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Preparation of Plant Material for Estimating a Wide Range of Elements

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FORESTRY COMMISSION OF N.S.W.

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SUMMARY

This report is concerned with the oxidation of plant material prior to chemical analysis for the elements—phosphorus, aluminium, calcium, magnesium, sodium, potassium, iron, zinc and manganese. The procedures used for the oxidation of plant material have been summarized. These fall into two categories—namely dry or wet ashing techniques. In order to obtain one solution which can be analysed for all nine elements, dry ashing has been shown to be preferable to wet ashing. Three dry ashing procedures have been fully investigated. A dry ashing procedure which involves removal of the insoluble siliceous residue by filtration, has been shown to give large errors for some elements. A commonly used dry ashing procedure in which the silica in the sample is destroyed, has also been investigated. It is a lengthy, tedious procedure and the results have shown that, since no greater precision is obtained, the removal of silica is unnecessary. A simple dry ashing procedure carried out at 420° C with a single solution of the ash in dilute hydrochloric acid is the most suitable procedure for the oxidation of plant material.

INTRODUCTION

A research study into tree nutrition with particular reference to *Pinus* spp. was commenced by the Chemistry Group, Forestry Commission of N.S.W. in 1961. Dry ashing was adopted as the method for the destruction of organic matter prior to chemical analysis for the following elements: phosphorus, aluminium, calcium, magnesium, sodium, potassium, iron, manganese and zinc. This dry ashing procedure is a very tedious and lengthy process involving the separation and removal of silica from the ash. A more simple ashing procedure with comparable accuracy and precision was therefore investigated.

This report is divided into three sections. The first part consists of a comprehensive literature survey covering various facets of dry ashing together with a discussion of the attributes of dry and wet ashing procedures. The second part of the report is concerned with the study of two simpler dry ashing procedures and their comparison with the dry ashing procedure which had been previously used. The third part is a critical appraisal of the simple dry ashing procedure which was found to be the most satisfactory.

I. OXIDATION OF ORGANIC MATTER

There is a vast literature on the subject of the destruction of organic matter; this mainly falls into two categories—wet and dry oxidation. Both methods have their devotees and this survey covers the theory of the two procedures and then deals with the elements separately or in related groups.

Dry Oxidation involves those procedures in which organic matter is oxidized by reaction with gaseous oxygen, with the application of energy in some form. The following series of processes is involved:

- (1) evaporation of moisture;
- (2) evaporation of volatile materials including those produced by thermal cracking or partial oxidation;

- (3) progressive oxidation of the non-volatile residue until all organic matter is destroyed.

The first two steps are usually carried out at a temperature much lower than that used to complete the oxidation. This is largely to prevent ignition of the volatile and flammable material and the subsequent uncontrolled rise in temperature and increased chance of loss of material. During the oxidation process the element to be determined will behave in one or more of a number of ways. Ideally it will remain quantitatively in the residue after oxidation and in a form in which it can be readily recovered, generally by solution of the ash in dilute acid. Some part of the element may occur in, or be converted to, a volatile form which may then escape from the vessel used to contain the organic sample. Some part of the element may combine with the vessel used to contain the sample, or with some other solid material in such a way as to be irrecoverable by the normal procedures used for the solution of the ash.

Wet Oxidation procedures involve the use of some combination of four reagents (sulphuric acid, nitric acid, perchloric acid and hydrogen peroxide). Problems can arise in wet digestion procedures from two main sources, adsorption on undissolved solid material, and volatilization. The temperatures involved in wet oxidation methods are generally very much lower than in dry ashing and retention losses caused by reaction between the desired elements and the apparatus are very much less likely. The same observation applies also to reaction with other solid constituents of the sample. One mechanism that is important is coprecipitation of the element being determined with a precipitate formed in the digestion mixture. The possibility of coprecipitation should be investigated. Problems can arise from reagent contamination and interference by acids present in solutions for subsequent atomic absorption analysis. Wet ashing usually requires considerable supervision.

Sulphuric acid is the most frequently used component of wet digestion mixtures, and is widely employed in conjunction with nitric acid, perchloric acid and hydrogen peroxide. In addition to its function in partially degrading organic materials by its own action, the presence of sulphuric acid in mixtures containing other oxidizing agents also serves to raise the boiling point of the mixture and so enhance the action of the other oxidants. The main disadvantages with the use of sulphuric acid are its tendency to form insoluble compounds and, ironically, its high boiling point. The high boiling point of sulphuric acid makes it difficult to remove the excess acid after completion of the oxidation and this is sometimes important in subsequent estimations. Nitric acid is much the most widely used primary oxidant for the destruction of organic matter. The normal concentrated acid boils at about 120° C, a factor which assists in its removal after oxidation, but which correspondingly limits its effectiveness. Nitric acid is commonly used in the presence of sulphuric acid, which partially degrades the more resistant material, or with perchloric acid, which continues the oxidation after nitric acid has been removed. Perchloric acid has been used for hundreds of thousands of oxidations without incident, however the occurrence of occasional explosions of quite stunning violence, has led to it being viewed with grave suspicion. A large proportion of the

explosions arose from the absorption of perchloric acid by some materials used in the construction of fume hoods. With this awareness, part of the risk has been removed. There is no doubt that, handled with knowledge and care, this reagent is extremely efficient in the destruction of organic material. From a technical rather than a safety viewpoint, a mixture of nitric and perchloric acids probably has fewer disadvantages than other common wet oxidation reagents, but for use in unskilled or inexperienced hands avoidance is perhaps better than regret. Hydrogen peroxide is also used, particularly in combination with sulphuric acid, and it is a powerful oxidizing agent.

Ashing Apparatus. The most commonly used material for the construction of laboratory apparatus is borosilicate glass, while silica, platinum and porcelain are also widely used for specific applications. Contamination can be introduced either through external contamination of the apparatus itself or through solution of the material of construction during the various manipulative processes. External contamination is generally removable by efficient acid cleaning before use. With regard to contamination from solution of the apparatus itself, the two factors of greatest importance are the composition of the material and its resistance to attack by the reagents employed. The purest material for general use in the destruction of organic matter is probably silica. In addition to its high purity, fused silica (vitreosil) is also a chemically resistant material, particularly at comparatively low temperatures.

Storage of Ash Solutions. There has been considerable discussion of the relative merits of glass and polyethylene for the storage of dilute solutions, and Theirs⁴¹ has concluded that unplasticized, high-pressure polyethylene is the best material of all. It has the advantage of freedom from contamination—although the same cannot be said of the now common low-pressure material—and it does not appear to be unduly subject to adsorption problems. However, it has been shown that loss of water vapour can occur from polyethylene ampoules, although these losses are unlikely to be serious when considering the accuracy of most trace element determinations. An interesting solution to the combined problems of glass and polyethylene has been the use of polythene bags as liners for glass bottles. In general, it is probably fair to say that either glass or polyethylene can be used for these dilute solutions provided that care is taken to achieve and maintain suitable conditions.¹⁵

Another important source of possible error is the loss of material on precipitates or other solid material produced or existing in the body of the solution. At the very lowest concentration levels it is possible that adsorption of ions on to particles of dust or of solid substances in colloidal solution might cause losses, but at the part per million level this is not likely to be serious. More serious is the loss by adsorption on precipitates formed in the solution, either intentionally or accidentally. The readiness with which precipitates which are formed in solutions of high pH, will carry down ions present in low concentration is well known, and this property is used in numerous purification and concentration processes. In general it is wise to avoid the formation of such precipitates in solutions containing only traces of ions, and indeed the formation of any precipitate must be viewed with suspicion.

Detailed Studies on Various Elements. Gorsuch¹² conducted an investigation into sample loss caused by volatilization and retention of various elements during wet and dry oxidation. Although he found evidence of both volatilization and retention of various trace elements, he was unable to come to a definite conclusion as to whether wet or dry oxidation of organic matter would give the most satisfactory results in a particular circumstance. In a later comprehensive publication¹⁵ he concluded that for the bulk of determinations, the relative advantages and disadvantages of wet or dry oxidation can be weighed against each other on the basis of convenience, but for some applications there are overriding reasons for using one or the other. For example, when volatilization will occur during dry ashing, as with mercury and selenium, then wet oxidation is almost essential. When very small traces are to be determined in large samples the same can be said of dry ashing. A very pertinent statement was made in his preface—"element losses which can occur during wet and dry oxidation are probably the single greatest source of error in the majority of trace element determinations."

Middleton and Stuckey²⁰ presented a critical review of methods that have been used for the destruction of organic matter of different biological origins preparatory to the determination of trace metals. They pointed out that the reactions involved in dry or wet ashing vary not only according to the reagents and methods used, but with the nature of the substances concerned, and that these must be taken into account. It was not surprising to find that methods for destroying organic matter that worked satisfactorily when applied to substances of one type became troublesome when used for materials having quite a different composition: for example, the destruction of organic matter of vegetable origin and consisting largely of carbohydrates (i.e., nearly all vegetable tissues except seeds) by methods of dry ashing or wet oxidation generally offered no special problems or particular difficulty; on the other hand, animal tissues (organs, meat products, blood, shell-fish and the like) often gave trouble.

In spite of the apparent theoretical advantages inherent in wet ashing, many workers have not used this procedure. Jones²⁴ reported on a survey of analytical methods for plant leaf tissue and found that seventeen out of the eighteen laboratories involved used dry ashing techniques with the majority reporting ashing times of 3 to 16 hours at 400° C to 500° C.

A very recent publication²¹ reported a collaborative study of wet and dry ashing techniques for the analysis of plant tissue by atomic absorption spectrophotometry. The elements determined were calcium, copper, iron, magnesium, manganese, potassium and zinc. The wet ashing was carried out with perchloric acid as the oxidizing agent. The dry ashing procedure involved ashing in a porcelain crucible for two hours at 500° C, digestion of the ash with dilute nitric acid and then solution in dilute hydrochloric acid. Both the dry and wet ashing techniques produced satisfactory results and the authors recommended either method for adoption as an official first action method.

In a comparison between dry ashing and wet digestion in the preparation of plant material for atomic absorption analysis, Giron¹¹

suggested that dry ashing is a better procedure for plant materials. This decision was based on the better precision for the dry ashing results combined with the non-significant differences between the averages of the two procedures.

Detailed Individual Element Studies on a large number of elements have been reported and the results for those elements of importance to forestry are summarized below. Reference to Gorsuch¹⁵ is not always acknowledged.

1. *Magnesium, calcium, strontium and barium.* The removal of organic matter from elements of this group is reasonably simple. Their common salts are comparatively non-volatile and stable and they have no marked tendency to form volatile organic compounds or complexes. The choice of ashing temperature is not critical, and temperatures between 430° and 620° C are probably quite satisfactory. One worker has reported no losses of magnesium on ashing grass samples at 430°, 530° and 620° C.¹⁰ The *A.O.A.C. official methods of analysis*³ quotes temperatures between 500° and 550° C. Losses of magnesium have been reported at very much lower temperatures and a loss of 10 per cent calcium at 400° C has been reported.¹⁶ A collaborative study³⁹ on the analysis of poultry feed showed no significant differences in the results obtained after ashing at 500°, 600°, and 700° C and all the results were a little higher than those obtained by the wet ashing referee method. Another A.O.A.C. collaborative study¹⁹ found no significant difference in the recoveries of calcium and magnesium from feeds after dry ashing at 550° C for 4 hours or after wet oxidation. Roach *et al.*³⁷ obtained excellent recoveries of magnesium from animal feeds ashed at 450° C. Investigations into the recovery of strontium and barium have been fewer, although Thiers⁴¹ obtained good recoveries of both at the one part per million level after careful ashing at 450° to 500° C.

Wet oxidation methods have been widely used for all of these elements and in general little trouble has been found although caution is required with sulphuric acid in the various oxidation mixtures. For magnesium there is no problem, and for calcium in small amounts the solubility of the sulphate is usually sufficient, but for samples containing large amounts of calcium and for strontium and barium at all times, it is probably wise to avoid the use of sulphuric acid altogether. Recoveries between 96 per cent and 100 per cent were achieved for strontium after oxidation with mixtures containing sulphuric acid.¹² The disadvantage of being unable to use sulphuric acid lies in the extent to which the rate of oxidation of the sample will thereby be decreased, because of the lower temperatures reached. This can be overcome by using a mixture of perchloric and nitric acids but the possible hazards and precautions necessary with this mixture must be taken into account.

