	1.40	1.44	0.04	2.9
Low in K (0.26%) and high in Mn (10820 p p m*)	0.26	0.24	0.02	7.7
GROWN IN SAND				
Normal	1.22	1.24	0.02	1.6
Normal	1.08	1.10	0.02	1.8
Normal	1.08	1.12	0.04	3.7
Normal	1.13	1.16	0.03	2.6
Normal	1.07	1.12	0.05	4.7
Normal	1.12	1.15	0.03	2.7
High in N (4.91%)	1.36	1.48	0.12	8.8
High in P (0.75%*)	1.22	1.23	0.01	0.8
High in P (0.73%*)	1.08	1.12	0.04	3.7
High in P (0.68%*)	1.12	1.14	0.02	1.8
Low in K (0.29%)	0.29	0.28	0.01	3.7
High in Mn (1165 p p m)	1.79	1.83	0.04	2.2
High in Mn (16013 p p m*)	0.98	1.07	0.09	9.2
High in P (0.64%*) and also in Mn (1165 p p m)	1.79	1.83	0.04	2.2

\* These high values are due to the deliberate addition of the respective elements to the wet digest

TABLE 2. COMPARISON OF CALCIUM CONCENTRATION VALUES OBTAINED BY CHEMICAL AND FLAME PHOTOMETRIC METHODS OF ANALYSES FOR LEAF SAMPLES TAKEN FROM RUBBER PLANTS GROWN IN THE FIELD AND IN SAND

The terms high and low are used only in relation to concentrations usually found in healthy laminae. All values expressed as percentage of oven dried laminae.

Description of Sample	Calcium % Chemical	Flame	Ca % Difference	% Error
FIELD GROWN				
Normal	0.74	0.76	0.02	2.7
Normal	0.79	0.80	0.01	1.3
Normal	1.05	1.06	0.01	1.0
Normal	0.71	0.76	0.05	7.0
Normal	0.52	0.57	0.05	1.0
Normal	0.84	0.85	0.01	1.2
Normal	0.80	0.84	0.04	5.0
Normal	1.04	1.03	0.01	1.0
High in Mg (0.44%)	0.51	0.52	0.01	2.0
Low in Mg (0.09%)	1.14	1.14	0.00	0.0
GROWN IN SAND				
Normal	0.62	0.66	0.04	6.5
Normal	0.73	0.74	0.01	1.4
Normal	0.65	0.64	0.01	1.5
Normal	0.72	0.73	0.01	1.4
Normal	0.56	0.57	0.01	1.8
Normal	0.59	0.64	0.05	8.5
Normal	0.65	0.65	0.00	0.0
Normal	1.11	1.10	0.01	0.9
Normal	1.08	1.09	0.01	0.9
Normal	0.40	0.40	0.00	0.0
Normal	0.91	0.85	0.06	6.6
High in N (5.39%)	0.68	0.70	0.02	2.9
High in P (0.45%)	0.37	0.40	0.03	8.1
High in P (0.51%)	0.54	0.56	0.02	3.7
High in P (0.49%)	0.71	0.68	0.03	4.2
High in P (0.53%)	0.96	0.89	0.07	7.3
Low in Ca (0.19%)	0.19	0.19	0.00	0.0
Low in Ca (0.26%)	0.26	0.28	0.02	7.7
High in Mo (1072 p.p.m)	1.24	1.15	0.09	7.3
High in Mo (611 p.p.m)	0.41	0.42	0.01	2.4
High in Mo (811 p.p.m)	0.70	0.66	0.04	5.7
High in N (5.64%), P (0.51%) and Fe (251 p.p.m)	0.83	0.86	0.03	3.6
High in N (5.50%), P. (0.57%) and Fe (258 p.p.m)	0.95	0.97	0.02	2.1
High in N (5.37%), P (0.47%) and Fe (294 p.p.m)	0.92	0.96	0.04	4.3
High in N (5.46%), P (0.52%) and Fe (286 p.p.m)	1.05	1.02	0.03	2.9
High in P (0.51%) and Fe (283 p.p.m)	0.96	0.99	0.03	3.1

Several methods for eliminating the interferences of other elements were tried. A method, in which calcium is precipitated as the oxinate and then dissolved in dilute acid before determination (BEAUFILS 1954), though a definite improvement, gave rather low results and the error often exceeded the 10% limit. The lower values (than those obtained by a chemical method) were attributed to the presence of magnesium and phosphate ions which were also precipitated with the calcium under the conditions of the oxinate separation.

Ion exchange resins may be employed to remove phosphate ions from solutions whose calcium content is due to be assayed by flame photometric means (HEMINGWAY 1956). However, our experiences showed that even the use of Zeokarb 215 and Amberlite IR120 permitted errors which exceeded 10% and 30%, respectively; generally, these errors were greater in the case of leaves obtained from soil grown plants than for those grown in sand. The use of an anion exchange resin, Deacidite B, gave abnormally low values and was also abandoned.