2. *Sodium and potassium.* Because the alkali metals occur widely in ionic form, it is often possible to separate them without the necessity of destroying the organic matrix first. At the simplest, many of their organic salts are water soluble and simple solution is an adequate pretreatment.

There appear to be no studies on ashing procedures for the determination of sodium and potassium in plant materials. Grove *et al.*¹⁷ found there was a lack of information concerning the loss of these elements during the dry ashing of large samples of animal and human tissue. They conducted an extensive investigation into the recovery of sodium and potassium from a variety of animal materials and showed that up to 500° C, the recovery was complete even after heating for periods of 72 hours. At 550° C the recovery was complete after 24 hours heating, but after 72 hours some were low, particularly with potassium, while at 650° C nearly all the recoveries were significantly low, even at 24 hours. When the ashing temperature was increased, silica crucibles were attacked by the sample probably due to the alkali compounds being converted to the alkali oxides in the temperature range 400°–700° C. Etching of the crucibles was observed at about 700° C and became more noticeable as temperature and time were increased. Another study²⁵ showed that different recoveries were obtained for potassium when ashing was carried out in silica (95 per cent) and porcelain (85 per cent) crucibles. Hamilton *et al.*¹⁸ reported negligible losses of sodium during the dry ashing of biological materials at 450° C.

Wet oxidation methods should be uniformly successful¹⁵ in obtaining complete recovery of the alkali metals. There is one report that Pyrex glass used in the construction of an oxidation apparatus adsorbed sodium, necessitating the use of silica⁵ but generally no such difficulties have been noted.

Coomber and Webb⁹ carried out a comparison of low temperature radio frequency ashing with other methods of organic sample oxidation for the determination of sodium in an acrylic fibre. Lower results were obtained by the two wet oxidation methods than by the dry ashing procedures and they were considered to be less reliable than the dry methods. The concentrated acids used in wet ashing were found to give lower values for sodium when present in subsequent analyses by atomic absorption. The two dry methods gave identical results with the more precise results from low temperature radio frequency ashing.

3. *Phosphorus.* The results of analyses for phosphorus using both wet and dry methods appear to be identical. As early as 1936, Ashton⁴ and Weissflog and Mengdehl⁴³ showed that dry ashing and wet combustion of plant material were equally satisfactory. Ashton carried out the dry ashings in the presence of magnesium nitrate at a temperature of 800° C. One of the objections to dry ashing had been the contention that the pyro- and metaphosphates resulting from the high temperatures required are converted with difficulty to orthophosphate. Mengdehl²⁸ showed however that ten minutes of boiling with dilute hydrochloric acid was sufficient to convert these salts to orthophosphate. Bertramson⁷ also obtained good recoveries by ashing at 600°–700° C in the presence of magnesium nitrate. Tusl⁴² studied a dry ashing procedure at 500° C (for animal feeding stuffs) with and without the addition of sodium carbonate to establish whether dry ashing without fixatives was adequate for the satisfactory recovery of phosphorus. He concluded that simple dry ashing without fixative can be used for determining phosphorus in biological materials.

4. *Aluminium*. Determinations of aluminium after destruction of the organic matter with both wet and dry oxidation procedures, have been reported and the adverse comments noted have virtually all been applied to the dry ashing methods. Dry ashing appears to have been used successfully at temperatures in the region of 500° C, although recovery data are sparse. In one survey⁴¹ recoveries of 95 per cent and 98 per cent were reported for concentrations of 1 and 3 ppm after ashing carefully under conditions designed to prevent contamination of the sample. The ash was dissolved in 6N hydrochloric acid, and no difficulty was experienced, although higher ashing temperatures might be troublesome due to the increasing insolubility of the alumina produced. Other difficulties in recovering aluminium, have been reported,³³ when silica-containing samples were ashed. The ash obtained was evaporated to dryness with acid, and then extracted with more acid. The dried residues were dissolved in hydrochloric acid, and the retained aluminium determined.

5. *Zinc*. For the estimation of zinc in organic matrices, most of the common oxidation procedures have been applied, but whereas wet digestions seem to give uniformly good recoveries, dry ashing methods have aroused an appreciable amount of controversy. Ashing temperatures up to 900° C have been reported in the literature and most zinc losses have been recorded at high ashing temperatures. The two types of loss possible in dry ashing, excluding purely mechanical losses, are losses by volatilization, and losses by retention, and both types have been postulated to explain low zinc recoveries. Gorsuch¹⁵ investigated zinc volatilization and found no evidence, even under an extremely rigorous heating program which involved 31 hours heating at temperatures up to 1 000° C, that there was any volatilization of zinc. However, there were losses of zinc by retention on the silica ashing vessels. This finding was explained by the formation of a stable zinc silicate and it has been shown that this effect is accentuated by the presence of sodium chloride which apparently acts by weakening the silica structure and facilitating the reaction with the zinc compound. Gorsuch concluded that the presence of large amounts of chlorine in any chemical form, must be regarded with caution but apart from this, there was no reason why dry ashing at a temperature of 500° C should not be quite satisfactory.

On the other hand Pijck, Hoste and Gillis³⁴ reported a zinc recovery of only 30 per cent after ashing for 3 hours at 90° C. Results published by other investigations are no less conflicting. Hamilton, Minski and Cleary¹⁸ observed that no loss of zinc occurred at 850° C but found that 45 per cent of it was adsorbed on the silica of the crucible. Knauer²⁶ observed that there was no loss at 800° C and Strohal *et al.*⁴⁰ obtained only a 56 per cent recovery of zinc at 800° C. Roach *et al.*³⁷ obtained mean recoveries of 99 per cent from dry ashing animal feeds at 450° C for the determination of zinc by atomic absorption spectrophotometry. Raaphorst *et al.*³⁶ also recently studied the loss of zinc during the dry ashing of biological material. After ashing at temperatures of up to 1 000° C, no significant loss of zinc by volatilization was observed. Also after ashing at 450° and 550° C, the added labelled zinc-65 was removed quantitatively from the crucibles with hydrochloric acid.

Baker and Smith⁶ conducted a comprehensive investigation into the preparation of solutions for atomic absorption analysis of trace elements in plant tissue. They concluded that the high concentrations of extraneous ions often present in plant ash solutions interfere with the determination of iron, manganese, zinc, and copper by atomic absorption. To overcome this, they proposed a procedure involving complete extraction of the elements into a separate organic phase. They stated that although individual elements can be determined in individual plant materials with little error by other procedures, their proposed procedure was the most reliable for all four trace elements studied. In the course of their work, they found dry ashing at 500° C resulted in only small decreases in zinc levels in most of the materials and the mean recovery was down about 5 per cent. The recovery of zinc was also reduced (by 5 per cent) when it was not separated from the extraneous inorganic salts after wet ashing.

6. *Manganese.* Both wet and dry oxidation methods have been applied with little adverse comment. Dry ashing temperatures up to at least 800° C have been used, but tracer recovery experiments have shown losses of 15 per cent at 700° C and 20 per cent at 800° C,⁴⁰ so that upper limits of 500° to 550° C seem to be advisable. Knauer²⁶ reported no losses over the temperature range 460° to 800° C. Baker and Smith⁶ found no significant loss of manganese with dry ashing at 550° C, but a small loss of 3 per cent when the manganese was not extracted from the residual inorganic salts after wet ashing. Loss of manganese by reaction with silica in the sample is sometimes considered to be a hazard, and the use of a low maximum temperature should help to minimize this type of loss.

Wet oxidations with mixtures of nitric, perchloric and sulphuric acids, and hydrogen peroxide have been applied successfully,¹⁵ but Bradfield⁸ warned that the use of sulphuric acid should be avoided whenever possible in sample preparation for the determination of manganese by atomic absorption spectrophotometry because low values are obtained and there is the potential danger of precipitation of alkaline earth sulphates and loss of manganese by adsorption.

An interlaboratory comparative study of wet oxidation and dry ashing at 550° C, prior to the determination of manganese in feeds, revealed no significant differences between the two techniques for samples containing from 50 to nearly 400 ppm of manganese.¹⁹

7. *Iron.* The preparation of biological materials for the estimation of iron is well documented because this element is widely distributed and usually plays an important role in biological materials. Both wet and dry methods have been extensively used. Wet oxidation has proved very successful in almost all cases, with most of the possible combinations of nitric, sulphuric and perchloric acids, and hydrogen peroxide being used. Recovery experiments by various workers, have nearly always given good and consistent results. Radio-chemical measurements of iron at the 1 ppm level, using combinations of nitric, sulphuric and perchloric acids¹³ have shown recoveries between 97 per cent and 102 per cent. These results are in good accord with other radio-chemical work which showed recoveries of 96 per cent to 106 per cent in the 30 to 400 ppm range³² and ordinary chemical determinations which gave overall recoveries of 100± 1 per cent²². A serious exception to

this general agreement on the suitability of wet digestion methods is to be found in the unsatisfactory precision obtained in a collaborative study by members of the A.O.A.C. in which six feed samples were analysed for iron after destruction of the organic matter by wet ashing.³

There is considerable disagreement however about the effectiveness of dry ashing. Many workers have used such methods with apparent satisfaction, or at least without comment, and some have reported quite adequate recoveries. On the other hand many have found the recovery varied according to the nature of the sample, the nature of the ashing aid and the temperature used. Satisfactory recoveries have been reported by a number of workers after ashing samples directly, without the use of an ashing aid, at temperatures in the range 450° to 550° C.^{44, 30, 12} Also in the collaborative work of the A.O.A.C. mentioned above, more satisfactory precision was obtained than by wet ashing. However there are a great many instances of difficulties occurring during or after the dry oxidation of samples.

Knauer²⁶ investigated various dry ashing parameters when determining iron in marine shrimp, and found the optimum muffling temperature range was 560°–600° C. No statistical differences were found when the samples were heated at 550° C for periods of time ranging from 4 to 16 hours. In their work, Baker and Smith⁶ obtained excellent recoveries for iron salts muffled at 550° C for 4 hours. However dry ashing of plant tissues resulted in a 10 per cent decrease in the mean recovery of iron. There was the same 10 per cent loss when the iron was not separated from the extraneous inorganic salts after wet ashing.

There are two possible mechanisms for the low recovery of iron after the dry ashing of organic materials—loss by volatilization or loss by reaction with some solid material. As in the case for zinc, the presence of sodium chloride can greatly influence the losses due to the reaction of iron with solid material present in the system. This is particularly the case when the ashing is carried out in silica or porcelain dishes in the presence of chloride ions which can greatly weaken the silicate structure and facilitate its reaction with iron.¹³ This has been demonstrated in a series of experiments in which iron with Fe-59 tracer was heated in silica crucibles with sodium chloride; up to 40 per cent of the iron was retained by the crucibles. This type of loss has also been described by Petersen³³ who considered that it became important at temperatures above 600° C. Other work³⁸ has been quoted in which the greatest retention losses of iron occurred on the residues of samples of straw and hay. The retention losses were highly correlated with the silica content of the samples.

8. *Copper.* The wet oxidation methods appear to have been almost uniformly successful and nearly every possible combination has been used, ranging from sulphuric acid and potassium sulphate to sulphuric, nitric and perchloric acids plus hydrogen peroxide. However, there is one collaborative study by members of the A.O.A.C., in which an investigation was made of the analysis of feedstuffs for a number of elements, including copper, by atomic absorption spectrophotometry. The results for samples prepared by wet oxidation were unsatisfactory and the dry ashing results were even worse.¹⁹

Dry oxidation has often been used with apparent success. However there are many reports which give consistently low recoveries of copper. When assessments have been made of the cause of these losses, the usual conclusion has been that it has occurred through interaction between the copper and the material of the crucible. The presence of silica in the samples themselves is also a serious hazard, and once fixation has occurred, recovery of the copper is a difficult matter. The normal method of solution of the ash, with hydrochloric acid, is not generally effective in removing the element from the ashing vessel or from silica present. It has been claimed a mixture of acids is more effective,²⁰ and using a 2:1 mixture of dilute hydrochloric and nitric acids, good recoveries and agreement with wet oxidation have been found after ashing at temperatures between 450° and 600° C. By comparison, recoveries after extraction with hydrochloric acid alone were found to be from 15 to 60 per cent lower. The contention that the mixture of acids is superior was not however borne out by other work¹³ using Cu-64 as a radioactive tracer, in which no differences were found between the recoveries with hydrochloric acid alone, and those obtained with the nitric and hydrochloric acid mixture. A plausible explanation for the retention of copper on silica crucibles is that the reduction of copper to the metal occurs in the presence of organic matter and it is in this form that reaction with the silica takes place. It appears that this type of interaction is made less likely by the use of low temperatures and by the inclusion of an ashing aid such as magnesium nitrate.¹⁵

When investigating ashing temperatures, Knauer²⁶ obtained maximum sample concentration for copper in marine shrimp, between 560° and 620° C. The report of the Analytical Methods Committee on the determination of small amounts of copper in organic matter by atomic absorption spectrophotometry pointed out that dry or wet ashing was suitable for the destruction of organic matter.² If dry ashing was used, the recovery of copper should be checked and the residue should be dissolved in dilute hydrochloric acid or *aqua regia*. Isaac and Johnson²¹ reported complete ashing of plant tissue (using temperatures in excess of 500° C) was important for copper recovery. Baker and Smith⁶ found a drastic loss of copper (90 per cent) on muffling plant tissues without the addition of phosphoric acid.

Conclusions

For certain elements (e.g., phosphorus, calcium and magnesium) both wet and dry oxidation procedures are satisfactory even when carried out under a wide range of conditions. For the complete recovery of such elements as copper, iron, aluminium, sodium, potassium, and manganese, precise conditions for both wet and dry oxidation procedures are necessary. Low ashing temperatures are necessary for most elements—in particular sodium, potassium and aluminium. In the latter case, problems have been experienced with high silica concentrations. There have also been some reports of losses of zinc, iron and copper by retention on silica ashing vessels. Detailed studies into wet ashing procedures have not been undertaken to the extent they have with dry ashing. Despite the apparent theoretical advantages of wet ashing, very few workers use it for plant material. Some losses of manganese in particular and sodium have been reported in the residue remaining after

wet ashing. Where the analysis of a wide range of elements is required on a particular sample, there is little doubt that dry ashing (as opposed to wet ashing) provides the necessary solution for analysis, with only a single oxidation of the sample.

II. COMPARISON OF DRY ASHING PROCEDURES

During the period 1961-70, an ashing procedure was followed by this laboratory in which the sample was charred before ashing at 420° C.¹ The ash was digested with 5N hydrochloric acid, a small quantity of nitric acid added, and then taken to dryness. The residue was heated for 1 hour, cooled, taken up in 5N hydrochloric acid and filtered. The insoluble residue was washed with hot dilute hydrochloric acid until the washings were free of dissolved salts. The filter paper was then dried, ignited at 600° C in a platinum crucible and the silica removed with hydrofluoric acid in the presence of a small quantity of sulphuric acid according to Piper.³⁵ The residue was dissolved in hot dilute hydrochloric acid and added to the first ash solution. This procedure is referred to later as method C. It was considered a necessary procedure in order to give the maximum accuracy for the various required elements, some of which were analysed for the conventional colorimetric or titrimetric methods. However, by the introduction of more sophisticated instrumentation, namely the atomic absorption spectrophotometer (in the late 1960's), and since this ashing procedure was both tedious and lengthy, the possibility of finding a simpler dry ashing procedure was investigated.

Destruction of organic matter by dry ashing has been reported to lead to the formation of insoluble silicates which may be removed from the sample solution during a filtering process. Also this residue can contain small amounts of the mineral constituents present in the organic matter sample and, for some of the trace elements, this fraction may represent a considerable proportion of the total amount present in the sample. The following study was undertaken to assess a procedure in which the residue was left in solution on the basis that the silica had been rendered insoluble and had retained minimum amounts of elements; the accuracy of a procedure in which the residue was filtered off; and the merits of a procedure in which the silica was dissolved in hydrofluoric acid and then eliminated by volatilization through heating.

Methods and Materials

Three ashing procedures were compared. They were assessed from the results obtained in the subsequent estimations of the following elements present in the ash solutions: phosphorus, aluminium, calcium, magnesium, sodium, potassium, iron, zinc and manganese. The procedures were:

- Method A. The ash solution was made to volume without separation of the insoluble siliceous material.
- Method B. The insoluble siliceous material was filtered off and the ash solution then made to volume.
- Method C. The insoluble siliceous material was filtered off, the silica removed with hydrofluoric acid, the residue solubilized, added to the original ash solution and made to volume.

Twelve samples of dried finely ground foliage were selected for this study, comprising nine samples of *Pinus radiata* (D. Don), two of *P. elliotii* (Engelm.) and one *P. taeda* (L.). The various samples were chosen to cover a wide range of individual elemental concentrations as well as varying interelement relationships (e.g., high sodium—low potassium and *vice versa*, etc.). Each sample was ashed in triplicate by each of the three ashing procedures (A, B and C).

Ashing Procedures

The following steps 1–9 in the procedure were carried out for the three methods:

- (1) a squat-shaped vitreosil crucible was oven dried for approximately 30 minutes at 105° C. A glazed, translucent crucible with capacity 50ml was used—No. C2 in Vitreosil silica catalogue;
- (2) the crucible was then cooled in a desiccator and weighed;
- (3) approximately 2g plant material was weighed accurately into the crucible and dried in an oven at 105° C overnight (16 hours);
- (4) the crucible and contents were cooled in a desiccator, weighed and then charred slowly under a watchglass on a hotplate or extraction heating unit for about 1½ hours;
- (5) the crucible and contents were then placed in a cool muffle furnace (<100° C) and the temperature raised slowly (about 100° C per hour) to 420° ± 5° C. This ashing temperature was maintained overnight to give a total ashing time of 16 hours;
- (6) in the majority of cases, the resulting ash was greyish white or grey. If there were large amounts of carbon still remaining in the ash the sample was muffled longer (2–4 hours) at the same temperature. After muffling, the crucible and contents were cooled and weighed to determine the weight of crude ash;
- (7) the crucible was covered with a watch glass and the ash was moistened with 1–2 drops of distilled water. 3ml 5N hydrochloric acid was pipetted under the lip of the watch glass with care to avoid any loss by effervescence. The covered crucible was then warmed on a boiling water bath for 30 minutes;
- (8) the cover was rinsed and removed, 0.2ml 15N nitric acid added and the solution evaporated to dryness. The crucible was then placed in an oven at 105° C for 1 hour to complete the dehydration. (This treatment insolubilizes the silica for removal by filtering and hydrolyzes complex polyphosphates which can sequester iron and make it unavailable);
- (9) the dried salts were moistened with 2ml 5N hydrochloric acid, 10ml distilled water added and warmed on a boiling

water bath until all salts were in solution (about 10 minutes).

The following steps in the procedure were carried out for the individual methods:

Method A

- (10) the solution was transferred quantitatively (using a rubber tipped glass rod) to a 250ml volumetric flask with distilled water. The solution was then made to volume with distilled water;

Method B

- (10) the solution was filtered through a Whatman No. 44 filter paper into a 250ml volumetric flask and the insoluble residue (mostly silica) was transferred using a rubber-tipped glass rod. The filter paper was well washed with warm 0.02N hydrochloric acid followed by distilled water;
- (11) the filter paper was discarded and the solution made to volume with distilled water.

Method C

- (10) as for method B.
- (11) the filter paper was placed in a platinum crucible and dried at 105° C. The filter paper was ignited by placing the crucible in a cool muffle and bringing it to 600° C quickly (about 200° C per hour). The temperature was maintained at 600° C for half an hour;
- (12) when cool, the contents of the crucible were moistened with 1–2 drops distilled water, 2–3 drops 36N sulphuric acid added and approximately 2ml concentrated hydrofluoric acid. This solution was heated gently until white fumes of sulphur trioxide appeared. Care was taken that the solution was not taken to dryness. More hydrofluoric acid may be required for samples with high silica content;
- (13) when cool, 2ml 5N hydrochloric acid and 10ml distilled water were added and heated gently to dissolve any insoluble material. The contents of the crucible were added to the original filtrate in the 250ml volumetric flask and made to volume with distilled water.

Chemical Analyses

The methods of chemical analysis for the estimation of phosphorus, aluminium, calcium, magnesium, sodium, potassium, iron, zinc and manganese, in the ash solutions are given in Appendices 1, 2 and 3.

Results and Discussion

The results of the chemical analyses from the solutions obtained by the three ashing procedures will be discussed separately for each element. The various element means obtained by each ashing procedure are given in table 1. The mean results obtained for one of the ashing procedures (method A) are given in table 2—these results give an indication of the wide variation in individual elements between the foliage samples selected for this study.

The results for phosphorus obtained by the three ashing methods are shown in table 3. Analysis of variance (table 3a) was used to investigate whether there were any statistical differences between the ashing methods. The results gave no statistical differences between methods, mainly because of overlap between all three methods. The above findings are in very good agreement with those of the literature in which dry ashing under a range of conditions was found to be suitable for the estimation of phosphorus. It is important to note from these results that there is no positive interference in the colorimetric estimation of phosphorus because of the presence of silica in the ash solution (method A). The ashing procedures involve a careful dehydration of the silica and ensure the silica is completely insoluble and hence in a form in which it does not interfere with the phosphorus estimation—it is only soluble silica which interferes. It is, therefore, apparently not necessary to remove the silica from solution such as is carried out in methods B and C and followed by many workers.

The three ashing methods also gave statistically inseparable results for calcium and potassium (tables 4, 4a and 5, 5a respectively). The findings for calcium were supported by the literature from which it was concluded that the oxidation of organic matter presents no problems prior to the analysis of calcium. Similarly there are no reported difficulties for potassium provided low ashing temperatures are used.

The results for aluminium, magnesium, sodium and manganese are tabulated in tables 6, 7, 8 and 9 respectively. Analysis of variance was carried out for each set of data (tables 6a, 7a, 8a and 9a respectively). Analysis of variance using all the aluminium results (table 6a) was complicated by a very significant interaction term. The magnitude of this interaction term was due largely to the results obtained for the samples from Murraguldrrie, Mannus and Barcoongere which were inconsistent with the results obtained for the other samples. However, there were significant differences between the three methods when they were statistically analysed in pairs—method C gave the highest results (mean = 776 ppm Al) followed by method A (mean = 739 ppm Al) with the lowest results being obtained by method B (mean = 708 ppm Al). In the case of magnesium, analysis of variance (table 7a) gave no significant difference between method A and method C whereas method B was significantly lower (the mean difference being 5 per cent) than the other two ashing methods. There was also very considerable variation between the replicates in method B, whereas the replicate variation in methods A and C was very small. A very significant interaction term (due to inconsistencies within methods B and C) was obtained in the analysis of the results for sodium (table 8a) and hence the results for the individual methods were statistically analysed, two methods at a time. For the majority of samples, the highest sodium values were obtained by method A with method C giving the lowest results. Although sometimes the results obtained for a particular sample were higher by method B than method C or *vice versa*, the mean results for the two methods were the same (313 ppm Na and 316 ppm Na respectively). However, there was a mean 12 per cent loss when compared with the results from method A. The results of the statistical analysis for manganese (table 9a) gave method C 3 per cent significantly higher than method A, with method B,

8 per cent lower than the other two methods. However, the interaction between the methods was significant mainly because of the large variation within the results obtained by method B.

For each of these four elements (aluminium, magnesium, sodium and manganese), method B gave the lowest results. The elements appear to fall into the one category, each being affected by the presence of silica in the samples. There is apparently some adsorption onto and/or occlusion of these elements by the silica and these are filtered off with the insoluble siliceous material. Both aluminium and manganese gave results by method C which were higher (by 5 and 3 per cent respectively) than even method A. This indicates that errors are incurred for these two elements when they are not fully separated from the silica. It was noted earlier than Sandell³⁸ reported difficulties in recovering aluminium when silica-containing samples were ashed. However, the mean errors between methods A and C can be considered acceptable particularly as it is possible to obtain occasional erroneous results by method C (particularly in the case of aluminium—see table 6). In the case of magnesium, the results obtained from method C were equal to those from method A. Here it seems that the small quantity of magnesium which is affected by the silica (5 per cent) although filtered off during the ashing procedure, dissolves on standing in the solution prepared according to method A. On the other hand, identical results were obtained for sodium by methods B and C. This is most probably due to the volatilization of the adsorbed sodium during the removal of silica with hydrofluoric acid in method C.