Success was finally achieved by precipitation of calcium from the wet digest, as the oxalate; this precipitate is dissolved in dilute hydrochloric acid and used for the flame analysis. A similar method has been reported (WILLIAMS & MORGAN 1953). Prior separation of phosphate is unnecessary and the procedure can be applied to both soil and sand grown leaf material. The values obtained deviate up to 10% from values obtained by a well tried purely chemical method. The flame method is now being used on a routine basis at a wavelength of 554 millimicrons and the detailed procedure finally evolved is as follows.

A 20 ml aliquot of the wet digest solution is transferred into a 100 ml centrifuge tube and the following reagents added: 2 drops of methyl red indicator, 5 ml of 30% (w/v) ammonium chloride solution and 2.5 ml of 10% (v/v) acetic acid. The tube is warmed on a boiling steam bath for about 10 minutes and 5 ml of warm 5% (w/v) ammonium oxalate solution added. Ammonium hydroxide (approximately 5N) is added dropwise until the solution is alkaline. The tube is warmed on a boiling steam bath for at least thirty minutes and subsequently cooled overnight. As much as possible of the supernatant liquid is withdrawn by suction, without disturbing the calcium oxalate precipitate; the suction tube is washed clean with hot water and the volume made up to approximately 30 ml. The tube is centrifuged at 2,000 r.p.m. for five minutes, the supernatant liquid removed and the precipitate dissolved in 2.0 ml of 5N hydrochloric acid. The solution is carefully transferred to a 10 ml graduated flask and made up to the mark with water. The solution is then ready for flame analysis. The Beckman spectrophotometer and flame attachment controls are set as follows:

Slit width .. .. .	0.25 mm
Panel oxygen pressure ..	10 p.s.i.
Panel acetylene pressure ..	5 p.s.i.
Phototube knob .. .. .	'all out' (blue phototube)
Load resistor switch at No 3 (10,000 megohms)	
Selector switch at .. .. .	0.1

The transmittance scale is set to 100%, using the luminosity of a 300 p.p.m. calcium standard; the setting is checked after every three determinations. The working range of the method extends between 0 and 300 p.p.m. of calcium but the best results are

usually obtained in the range between 100 to 200 p.p.m. The method, as outlined above, applies to the wet digest solutions derived from leaf material; for digests prepared from roots, which often contain a large amount of iron, a preliminary removal of iron is necessary to achieve correct values.

Table 2 gives a comparison of values obtained by both chemical and flame photometric methods of analyses on the same wet digests, derived from leaf samples of varied mineral status. The correspondence between methods is good. The chemical method involves a dry ashing of the laminae, followed by solution in hydrochloric acid; the calcium is precipitated as oxalate, separated, and estimated by titration against permanganate. The whole operation from the dry leaf powder stage usually permits the completion of two dozen estimations in about one and a half working days. The flame photometric procedure reduces the time needed to assay the calcium, from the calcium oxalate stage. But the biggest advantage is that it can be carried out directly on the wet digest whereas the chemical method requires an independent dry ashing procedure, for which a minimum 0.5 gm of dry lamina powder is needed.

#### ESTIMATION OF PHOSPHORUS

Phosphorus is determined as described by TRUOG & MEYER (1929); the estimation is based on the measurement of the light absorption of the reduced phosphomolybdate blue complex. One modification which has been introduced is to bring the wet digest to a pH of 4.0 before adding the reagents listed by Truog and Meyer. The method covers a range of 0 to 30 micrograms of phosphorus and requires 0.5 to 1.0 ml aliquot of the wet digest. Almost invariably it has been found that if a known amount of phosphorus is added to leaf material, before wet digestion, the proportion recovered by estimation ranges between 95 to 105% of the amount added.

The preparation of reagents is given by TRUOG & MEYER (1929). The principal details of the method, as adopted by us, are as follows. One millilitre of the wet digest solution is diluted to approximately 75 ml, with water, in a 100 ml graduated flask. Two drops of beta-dinitrophenol indicator (0.1 gm in 2.5 ml of ethanol plus 97.5 ml of water) are added and brought to neutrality with, approximately, 2N ammonium hydroxide. Two millilitres of the ammonium molybdate and sulphuric acid reagent are added and the solution made up to 100 ml before 0.4 ml of the stannous chloride reagent is added. The flask is well shaken and allowed to stand for precisely three minutes. The light absorption of the blue solution is measured by means of Hilger Biochem absorptiometer, using a red filter (No 70) and 2 cm cells.

Usually one laboratory assistant can complete comfortably, from the wet digest stage, two dozen estimations within one half of a working day.

#### ESTIMATION OF MAGNESIUM

A method of magnesium estimation (HUNTER 1950), based on the formation of a coloured complex of magnesium hydroxide with thiazol yellow dye, has been found to be fully satisfactory and is used by us without modification. A 3 to 5 ml aliquot of the wet digest is required for the estimation and a working range of 20 to 100 micrograms of magnesium is covered. Known amounts of magnesium added to the leaf material, before acid digestion, can be recovered to within 5% of the amount added.