These above findings are not exactly in agreement with Jones and Milne,²³ who determined the chemical contents in the silica residue obtained from dry ashing oats for 12 hours, one lot at 450° C and another at 550° C. The silica residue had been separated according to the method of Piper³⁵ and substantial amounts of sodium, potassium, calcium, magnesium, iron and manganese were detected in this residue. For example the siliceous residue from ashing at 450° C contained 28 per cent of the total manganese. The conclusions of Jones and Milne²³ agreed with those of Piper,³⁵ that is, while both major and trace elements are retained with the silica, the proportion of the total is small for the major elements but large for the trace elements. However it is worth noting here that the high retention of trace elements on the silica in the samples which were studied by Jones and Milne is probably a factor of the high silica content of the plants (where silica represents up to 85 per cent of the ash) particularly in comparison to pines (where silica is 5–10 per cent of the ash).

Good agreement was obtained for iron between the three ashing methods (table 10). This was unexpected because of the numerous difficulties previously reported in the preparation of biological materials for the estimation of iron. The analysis of variance of the iron results gave a very significant difference between method C (mean = 189 ppm Fe) and methods A (mean = 182 ppm Fe) and B (mean = 182 ppm Fe). However the difference is only 3 per cent. There are two possible mechanisms for the low recovery of iron after the dry ashing of organic matter¹⁶—loss by volatilization (which will be discussed in the experiments outlined in section C) and loss by reaction with some solid material. This latter case is a possible explanation of

these results for iron. The lack of difference between methods A and B indicates either that there is no adsorption of iron on the silica in the sample or more likely that it is adsorbed and insoluble in the solution prepared according to method A, and then filtered off by method B. Hence the results from these two methods were the same. With the separation from silica in method C, higher iron results were then obtained. It is worth noting however, that for some samples there was considerable variation in the results obtained for the replicates by method C. Also not all samples gave differences between methods—for 25 per cent of the samples there was no difference in the mean results for methods A and C.

The mean results obtained for zinc by the three methods were very similar (table 11). Methods A and B both had mean levels of 65 ppm zinc, with method C 5 per cent lower (table 11a) at 62 ppm zinc. However, the results within method B were extremely variable and had to be excluded to give the above finding without the complication of a significant interaction term. There is a vast amount of literature on the subject of the preparation of biological materials for the analysis of zinc and the majority of workers found no problems over a range of ashing temperatures. The difference found for method C, although small, is hard to explain but is most probably a combination of the extra variation obtained with the filtering step and the second ashing at 600° C.

Some further results of analyses of ash solutions prepared (from foliage samples from Nalbaugh State Forest) according to methods A and C are given in table 12. The results for all the elements are in agreement with those discussed above.

Conclusions

The results of the investigation into the three dry ashing procedures have shown that the simple foliar ashing procedure referred to as method A, gave a solution of the ash which when analysed for the nine elements was found to give more consistent results than either the other simple ashing procedure method B, or the more tedious, lengthy procedure of method C.

For methods A and C, the analyses for the elements phosphorus, calcium, magnesium and potassium gave identical results. The analyses for sodium and zinc were found to be 5 per cent more accurate by method A, whereas those for aluminium, iron and manganese were 5, 3 and 3 per cent respectively underestimated by method A. Every step in an analytical procedure is a potential source of error and it must not be overlooked that apart from the saving in time (50 per cent) of the simplified ashing procedure when compared to the more tedious one, the simple procedure has been shown to be more consistent. There is minimal variation between replicates and this is in contrast to the lengthy silica removal procedure or the filtration procedure in particular which resulted in considerable variation—particularly for certain elements such as aluminium, sodium, iron and manganese.

Hence, although the results from methods A and C were very similar over the range of the nine elements studied, the results obtained

from method B were often quite markedly different. Results comparable with methods A and C were obtained by method B for phosphorus, calcium, and potassium, but sodium, iron and zinc were underestimated by 5, 3 and 3 per cent respectively, and there were even larger errors with aluminium, manganese and magnesium being 9, 9 and 5 per cent respectively. The worst aspect of this method was that a large variation was obtained between replicates. Many workers follow an ashing procedure in which the ash solution is filtered and the silica residue discarded and as shown by the preceding results this could give a solution for analysis which would be far from satisfactory. However by leaving the silica in solution (method A), minimal errors occur in that the elements (particularly magnesium) can solubilise on standing in the acid solution. In this case, the error incurred is less than the variation which can be expected with the lengthy silica removal procedure of method C.

It is recommended that the ashing procedure of method A be adopted for the destruction of organic matter in plant material. The next section of this report is a critical appraisal of method A.

III. CRITICAL APPRAISAL OF THE SIMPLE ASHING PROCEDURE—METHOD A

The following investigations were undertaken in order to assess the accuracy of method A as an ashing procedure for the destruction of organic matter in plant material. In all cases the procedure outlined as method A in section II was followed.

Methods and Materials

1. Effect of Ashing Temperature

The simple ashing procedure was assessed over the temperature range 350° to 550° C to determine whether there were any element losses dependent on the ashing temperature. These losses were considered possible by volatilization or by such means as adsorption on the silica crucibles. A homogeneous sample of *P. radiata* foliage was prepared for this study and samples were ashed in triplicate at various temperatures—350° C, 400° C, 425°, 450° C, 500° C, 525° C, 550° C.

2. Ash-dissolving Solutions

From the literature it appeared evident that the degree of adsorption of certain elements on the silica crucible could be affected by the nature of the ash-dissolving solutions. Four different ash-dissolving solutions were investigated. The bulk foliage sample used for this investigation was the same as that above. The various samples were ashed following the procedure of method A, however a muffling temperature of 550° C was used rather than 420° C. This ashing temperature was chosen since the adsorption of some elements on the silica of the crucible appears to be enhanced at high temperatures, therefore any significant differences in the ash-dissolving solutions would be more

evident at this temperature. In step 7 of the ashing procedure, the following ash-dissolving solutions were used:

- (a) 3ml 5N HCl;
- (b) 3ml 5N HNO₃;
- (c) 3ml dilute HCl/HNO₃ (prepared by mixing equal volumes of 5N acids);
- (d) two digestions (followed by evaporation) of the dried salts with 3ml 5N HCl.

In each case, for step 9, the residue was dissolved in 2ml of the same acid as that used to dissolve the ash.

3. Recovery Experiments

The accuracy of the simple ashing procedure was investigated by carrying out recovery experiments. The recoveries of phosphorus, aluminium, calcium, magnesium, sodium, potassium, iron, zinc and manganese were assessed over a range of ashing temperatures. They were also assessed with and without the presence of added silica, since it was considered possible that the quantity of silica within a sample could affect the quantitative estimation of some elements.

The recovery tests were carried out on 2g subsamples of the homogeneous sample of *P. radiata* foliage. A 5ml aliquot of a standard solution (see below) was added to particular subsamples. The relative levels of the various elements in the standard solution were chosen to correspond with those usually obtained from healthy *P. radiata* foliage. After the addition of the standards, the solutions were evaporated to dryness before proceeding with the ashing procedure (step 4). The standard solution was prepared so that a 5ml aliquot contained the following elemental concentrations:

Al	2 000 μg per 5ml.
Ca	10 000 μg per 5ml.
Mg	10 000 μg per 5ml.
Mn	500 μg per 5ml.
Zn	100 μg per 5ml.
Fe	500 μg per 5ml.

Phosphorus and potassium were added as individual weights of crystalline KH₂PO₄ to give phosphorus additions of between 4 000 μg and 5 000 μg P. 3 000 μg potassium was added to each sample and with the potassium contribution from the KH₂PO₄, the potassium additions were in the range 8 030 μg to 9 300 μg . The silica additions were made with 1g weights of pure silica. The standard solution was prepared to contain 500 μg sodium per 5ml solution. However, this was estimated by atomic absorption to be actually 583 μg sodium. This was expected as sodium is an impurity in many A.R. inorganic reagents. The actual concentrations of all elements in the standard solution were determined accurately (see appendix 1 for analytical methods).

The conditions for the various recovery experiments were as follows:

No.	Sample	Ashing temperature °C
1	Standard Solution	Not muffled Not muffled
2	Standard Solution + Silica	
3	Standard Solution	420
4	Standard Solution + Silica	420
5	Foliage	420
6	Foliage + Standard Solution	420
7	Foliage + Standard Solution + Silica	420
8	Standard Solution	500
9	Standard Solution + Silica	500
10	Foliage	500
11	Foliage + Standard Solution	500
12	Foliage + Standard Solution + Silica	500
13	Standard Solution	550
14	Standard Solution + Silica	550
15	Foliage	550
16	Foliage + Standard Solution	550
17	Foliage + Standard Solution + Silica	550
18	Standard Solution	600
19	Standard Solution + Silica	600
20	Foliage	600
21	Foliage + Standard Solution	600
22	Foliage + Standard Solution + Silica	600

Results and Discussion

1. Effect of Ashing Temperature

The results obtained for the nine elements are shown in tables 13–22. Over the ashing temperature range of 350° to 550° C, significant differences between temperatures were only obtained for the elements calcium and potassium. In the case of calcium, significantly lower results were obtained at 350° and 550° C, while the same results were obtained for all the other ashing temperatures. The losses only amounted to 2 per cent and this is considerably less than the 10 per cent loss at 400° C reported by Griggs *et al.*¹⁶ There was a significant loss of potassium for all ashing temperatures above and including 450° C. This is most probably by volatilization. The difference obtained by increasing the temperature from 425° to 450° C amounted to a loss of 8 per cent potassium but as the ashing temperature was raised to 550° C there was no further loss. There is only one report of losses of potassium up to 500° C where, at the trace level, the various chemical forms of the five alkali metals showed considerable losses up to 600° C.²⁷ In a study on ashing grass samples, Davidson¹⁰ reported losses of 7 per cent at 740° C whereas temperatures of 430°, 530° and 620° C caused no losses.

For some of the other elements, although there were no statistical differences between ashing temperatures, there are other pertinent

observations. Considerable variation and lower results (almost significant) were obtained for aluminium at the lower temperatures of 350°, 400° and also 550° C. It has been considered⁴¹ that higher ashing temperatures might be troublesome due to the decreasing solubility of the alumina produced. There was a tendency towards higher iron results with increasing temperature. This is in agreement with Knauer²⁶ and although not significant, the higher results obtained at 500–550° C do offer a possible explanation for the higher iron results obtained by method C when compared with method A (in section B).

Muller⁸¹ investigated the effect of different ashing temperatures on the macroelements in pine needles and chose a maximum of 450° or preferably $425^\circ \pm 25^\circ$ C as a suitable ashing temperature.

Since at ashing temperatures below 425° C calcium is underestimated, and above 450° C there are considerable losses of potassium which outweigh the small increases in iron above 550° C, it can be concluded from this investigation of ashing temperatures that the optimum temperatures for ashing plant material are between 425° and 450° C.

2. Ash-dissolving Solutions

These results are given in tables 23–32. The only element for which there was a significant difference between the four ash-dissolving solution was zinc. Every other element gave statistically inseparable results. There have been reports of losses of zinc by retention on the silica ashing vessels.¹⁵ This present work has shown that a single digestion with dilute hydrochloric acid is the most effective means of minimizing this retention. However it would appear that with repeated digestions with dilute hydrochloric acid or digestion with dilute nitric acid, enhanced retention of the zinc will occur. In these cases, errors of between 5 and 10 per cent were obtained. It does appear that the lack of difference between ash-dissolving solutions for the other elements indicates there is minimal adsorption of these elements on to the silica ashing crucible.

3. Recovery Experiments

The results of the recovery work are given in table 33. Those for the foliage (only) samples have been reported as actual determinations for each element while those for the standard solution (elements) are expressed as percentage recoveries based on the added amounts.

The results to be considered firstly are those from the foliage-only samples. As would be expected comparable results were obtained, over the studied temperature range, with the work reported earlier on ashing temperatures. That is, with increasing ashing temperature, losses occurred for calcium, potassium, aluminium and zinc. However the major losses were primarily at 600° C—a higher ashing temperature than studied earlier.

The accuracy of method A can be assessed from the results obtained at the ashing temperature of 420° C. Iron is the only element which appears to give less than satisfactory recovery but the results for iron are variable over the whole of the temperature range studied.

With the lack of replicates at each temperature it is difficult to draw definite conclusions about this element. The basic uniformity of the results for the other elements overcomes the lack of replicates, since each temperature level represents a replicate.

Different recoveries for some elements have been obtained depending on the presence or absence of added silica. Considering the large amount of silica added (1g), the recoveries are remarkably good. Over the range of ashing temperature from 420° to 600° C, the elements most affected by added silica were calcium, sodium, iron, zinc, and manganese. The general trend was for losses to increase with increasing ashing temperature. The findings for zinc and manganese were in agreement with reports in the literature on the possible retention of these elements on silica present in the foliage sample. However these present recovery tests were carried out in the presence of a very large amount of added silica which is far in excess of that present in pine foliage material. It is obvious that the recoveries of the various elements in the presence of foliage are excellent and hence the concentration of silica normally present in pine foliage presents no problems. When samples known to contain high concentrations of silica (e.g., 3 to 4-year-old needles from some coniferous species) are to be ashed, there is the possibility of some losses.

The most important finding from this recovery work is that the accuracy of ashing method A is excellent and hence there is no need to follow an ashing procedure which involves the removal of silica for pine foliage. It can also be generalized that possible losses by retention of some elements on the silica of the ashing vessel are minimal—particularly in comparison to possible losses by adsorption on silica in high silica-containing samples.

Conclusions

It has been shown that the accuracy of the simple ashing procedure referred to as method A is excellent. The optimum ashing temperature is 425°C and provided the ash is dissolved in a single digestion of dilute hydrochloric acid, optimum accuracy is obtained in the determination of the required inorganic constituents in the plant material—namely phosphorus, aluminium, calcium, magnesium, sodium, potassium, iron, zinc and manganese. The accuracy of the procedure has been verified by recovery tests, from which it was also concluded that some losses can possibly occur during the dry ashing of plant material which is known to contain high silica concentrations (e.g., this may be possible with some coniferous species other than pine).

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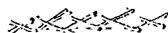


TABLE 1

Mean foliar chemical analyses obtained for the three ashing procedures

Element	ppm			Significance Level between Methods	Significant difference required between means (ppm)
	Method A	Method B	Method C		
Phosphorus	1 551	1 519	1 546
Aluminium	739	708	776	*	31
Calcium ..	2 161	2 140	2 160
Magnesium	1 657	1 568	1 644	*	36
Sodium ..	357	313	316	*	10
Potassium	6 688	6 611	6 519
Iron ..	182	182	189	*	5
Zinc ..	65	65	62	*	1
Manganese	237	223	245	*	7

* Significant at 1 per cent level.

TABLE 2

Mean foliar chemical analyses obtained for the separate samples ashed according to method A

Forest	Species	ppm								
		P	Al	Ca	Mg	Na	K	Fe	Zn	Mn
Mullions Range ..	<i>P. radiata</i> ..	796	791	945	1 075	96	6 348	255	53	101
Old Depot ..	<i>P. radiata</i> ..	2 006	556	2 164	1 107	662	5 483	234	79	206
Penrose ..	<i>P. radiata</i> ..	978	867	4 033	2 234	157	6 002	199	85	284
Jervis Bay ..	<i>P. radiata</i> ..	1 801	781	1 191	955	1 933	8 780	344	37	36
Murraguldrrie I ..	<i>P. radiata</i> ..	3 410	733	3 234	3 076	29	5 521	183	102	317
Belanglo ..	<i>P. radiata</i> ..	1 063	403	1 820	1 684	157	6 713	211	35	139
Mannus ..	<i>P. radiata</i> ..	2 581	902	2 927	3 107	58	5 977	169	95	448
Lidsdale ..	<i>P. radiata</i> ..	1 668	942	2 351	1 941	181	8 919	149	84	376
Newnes ..	<i>P. radiata</i> ..	1 212	746	796	890	92	9 246	208	29	149
Whiporie ..	<i>P. elliotti</i> ..	1 163	639	2 367	1 718	152	5 263	80	66	317
Olney ..	<i>P. elliotti</i> ..	1 041	649	2 742	1 120	422	6 889	83	44	104
Barcoongere ..	<i>P. taeda</i> ..	890	861	1 365	982	343	5 112	70	67	362

TABLE 3

Foliar phosphorus results from the three ashing methods

Forest	Species	Phosphorus (ppm)											
		Method A				Method B				Method C			
		Replicates			Mean	Replicates			Mean	Replicates			Mean
Mullions Range	<i>P. radiata</i>	796	777	816	796	732	777	750	753	768	787	758	771
Old Depot	<i>P. radiata</i>	2 010	2 001	2 007	2 006	1 949	1 926	1 971	1 949	2 008	2 033	1 956	1 999
Penrose	<i>P. radiata</i>	978	999	958	978	960	986	971	972	991	1 001	989	994
Jervis Bay	<i>P. radiata</i>	1 891	1 832	1 679	1 801	1 863	1 748	1 746	1 786	1 863	1 826	1 837	1 842
Murraguldrie	<i>P. radiata</i>	3 394	3 400	3 436	3 410	3 307	3 417	3 258	3 327	3 431	3 367	3 368	3 389
Belanglo	<i>P. radiata</i>	1 081	998	1 110	1 063	1 063	1 005	1 087	1 052	1 079	1 069	1 019	1 056
Mannus	<i>P. radiata</i>	2 574	2 513	2 655	2 581	2 504	2 561	2 440	2 502	2 531	2 568	2 566	2 555
Lidsdale	<i>P. radiata</i>	1 701	1 660	1 643	1 668	1 692	1 635	1 635	1 673	1 671	1 659	1 690	1 673
Newnes	<i>P. radiata</i>	1 240	1 220	1 177	1 212	1 144	1 152	1 131	1 142	1 203	1 168	1 156	1 176
Whiporie	<i>P. elliotii</i>	1 132	1 171	1 185	1 163	1 179	1 126	1 119	1 141	1 169	1 159	1 139	1 156
Olney	<i>P. elliotii</i>	1 067	1 018	1 038	1 041	1 029	1 115	1 016	1 053	1 064	1 073	1 090	1 076
Barcoongere	<i>P. taeda</i>	879	879	912	890	878	887	862	876	846	880	881	869
Grand Mean				1 551				1 519				1 546

TABLE 3A

Analysis of variance for foliar phosphorus results from the three ashing methods

Dispersion								Degrees of freedom	Sum of squares	Variances	F ratio
31	Between ashing methods	2	15 660.4	7 830.19	..
	Between forests	11	61 849 000	5 622 640	492.0**
	Interaction of methods	22	284 097	12 913.5	..
	Inter-method reproducibility (error)	72	822 877.4	11 428.9	..
	Total	107	62 971 700

** Significant at 1 per cent level.

TABLE 4

Foliar calcium results from the three ashing methods

Forest	Species	Calcium (ppm)											
		Method A				Method B				Method C			
		Replicates			Mean	Replicates			Mean	Replicates			Mean
32 Mullions Range	<i>P. radiata</i>	895	992	948	945	854	907	808	856	958	951	908	939
Old Depot	<i>P. radiata</i>	2 176	2 061	2 254	2 164	2 249	2 215	2 169	2 211	1 964	2 134	2 128	2 075
Penrose	<i>P. radiata</i>	4 003	4 038	4 058	4 033	3 756	3 824	3 772	3 784	4 065	4 024	3 993	4 027
Jervis Bay	<i>P. radiata</i>	1 185	1 156	1 231	1 191	1 113	1 175	1 180	1 156	1 155	1 189	1 175	1 173
Murraguldrie I	<i>P. radiata</i>	3 312	3 083	3 306	3 234	3 148	3 255	3 267	3 223	3 263	3 315	3 017	3 198
Belanglo	<i>P. radiata</i>	1 786	1 714	1 960	1 820	1 843	1 885	1 831	1 853	1 849	1 769	1 999	1 872
Mannus	<i>P. radiata</i>	2 938	2 921	2 922	2 927	3 021	3 066	3 005	3 031	2 959	3 000	3 087	3 015
Lidsdale	<i>P. radiata</i>	2 221	2 418	2 413	2 351	2 485	2 366	2 323	2 391	2 338	2 343	2 405	2 362
Newnes	<i>P. radiata</i>	842	747	798	796	821	727	722	757	900	918	842	887
Whiporie	<i>P. elliotii</i>	2 348	2 394	2 360	2 367	2 221	2 231	2 608	2 387	2 527	1 914	2 516	2 319
Olney	<i>P. elliotii</i>	2 751	2 707	2 767	2 742	2 756	2 673	2 635	2 688	2 576	2 709	2 833	2 706
Barcoongere	<i>P. taeda</i>	1 315	1 329	1 452	1 365	1 407	1 288	1 333	1 343	1 254	1 403	1 381	1 346
Grand Mean					2 161				2 140				2 160

TABLE 4A

Analysis of variance for foliar calcium results from the three ashing methods

Dispersion	Degrees of freedom	Sum of squares	Variances	F ratio
Between ashing methods	2	171 388	85 694.1	1.41
Between forests	11	94 016 327	8 546 940	140.9**
Interaction of methods	22	1 123 900	51 086.5	..
Inter-method reproducibility (error)	72	2 851 120	60 663.9	..
Total	107	99 679 420

** Significant at 1 per cent level.

TABLE

Foliar potassium results from the three ashing procedures

Forest	Species	Potassium (ppm)											
		Method A				Method B				Method C			
		Replicates			Mean	Replicates			Mean	Replicates			Mean
Mullions Range	<i>P. radiata</i>	5 749	6 363	6 932	6 348	5 613	6 066	6 148	5 942	6 425	6 363	6 377	6 388
Old Depot	<i>P. radiata</i>	5 646	5 360	5 442	5 483	5 460	5 266	5 322	5 349	5 545	5 232	5 296	5 358
Penrose	<i>P. radiata</i>	5 557	6 179	6 270	6 002	6 174	6 037	5 919	6 043	6 182	5 903	6 110	6 065
Jervis Bay	<i>P. radiata</i>	8 121	9 055	9 163	8 780	8 967	9 342	8 903	9 071	8 175	8 930	8 837	8 647
Murraguldrie I	<i>P. radiata</i>	5 222	5 691	5 651	5 521	5 391	5 085	5 379	5 285	5 483	5 572	5 175	5 410
Belanglo	<i>P. radiata</i>	6 834	7 094	6 210	6 713	7 072	6 585	6 672	6 776	7 043	6 354	6 615	6 671
Mannus	<i>P. radiata</i>	5 730	6 069	6 133	5 977	6 059	5 983	5 903	5 982	6 085	5 874	5 870	5 943
Lidsdale	<i>P. radiata</i>	8 932	8 897	8 929	8 919	8 956	8 854	8 810	8 873	8 342	8 801	9 071	8 738
Newnes	<i>P. radiata</i>	9 396	9 338	9 004	9 246	9 392	7 889	8 473	8 585	7 389	7 963	6 881	7 411
Whiporie	<i>P. elliotii</i>	5 539	4 872	5 377	5 263	4 797	5 196	6 181	5 391	5 274	5 231	5 131	5 212
Onley	<i>P. elliotii</i>	6 836	6 857	6 974	6 889	7 424	7 556	5 928	6 969	6 913	7 130	7 377	7 140
Barcoongere	<i>P. taeda</i>	4 425	5 311	5 600	5 112	5 311	4 713	5 156	5 060	4 735	5 590	5 422	5 249
Grand Mean				6 688				6 611				6 519

TABLE 5A

Analysis of variance for foliar potassium results from the three ashing methods

Dispersion							Degrees of freedom	Sum of squares	Variances	F ratio
35	Between ashing methods	2	511 591	255 796	1.81
	Between forests	11	189 577 903	17 234 355	122.13**
	Interaction of methods	22	5 720 541	260 025	1.84
	Inter-method reproducibility (error)	72	10 160 174	141 114	..
Total							107	205 970 209