The reagents and their preparation have been listed by HUNTER (1950). Briefly the method of estimation is as follows. A suitable wet digest aliquot is pipetted into a 100 ml capacity conical flask, evaporated to dryness and the residue dissolved

in 15 ml of Morgan's reagent. Oxalic acid is added and the flask stood for one hour before the tartarate reagent, dye reagent and sodium hydroxide are added successively; the flask is shaken between each addition, and the final solution is allowed to stand overnight. The dye, which is not complexed by magnesium, is removed by shaking with a mixture of butan-1-ol and ethanol; the light absorption of this unused dye solution is measured in a Spekker absorptiometer. Blue green filters (No 3) and 4 cm cells are used for this purpose.

One laboratory assistant is able to complete two dozen estimations of magnesium, on the wet digest solutions, within the space of one to one and a half working days.

#### ESTIMATION OF MANGANESE

This estimation requires a larger aliquot of the wet digest than for any of the other elements discussed here. The method is based on procedures described by WILLARD & GREATHOUSE (1917) and RICHARDS (1930) and depends on the oxidation of manganese to permanganate, which is then estimated absorptiometrically. The method has been well tried and recovery values obtained for known amounts of manganese added to leaf material, before digestion, have been consistently good (95% to 105%). A range of 0 to 100 micrograms of manganese is covered by the method. A suitable aliquot (25 to 50 ml) of the wet digest solution is placed in a 100 ml beaker flask, 3 ml of syrupy phosphoric acid (sp. gr. 1.75) added, and the mixture evaporated on a hot plate until most of the fumes of perchloric acid (left over from the wet digestion) are driven off. The flask is cooled, about 25 ml of water added, followed by 0.2 gm of potassium periodate, and the solution boiled on a steam bath. Boiling is continued for one hour during which period the maximum purple colour development occurs. The flask is cooled and its contents diluted to 25 ml in a graduated flask. Manganese standards are prepared simultaneously in identical fashion and their light absorption values measured by means of Hilger Biochem absorptiometer, using a blue green filter (No 52) and 4 cm cells.

The completion of two dozen determinations requires the attention of one laboratory assistant for one working day but during that period he is not fully occupied and can carry out other duties.

#### DISCUSSION

The aim of this work was to evolve or modify methods by means of which it is possible to analyse about four dozen samples of rubber leaves for potassium, calcium, phosphorus, magnesium and manganese within the period of four days. This limited objective was achieved; three laboratory assistants are necessary for the routine implementation of this objective. One assistant is responsible for the wet digestion of the leaf samples and the analysis of the digests for phosphorus; another assistant is responsible for the estimation of potassium and calcium; and the third assistant is concerned entirely with the estimation of magnesium and manganese. The improvement in the rate of production of analytical results, which amounted to a 50% increase over the previous rate, was due entirely to the introduction of flame photometric methods of assay for potassium and calcium. The use of the flame photometer not only reduced the time required for each estimation but enabled all the elements to be determined on the basis of one wet digest rather than having a separate dry ashing procedure for calcium.

The methods, as described here, give consistent and reproducible results. The chemical methods satisfy the criterion that a weighed amount of standard when added

to the dry leaf material can be recovered to the extent of  $\pm 5\%$  of the amount added. The adaptation of the flame photometric methods for routine purposes was adjudged on whether or not they gave an analysis value, for a leaf sample, to within 10% of that obtained by the chemical method formerly employed. Many papers are published which describe methods of estimating various elements in plant material; frequently we find that we cannot achieve the precision and accuracy claimed by the authors of such papers under routine conditions. The value of our present paper lies in the fact that methods are described which have been well tested and are used by us under routine circumstances, for the analysis of rubber leaves. For all our purposes we regard values which are within 10% of the true values as being of sufficient accuracy in the analysis of hevea leaf material. While more accurate but slower procedures may be available, it is satisfactory to us that three laboratory assistants can analyse four dozen leaf samples, for five different elements in less than a week.

All the methods have been mainly directed towards the analysis of hevea leaf material. It is not expected that difficulties would be encountered with any other type of leaf material but it is known, from our experience, that for roots, iron must be removed before calcium may be estimated accurately by the flame photometric method.

Besides the estimation of the five elements discussed above, the following elements are also determined by us when required: nitrogen, sulphur, iron, boron, copper, zinc and molybdenum. Routine chemical methods are available for each of these estimations but each procedure demands a separate dry or wet ashing of the leaf material. These procedures require the following weights of lamina powder: nitrogen 0.05 gm, boron and iron 0.5 gm each, zinc 1gm, sulphur, copper and molybdenum 2 gm each. Thus these elements represent a heterogeneous group and require varying sub-samples of the original lamina powder, whereas the five elements discussed herein represent a discrete group and are all determined on the same wet digest solution.

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