** Significant at 1 per cent level.

TABLE 6

Foliar aluminium results from the three ashing methods

Forest	Species	Aluminium (ppm)											
		Method A				Method B				Method C			
		Replicates			Mean	Replicates			Mean	Replicates			Mean
Mullions Range	<i>P. radiata</i>	793	766	814	791	625	646	637	636	935	946	933	938
Old Depot	<i>P. radiata</i>	575	541	551	556	428	404	410	414	688	633	612	644
Penrose	<i>P. radiata</i>	893	864	843	867	736	758	820	771	950	897	932	926
Jervis Bay	<i>P. radiata</i>	738	802	804	781	736	703	708	716	738	738	745	740
Murraguldrrie I	<i>P. radiata</i>	716	746	738	733	814	854	813	827	741	748	783	757
Belanglo	<i>P. radiata</i>	374	370	465	403	358	415	434	402	505	442	512	486
Mannus	<i>P. radiata</i>	923	904	879	902	982	1 079	969	1 010	955	953	956	955
Lidsdale	<i>P. radiata</i>	925	1 057	843	942	918	957	881	919	995	1 010	959	988
Newnes	<i>P. radiata</i>	717	762	759	746	817	699	684	733	655	808	745	736
Whiporie	<i>P. elliotii</i>	646	648	624	639	517	510	540	522	889	631	681	734
Olney	<i>P. elliotii</i>	647	671	628	649	610	627	636	624	639	650	643	644
Barcoongere	<i>P. taeda</i>	881	834	869	861	856	980	928	921	647	808	827	761
Grand Mean					739				708				776

TABLE 6A

Analysis of variance for foliar aluminium results from the three ashing methods

Dispersion	Degrees of freedom	Sum of squares	Variances	F ratio
Between ashing methods	2	56 040.2	28 020.1	6.25**
Between forests	11	2 783 190	253 018	56.41**
Interaction of methods	22	359 914	16 359.7	3.65**
Inter-method reproducibility (error)	72	322 950.6	4 485.4	..
Total	107	3 522 100

** Significant at 1 per cent level.

Significant difference required between ashing method means = 31.6.

TABLE 7

Foliar magnesium results from the three ashing methods

Forest	Species	Magnesium (ppm)											
		Method A				Method B				Method C			
		Replicates		Mean	Replicates		Mean	Replicates		Mean			
38 Mullions Range	<i>P. radiata</i>	1 078	1 066	1 082	1 075	973	966	985	975	1 079	998	1 154	1 077
Old Depot	<i>P. radiata</i>	1 094	1 200	1 027	1 107	1 089	1 075	1 047	1 070	1 232	1 130	964	1 109
Penrose	<i>P. radiata</i>	2 222	2 242	2 239	2 234	2 179	2 250	2 228	2 219	2 331	2 107	2 107	2 248
Jervis Bay	<i>P. radiata</i>	957	985	984	955	877	911	914	901	967	848	934	916
Murraguldrie I	<i>P. radiata</i>	2 952	3 120	3 156	3 076	2 907	3 017	2 628	2 851	2 968	3 002	3 058	3 009
Belanglo	<i>P. radiata</i>	1 714	1 598	1 741	1 684	1 509	1 632	1 540	1 560	1 603	1 656	1 568	1 609
Mannus	<i>P. radiata</i>	3 159	3 001	3 161	3 107	3 069	3 210	2 849	3 042	3 088	2 958	3 033	3 026
Lidsdale	<i>P. radiata</i>	1 922	1 891	2 011	1 941	1 707	1 541	1 625	1 624	1 798	1 806	1 861	1 822
Newnes	<i>P. radiata</i>	938	905	947	890	907	1 004	1 001	971	1 036	1 056	959	1 017
Whiporie	<i>P. elliotii</i>	1 732	1 714	1 709	1 718	1 588	1 522	1 754	1 621	1 719	1 767	1 781	1 756
Olney	<i>P. elliotii</i>	1 176	1 069	1 115	1 120	1 015	1 152	1 080	1 082	1 103	1 179	1 195	1 159
Barcoongere	<i>P. taeda</i>	943	942	1 060	982	924	919	870	904	1 002	960	977	980
Grand Mean				1 657				1 568				1 644

TABLE 7A

Analysis of variance for foliar magnesium results from the three ashing methods

	Dispersion	Degrees of freedom	Sum of squares	Variances	F ratio
29	Between ashing methods	2	174 491	87 245·5	14·76**
	Between forests	11	58 566 767	5 324 250	900·95**
	Interaction of methods	22	188 533	8 569·7	1·45
	Inter-method reproducibility (error)	72	425 492	5 909·6	..
	Total	107	59 355 300

** Significant at 1 per cent level.

Significant difference required between ashing method means = 36·2.

TABLE 8

Foliar sodium results from the three ashing methods

Forest	Species	Sodium (ppm)											
		Method A				Method B				Method C			
		Replicates			Mean	Replicates			Mean	Replicates			Mean
Mullions Range	<i>P. radiata</i> ..	115	102	70	62	69	69	57	62	70	64	71	68
Old Depot	<i>P. radiata</i> ..	676	673	639	662	558	569	573	566	580	584	593	586
Penrose	<i>P. radiata</i> ..	152	159	159	157	151	144	135	143	137	141	143	140
Jervis Bay	<i>P. radiata</i> ..	1 960	1 900	1 940	1 933	1 620	1 761	1 700	1 694	1 740	1 600	1 600	1 647
Murraguldrrie I	<i>P. radiata</i> ..	18	39	31	29	33	28	34	32	34	38	51	41
Belanglo	<i>P. radiata</i> ..	149	148	173	157	130	137	120	129	147	140	162	150
Mannus	<i>P. radiata</i> ..	66	59	49	58	29	29	33	30	36	35	34	35
Lidsdale	<i>P. radiata</i> ..	186	185	171	181	177	175	174	175	185	211	251	216
Newnes	<i>P. radiata</i> ..	82	97	97	92	97	97	106	102	112	102	98	104
Whiporie	<i>P. elliotti</i> ..	151	161	145	152	134	143	142	140	125	120	134	126
Olney	<i>P. elliotti</i> ..	425	431	410	422	358	370	375	368	356	384	371	370
Barcoongere	<i>P. taeda</i> ..	327	354	349	343	311	313	307	310	325	297	306	309
Grand Mean					357				313				316

TABLE 8A

Analysis of variance for foliar sodium results from the three ashing methods

	Dispersion	Degrees of freedom	Sum of squares	Variances	F ratio
41	Between ashing methods	2	43 607	21 804	47.19**
	Between forests	11	22 772 614	2 070 238	4 481.03**
	Interaction of methods	22	130 851	5 948	12.87**
	Inter-method reproducibility (error)	72	33 255	462	..
	Total	107	22 980 327

** Significant at 1 per cent level.

Significant difference required between ashing method means = 10.1.

TABLE 9

Foliar manganese results from the three ashing methods

Forest	Species	Manganese (ppm)											
		Method A				Method B				Method C			
		Replicates			Mean	Replicates			Mean	Replicates			Mean
Mullions Range	<i>P. radiata</i>	101	101	101	101	102	109	109	107	107	106	109	107
Old Depot	<i>P. radiata</i>	203	208	207	206	207	209	206	207	208	211	213	211
Penrose	<i>P. radiata</i>	283	277	291	284	281	294	288	288	294	299	305	299
Jervis Bay	<i>P. radiata</i>	35	36	37	36	31	33	36	33	35	37	34	35
Murraguldrrie I	<i>P. radiata</i>	313	323	316	317	315	319	318	317	334	334	335	334
Belanglo	<i>P. radiata</i>	142	137	139	139	141	145	145	142	139	141	139	140
Mannus	<i>P. radiata</i>	456	458	429	448	443	428	448	440	451	458	447	452
Lidsdale	<i>P. radiata</i>	376	374	379	376	367	345	357	356	391	401	398	397
Newnes	<i>P. radiata</i>	154	145	148	149	142	145	152	146	175	174	160	170
Whiporie	<i>P. elliottii</i>	318	321	313	317	303	210	162	225	337	332	340	336
Olney	<i>P. elliottii</i>	103	103	106	104	96	55	47	66	106	113	115	111
Barcoongere	<i>P. taeda</i>	363	352	372	362	352	354	351	352	349	350	353	351
Grand Mean				237				223				245

TABLE 9A

Analysis of variance for foliar manganese results from the three ashing methods

Dispersion							Degrees of freedom	Sum of squares	Variances	F ratio
43	Between ashing methods	2	8 798	4 399	23.03**
	Between forests	11	1 676 789	152 435	798.09**
	Interaction of methods	22	21 034	956	5.01**
	Inter-method reproducibility (error)	72	13 759	191	..
	Total	107	1 720 380

** Significant at 1 per cent level.

Significant difference required between ashing method means = 7.

TABLE 10

Foliar iron results from the three ashing methods

Forest	Species	Iron (ppm)													
		Method A				Method B				Method C					
		Replicates			Mean	Replicates			Mean	Replicates			Mean		
Mullions Range	<i>P. radiata</i>	252	257	255	255	251	268	265	261	278	281	282	280		
44 Old Depot	<i>P. radiata</i>	239	233	231	234	233	234	231	233	252	248	258	253		
Penrose	<i>P. radiata</i>	197	193	206	199	208	209	204	207	225	232	204	220		
Jervis Bay	<i>P. radiata</i>	354	352	325	344	366	327	325	339	325	328	331	328		
Murraguldrie I	<i>P. radiata</i>	185	185	180	183	191	185	189	188	181	196	192	190		
Belanglo	<i>P. radiata</i>	200	225	207	211	222	253	200	225	225	224	218	222		
Mannus	<i>P. radiata</i>	172	164	170	169	159	160	158	159	174	172	158	168		
Lidsdale	<i>P. radiata</i>	153	155	139	149	148	143	150	147	140	176	168	161		
Newnes	<i>P. radiata</i>	211	205	207	208	201	173	206	193	190	187	171	183		
Whiporie	<i>P. elliotii</i>	72	83	86	80	87	83	65	78	90	85	92	89		
Olney	<i>P. elliotii</i>	91	79	80	83	101	59	71	77	80	100	109	96		
Barcoongere	<i>P. taeda</i>	64	75	70	70	84	77	73	78	77	71	81	76		
Grand Mean182					182				
													189		

TABLE 10A

Analysis of variance for foliar iron results from the three ashing methods

Dispersion		Degrees of freedom	Sum of squares	Variances	F ratio
45	Between ashing methods	2	1 117	559	4.82**
	Between forests	11	615 839	55 985	482.63**
	Interaction of methods	22	4 584	208	1.79
	Inter-method reproducibility (error)	72	8 339	116	..
	Total	107	629 870

** Significant at 1 per cent level.

Significant difference required between ashing method means = 5.1.

TABLE 11

Foliar zinc results from the three ashing methods

Forest	Species	Zinc (ppm)											
		Method A				Method B				Method C			
		Replicates			Mean	Replicates			Mean	Replicates			Mean
46 Mullions Range	<i>P. radiata</i> ..	54	49	55	53	47	47	47	47	45	46	50	47
Old Depot	<i>P. radiata</i> ..	81	78	78	79	79	77	75	77	76	77	75	76
Penrose	<i>P. radiata</i> ..	85	83	86	85	84	92	81	86	81	77	81	80
Jervis Bay	<i>P. radiata</i> ..	36	37	39	37	47	40	39	42	39	39	36	38
Murraguldrie I	<i>P. radiata</i> ..	101	107	98	102	99	98	102	100	87	89	90	89
Belanglo	<i>P. radiata</i> ..	37	32	35	35	37	40	37	38	36	35	38	36
Mannus	<i>P. radiata</i> ..	94	94	98	95	99	95	100	98	87	84	97	89
Lidsdale	<i>P. radiata</i> ..	85	85	82	84	80	77	81	79	86	78	83	82
Newnes	<i>P. radiata</i> ..	30	28	29	29	29	29	29	29	31	28	26	28
Whiporie	<i>P. elliotii</i> ..	67	66	65	66	70	69	68	69	67	64	66	66
Olney	<i>P. elliotii</i> ..	45	45	43	44	48	49	48	48	43	44	47	45
Barcoongere	<i>P. taeda</i> ..	67	67	66	67	65	62	62	62	67	65	68	67
Grand Mean				65				65				62

TABLE 11A

Analysis of variance for foliar zinc results from the three ashing methods

	Dispersion	Degrees of freedom	Sum of squares	Variances	F ratio
47	Between ashing methods	2	162	81	11.57**
	Between forests	11	54 524	4 957	708.14**
	Interaction of methods	22	571	26	3.71**
	Inter-method reproducibility (error)	72	491	7	..
	Total	107	55 748

** Significant at 1 per cent level.

Significant difference required between ashing method means = 1.2.

TABLE 12

Foliar chemical analyses obtained from ash solutions (of foliage samples from Nalbaugh S.F.) prepared according to ashing methods A and C

Ashing procedure	Sample Plot No.	ppm								
		P	Al	Ca	Mg	Na	K	Fe	Mn	Zn
Method C	3	3 079	509	1 937	2 792	387	8 857	133	161	98
	5	3 057	533	1 921	2 085	443	8 583	135	113	85
	7	2 960	515	2 130	2 576	501	6 414	190	121	80
	12	1 646	612	2 056	2 731	407	7 419	160	119	77
	14	2 497	811	1 157	1 646	550	11 804	167	158	77
	15	1 576	680	3 479	4 434	425	4 342	141	229	131
	18	1 782	457	872	1 161	639	5 567	123	137	48
	22	1 445	444	1 395	1 674	767	6 027	143	318	69
	Mean	2 255	570	1 868	2 387	514	7 376	149	169	83
	Method A	3	3 020	513	2 035	3 070	397	9 497	128	170
5		2 868	443	1 733	1 939	327	7 961	142	108	73
7		3 004	642	2 188	2 670	541	6 788	214	125	96
12		1 671	558	1 969	2 599	342	7 462	148	120	82
14		2 460	762	1 020	1 607	525	12 244	135	177	81
15		1 669	630	3 388	4 230	439	4 451	127	225	94
18		1 792	337	1 036	1 315	556	6 451	134	168	55
22		1 469	449	1 524	1 730	678	6 517	133	299	75
Mean		2 244	541	1 861	2 395	476	7 671	145	174	83

TABLE 13

Mean chemical analyses obtained from ash solutions prepared by method A at various ashing temperatures

Ashing temperature (°C)	ppm								
	P	Al	Ca	Mg	Na	K	Fe	Zn	Mn
350	800	674	2 926	1 420	192	3 860	52	27	142
400	794	670	2 987	1 436	189	3 774	50	27	146
425	786	720	2 968	1 422	187	3 867	51	26	147
450	786	721	2 994	1 403	189	3 587	52	26	147
475	786	722	2 987	1 387	187	3 578	50	26	148
500	787	722	2 977	1 385	189	3 519	60	25	149
525	802	719	2 997	1 356	189	3 618	57	26	148
550	801	678	2 948	1 368	187	3 610	55	27	145
LSD 0.05	40	131

TABLE 14

Phosphorus results obtained from ash solutions prepared by method A at various ashing temperatures

Ashing temperature (°C)	Foliar phosphorus (ppm)			
	Replicates			Mean
350	813	786	802	800
400	814	776	792	794
425	785	801	772	786
450	800	781	777	786
475	782	776	800	786
500	793	772	796	787
525	794	811	801	802
550	802	814	787	801

No significant differences between ashing temperatures.

TABLE 15

Foliar aluminium results obtained from ash solutions prepared by method A at various ashing temperatures

Ashing temperature (°C)	Foliar aluminium (ppm)			
	Replicates			Mean
350	661	650	711	674
400	692	670	649	670
425	734	715	711	720
450	714	732	716	721
475	755	717	694	722
500	733	720	714	722
525	757	661	739	719
550	712	646	676	678

No significant differences between ashing temperatures.

TABLE 16

Foliar calcium results obtained from ash solutions prepared by method A at various ashing temperatures

Ashing temperature (°C)	Foliar calcium (ppm)			
	Replicates			Mean
350	2 948	2 912	2 918	2 926
400	2 992	2 952	3 017	2 987
425	2 957	2 991	2 956	2 968
450	2 987	2 996	2 999	2 994
475	3 002	2 997	2 962	2 987
500	2 985	2 991	2 955	2 977
525	3 012	2 998	2 981	2 997
550	2 948	2 912	2 984	2 948

LSD_{0.05} = 40.

TABLE 17

Foliar magnesium results from ash solutions prepared by method A at various ashing temperatures

Ashing temperature (°C)	Foliar magnesium (ppm)			Mean
	Replicates			
350	1 438	1 392	1 430	1 420
400	1 382	1 442	1 484	1 436
425	1 387	1 449	1 430	1 422
450	1 436	1 384	1 389	1 403
475	1 368	1 426	1 367	1 387
500	1 372	1 363	1 420	1 385
525	1 428	1 335	1 305	1 356
550	1 352	1 410	1 342	1 368

No significant differences between ashing temperatures.

TABLE 18

Foliar sodium results from ash solutions prepared by method A at various ashing temperatures

Ashing temperature (°C)	Foliar sodium (ppm)			Mean
	Replicates			
350	203	183	190	192
400	191	183	193	189
425	195	183	183	187
450	188	189	191	189
475	186	188	188	187
500	196	186	185	189
525	189	188	189	189
550	184	191	186	187

No significant differences between ashing temperatures.

TABLE 19

Foliar potassium results obtained from ash solutions prepared by method A at various ashing temperatures

Ashing temperature (°C)	Foliar potassium (ppm)			
	Replicates			Mean
350	3 910	3 802	3 868	3 860
400	3 878	3 710	3 734	3 774
425	3 967	3 831	3 801	3 867
450	3 678	3 510	3 573	3 587
475	3 598	3 475	3 661	3 578
500	3 536	3 573	3 448	3 519
525	3 637	3 560	3 658	3 618
550	3 662	3 628	3 539	3 610

LSD_{0.05} = 131.

TABLE 20

Foliar iron results obtained from ash solutions prepared by method A at various ashing temperatures

Ashing temperature (°C)	Foliar iron (ppm)			
	Replicates			Mean
350	54	52	50	52
400	50	50	51	50
425	53	50	50	51
450	55	51	50	52
475	54	49	47	50
500	68	52	59	60
525	66	50	55	57
550	62	51	52	55

No significant differences between ashing temperatures.

TABLE 21

Foliar zinc results obtained from ash solutions prepared by method A at various ashing temperatures

Ashing temperature (°C)	Foliar zinc (ppm)			
	Replicates			Mean
350	30	28	23	27
400	25	28	28	27
425	26	27	26	26
450	27	26	26	26
475	27	26	26	26
500	25	24	25	25
525	27	24	27	26
550	26	27	27	27

No significant differences between ashing temperatures.

TABLE 22

Foliar manganese results obtained from ash solutions prepared by method A at various temperatures

Ashing temperature (°C)	Foliar manganese (ppm)			
	Replicates			Mean
350	143	134	149	142
400	142	144	152	146
425	144	147	149	147
450	148	147	146	147
475	149	151	144	148
500	152	147	149	149
525	157	141	147	148
550	146	146	144	145

No significant differences between ashing temperatures.

TABLE 23

Mean foliar analysis obtained using method A with various ash-dissolving solutions

Ash-dissolving solutions	ppm								
	P	Al	Ca	Mg	Na	K	Fe	Zn	Mn
1:1 HCl ..	801	676	2 948	1 368	187	3 610	55	26.7	145
1:1 HCl—three digestions ..	778	700	2 917	1 377	188	3 577	58	24.0	146
1:1 HNO ₃ ..	801	688	2 955	1 372	188	3 516	56	25.0	143
1:1 HCl—HNO ₃ (50% v/v) ..	787	673	2 941	1 337	189	3 639	54	26.0	146
LSD _{0.05}	1.4	..

TABLE 24

Foliar phosphorus results obtained using method A with various ash-dissolving solutions

Ash-dissolving solutions	Phosphorus (ppm)			
	Replicates			Mean
1:1 HCl	802	814	787	801
1:1 HCl—three digestions ..	773	793	769	778
1:1 HNO ₃	808	784	811	801
1:1 HCl—HNO ₃ (50% v/v) ..	799	783	778	787

No significant difference between treatments.

TABLE 25

Foliar aluminium results obtained using method A with various ash-dissolving solutions

Ash-dissolving solutions	Aluminium (ppm)			
	Replicates			Mean
1:1 HCl	712	646	676	676
1:1 HCl—three digestions	709	723	667	700
1:1 HNO ₃	721	688	655	688
1:1 HCl—HNO ₃ (50 per cent v/v) ..	705	643	672	673

No significant differences between treatments.

TABLE 26

Foliar calcium results obtained using method A with various ash-dissolving solutions

Ash-dissolving solutions	Calcium (ppm)			
	Replicates			Mean
1:1 HCl	2 948	2 912	2 984	2 948
1:1 HCl—three digestions	2 920	2 944	2 887	2 917
1:1 HNO ₃	2 948	2 980	2 937	2 955
1:1 HCl—HNO ₃ (50 per cent v/v) ..	2 910	2 978	2 935	2 941

No significant differences between treatments.

TABLE 27

Foliar magnesium results obtained using method A with various ash-dissolving solutions

Ash-dissolving solutions	Magnesium (ppm)			
	Replicates			Mean
1:1 HCl	1 352	1 410	1 342	1 368
1:1 HCl—three digestions	1 368	1 367	1 396	1 377
1:1 HNO ₃	1 362	1 390	1 364	1 372
1:1 HCl—HNO ₃	1 371	1 335	1 305	1 337

No significant differences between treatments.

TABLE 28

Foliar sodium results obtained using method A with various ash-dissolving solutions

Ash-dissolving solutions	Sodium (ppm)			
	Replicates			Mean
1:1 HCl	184	191	186	187
1:1 HCl—three digestion	186	186	191	188
1:1 HNO ₃	188	189	188	188
1:1 HCl—HNO ₃ (50 per cent v/v)..	190	188	190	189

No significant differences between treatments.

TABLE 29

Foliar potassium results obtained using method A with various ash-dissolving solutions

Ash-dissolving solutions	Potassium (ppm)			
	Replicates			Mean
1:1 HCl	3 662	3 628	3 539	3 610
1:1 HCl—three digestions	3 743	3 523	3 466	3 577
1:1 HNO ₃	3 527	3 556	3 466	3 516
1:1 HCl—HNO ₃ (50 per cent v/v) ..	3 495	3 745	3 676	3 639

No significant differences between treatments.

TABLE 30

Foliar iron results obtained using method A with various ash-dissolving solutions

Ash-dissolving solutions	Iron (ppm)			
	Replicates			Mean
1:1 HCl	62	51	52	55
1:1 HCl—three digestions	65	56	52	58
1:1 HNO ₃	65	52	52	56
1:1 HCl—HNO ₃ (50 per cent v/v) ..	51	53	58	54

No significant differences between treatments.

TABLE 31

Foliar zinc results obtained using method A with various ash-dissolving solutions

Ash-dissolving solution	Zinc (ppm)			
	Replicates			Mean
1:1 HCl	26	27	27	26.7
1:1 HCl—three digestions	25	24	23	24.0
1:1 HNO ₃	25	25	25	25.0
1:1 HCl—HNO ₃ (50 per cent v/v)..	27	25	26	26.0

LSD_{0.05} = 1.4.

TABLE 32

Foliar manganese results obtained using method A with various ash-dissolving solutions

Ash-dissolving solutions	Manganese (ppm)			
	Replicates			Mean
1:1 HCl	146	146	144	145
1:1 HCl—three digestions	148	144	145	146
1:1 HNO ₃	140	145	143	143
1:1 HCl—HNO ₃ (50 per cent v/v)..	148	144	145	146

No significant differences between treatments.

TABLE 33

Results from recovery experiments. In the case of the foliage only results—these are actual determinations. The other results are percentage recoveries based on the non-muffled samples

Number	Sample	Ashing Temperature (°C)	P	Al	Ca	Mg	Na	K	Fe	Zn	Mn
3	Elements	420	99.9	100.4	96.2	99.8	97.2	100.4	99.4	100.2	98.6
4	Elements + Si	420	97.0	100.8	93.0	96.6	113.1	101.5	99.1	101.5	97.8
5	Foliage (p.p.m.)	420	97.5	1 086	1 943	1 344	498	3 556	153	39	387
6	Foliage + Elements	420	99.9	100.1	102.9	100.5	98.1	103.2	72.1	101.5	99.1
7	Foliage + Elements + Si ..	420	96.6	102.8	102.4	101.2	79.9	101.3	63.2	100.0	95.3
8	Elements	500	101.3	100.8	94.3	101.0	95.7	98.5	106.0	101.5	99.7
9	Elements + Si	500	99.4	98.8	92.3	94.4	103.3	98.4	99.1	94.0	90.5
10	Foliage (p.p.m.)	500	97.4	1 102	1 950	1 354	504	3 464	157	38	385
11	Foliage + Elements	500	101.7	99.7	105.2	99.8	95.0	102.2	93.0	95.3	100.0
12	Foliage + Elements + Si ..	500	100.9	102.0	98.4	100.9	83.7	102.0	89.3	76.4	92.4
13	Elements	550	100.0	98.8	93.0	98.5	85.2	105.7	97.9	100.6	91.8
14	Elements + Si	550	100.6	97.3	93.8	94.5	101.6	92.4	99.1	99.2	92.4
15	Foliage (p.p.m.)	550	95.8	1 048	1 915	1 360	473	3 557	125	38	367
16	Foliage + Elements	550	100.1	103.8	99.1	100.2	64.8	100.2	87.6	88.2	93.3
17	Foliage + Elements + Si ..	550	97.6	98.5	97.7	95.0	80.6	98.5	51.5	79.6	87.0
18	Elements	600	100.6	99.0	96.5	100.3	95.2	101.1	107.2	105.3	99.1
19	Elements + Si	600	96.6	101.5	91.3	94.8	93.2	84.0	99.1	84.7	97.2
20	Foliage (p.p.m.)	600	97.2	1 033	1 904	1 353	465	3 257	140	35	388
21	Foliage + Elements	600	102.1	106.3	98.1	100.4	87.5	92.5	96.6	86.9	94.1
22	Foliage + Elements + Si ..	600	100.7	101.4	96.7	97.5	93.6	90.6	67.8	69.7	72.4

APPENDIX 1. Determination of phosphorus in foliage material.

REAGENTS

Reagent A—76.8g ammonium molybdate (A.R.) is dissolved in approx. 200ml distilled water. 1.755 g antimony potassium tartrate is dissolved in approx. 100ml distilled water. 896ml 36N H_2SO_4 is dissolved in 500ml distilled water. The first two reagents are added to the dilute sulphuric acid and the solution diluted to 2 litres with distilled water. This solution is stored in a refrigerator.

Reagent B—1.70g ascorbic acid is dissolved in 100ml distilled water, 50ml reagent A are added and diluted to 200ml with distilled water. This reagent must be prepared fresh daily.

2N H_2SO_4 —Approximately 55ml 36N H_2SO_4 are added to 500ml distilled water and diluted to 1 litre with distilled water.

2N NaOH—80g NaOH are dissolved in distilled water and diluted to 1 litre.

2, 6-dinitrophenol indicator—0.25g indicator is dissolved in a few drops of alcohol (1ml) and diluted to 100ml with distilled water.

Standard phosphorus solution—(50 ppm and 5 ppm) 0.219 5g KH_2PO_4 is dissolved in approximately 100ml distilled water, 5ml 36N H_2SO_4 added and the solution diluted to 1 litre with distilled water. This stock solution contains 50 ppm P. 50ml of this solution are diluted to 500ml to give a working standard of 5 ppm P.

PROCEDURE

An aliquot is taken from each of the ash solutions obtained by the ashing methods. It is preferable for the aliquot to contain 30–50 μg P and hence a 2ml or 5ml aliquot is the most suitable. The aliquot is placed in a 50ml volumetric flask and distilled water added to about 20ml. If the solution is more acid than usual, the solution is adjusted to about pH 3 by the addition of 2N NaOH until 2, 6-dinitrophenol indicator turns yellow and then is brought back to colourless by the addition of 2N H_2SO_4 . Distilled water is then added to about 45ml and the solution mixed. 5ml reagent B is added, the solution mixed and made up to volume. The solution is then heated at 70° C in a constant temperature water bath for 10 minutes. When cool, the solution is read on a spectrophotometer at 880 $m\mu$ against a reagent blank prepared using distilled water. The colour is stable for approximately 8 hours and should be read on the day the colour is developed.

If an automatic dilutor is available, the sample syringe is set to take a 2ml aliquot from the ash solution and delivered into a 4-inch specimen tube, with 12.5ml distilled water from the diluent syringe. In the next operation, 3ml reagent B is taken up by the sample syringe and also delivered into the tube with 12.5ml distilled water from the diluent syringe. This gives a final volume of 30ml. The solution is mixed, heated and the colour read as above.

STANDARD CURVE

Aliquots containing 10, 20, 30, 40, 50 μg P are placed in separate 50ml volumetric flasks. If an automatic dilutor has been used for the samples, then the same procedure should be followed for the standards by taking 2ml aliquots by the sample syringe from solutions containing 5, 10, 15, 20, 25, 30 ppm P. The procedure for colour development is then followed as above.

It is not necessary to determine all the points on the standard curve with each set of foliar ash solutions. Only one or two points on the graph need be checked with each set of estimations. A new curve is determined when a new batch of reagent A is prepared.

The absorbances obtained are plotted against the μg P in the aliquots. A straight line is drawn through the points and should pass through the origin. The equation of the line is determined for ease of calculation.

CALCULATION

$$\text{ppm. P} = \frac{(\mu\text{g P in aliquot}) \times \text{Ash Volume}}{\text{Aliquot} \times \text{O.D. wt foliage}}$$

APPENDIX 2. Determination of aluminium in foliage material.

REAGENTS

10 per cent Ammonium Acetate—100g ammonium acetate (A.R.) is dissolved in 1 litre distilled water and the pH of the solution is adjusted to 5.3.

0.2 per cent Ferron—2g ferron (8-hydroxy-7-iodoquinoline-5-sulphonic acid) is dissolved in 1 litre distilled water.

Standard Aluminium Solution—0.175 9g aluminium potassium sulphate (A.R.— $\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$) which has been stored in a desiccator over dry silica gel for a few days, is made up to 1 litre with distilled water. This solution contains 10 ppm Al.

Standard Iron Solution—0.864 0g ferric ammonium sulphate (A.R.— $\text{FeNH}_4(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$) is dissolved in sufficient water, 10 ml 10N HCl is added and diluted to 1 litre with distilled water. This stock solution contains 100 ppm Fe. 50ml of this solution are diluted to 500ml to give a solution of 10 ppm Fe.

PROCEDURE

An aliquot (usually 5ml) of the ash solution containing no more than 50 μg Al, is placed in a 25 ml volumetric flask. 5ml ammonium acetate is added, followed by 2ml ferron. The solution is made to volume with distilled water and the absorbances determined at 370 $\text{m}\mu$ and 600 $\text{m}\mu$ after standing for 1 hour. The developed colour is stable for approximately 24 hours. The absorbances are read against a blank containing distilled water to which reagents have been added as above.

If an automatic dilutor is available, the sample syringe is set to take a 5ml aliquot from the ash solution and this is delivered into a 4" specimen tube with 5ml ammonium acetate from the diluent syringe. In the next operation, 2ml ferron is taken up by the sample syringe and delivered into the tube with 13ml distilled water. The solution is mixed and read as above.

STANDARD CURVES

Aliquots containing 10 to 60 μg Al are placed in 25ml volumetric flasks and additional aliquots containing 10 to 60 μg Fe are placed in separate flasks. 5ml 10 per cent ammonium acetate is added followed by 2ml ferron. The solutions are made to volume with distilled water and the absorbances read at 370 $\text{m}\mu$ and 600 $\text{m}\mu$ against a reagent blank.

If the automatic dilutor has been used, 5ml aliquots are taken from the appropriate standard solution and the procedure followed as for ash solutions.

The total μg of Al or Fe in the 25ml volume is plotted against the E. reading. Straight lines are drawn through the points and pass through the origin. The equation of each line is calculated, e.g.,

$$\mu\text{g Fe (370 m}\mu) = C \times \text{E. reading}$$

$$\mu\text{g Fe (600 m}\mu) = D \times \text{E. reading}$$

$$\mu\text{g Al (370 m}\mu) = A \times \text{E. reading}$$

(N.B. The E. readings obtained for Al and for Fe at 370 $\text{m}\mu$ are perfectly additive.)

CALCULATIONS

$$\text{Comparable E. reading (B)} = (\text{E. reading at 600 m}\mu) \times \frac{D}{C}$$

for Fe at 370 $\text{m}\mu$

$$\text{E. reading for Al only} = (\text{E. reading at 370 m}\mu) - (\text{B}) 370 \text{ m}\mu.$$

$$\text{pm Al} = \frac{\mu\text{g Al} \times \text{Ash Volume}}{\text{Aliquot} \times \text{O.D. wt foliage}}$$

APPENDIX 3. Determination of Ca, Mg, K, Na, Fe, Zn and Mn in foliage material.

REAGENTS

Strontium Chloride Solution—10.1440 g strontium chloride (A.R.) is dissolved in distilled water and diluted to 1 litre with distilled water. This solution contains 3 333 ppm Sr.

Standard Solutions

1 000 ppm. Stock Calcium Solution—2.4975 g calcium carbonate A.R. (dried at 550° C for 4 hours) is dissolved in hydrochloric acid and diluted to 1 litre with distilled water.

1 000 ppm. Stock Magnesium Solution—1g magnesium ribbon (99.9 per cent purity) is dissolved in hydrochloric acid and diluted to 1 litre with distilled water.

1 000 ppm. Stock Sodium Solution—2.5419g sodium chloride A.R. (dried at 110° C for 4 hours) is dissolved in 1 litre distilled water containing 1ml 1ON HCl.

1 000 ppm. Stock Potassium Solution—1.9069g potassium chloride A.R. (dried at 500° C for 4 hours) is dissolved in 1 litre distilled water containing 1ml 1ON HCl.

1 000 ppm. Stock Manganese Solution—2.8768g potassium permanganate A.R. (99.9 per cent purity) is dissolved in distilled water and decolourized with oxalic acid. The solution is then diluted to 1 litre with distilled water containing 1ml 1ON HCl. (The concentration is checked by standardization against arsenious oxide.)

1 000 ppm. Stock Iron Solution—7.0225g ferrous ammonium sulphate A.R. ($\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$) is dissolved in 1 litre distilled water containing 1ml 1ON HCl.

1.000 ppm. Stock Zinc Solution—1g zinc metal (99.9 per cent purity) is dissolved in hydrochloric acid and diluted to 1 litre with distilled water.

(BDH, Merck or similar stock standard solutions (1 000 ppm) are also appropriate.)

Working Standard Solutions

Working standard solutions are prepared for calcium, magnesium and potassium to contain these three elements together in the one solution. These solutions are so prepared to also contain 3 000 ppm. Sr (as SrCl_2). Working standard solutions are prepared for each of the other elements by taking suitable aliquots from the stock standard solutions, adding 1ml 1ON HCl and diluting to 250ml with distilled water.

PROCEDURE

For the determination of calcium, magnesium and potassium, a 5ml aliquot is taken from the ash solution and diluted to 50ml with the strontium chloride solution. This gives a solution with a final concentration of 3 000 ppm Sr. This solution is aspirated into the burner of the A.A.

Sodium, iron, zinc and manganese are determined directly on the ash solution.

OPERATING CONDITIONS FOR VARIAN AA6, ATOMIC ABSORPTION
SPECTROPHOTOMETER

	Lamp current (mA)	Slit (μ)	Wave- length (\AA)	Fuel
Calcium	5	100	4227	N ₂ O/Acet.
Magnesium	4	50	2852	Air/Acet.
Sodium	5	50	5890	Air/Propane.
Potassium	10	300	7665	Air/Propane.
Iron	5	100	2483	Air/Acet.
Zinc	10	300	2139	Air/Propane.
Manganese	10	100	2975	Air/Acet.

The burner height, fuel flow and lateral position of the burner are so adjusted to give maximum sensitivity for each element.

CALCULATION

$$\text{ppm Element (in foliage)} = \frac{(\text{ppm in soln}) \times \text{Ash Volume} \times \text{Dilution}}{\text{O.D. wt foliage}}$$

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