
Rowland, A. P.. 1987 Reference materials for ecological analysis. In: Rowland, A. P., (ed.) *Chemical analysis in environmental research.* Abbotts Ripton, NERC/ITE, 88-91. (ITE Symposium, 18).

Reference materials for ecological analysis

A P ROWLAND
ITE, Merlewood Research Station, Grange-over-Sands

1 Introduction

Quality control is an essential feature of analytical procedures, but in the chemical analysis of ecological samples it is difficult to select ideal control materials when there are a large number of determinands. Certified vegetation reference materials are available to check at intervals the accuracy of methods which will initially have been verified by comparison with existing procedures or by standard addition tests. In routine use, reagent blanks will also need to be included to assess the bias from background levels or contamination. Replicate analyses will indicate the elemental precision.

The use of certified vegetation materials as routine control samples can raise problems. Only a few have been made available in the USA and Europe, and large quantities are expensive or even unobtainable. The levels of some nutrients may be far from ideal for the work of a particular laboratory, and some elements of interest may not be certified even provisionally. Under these circumstances, there is a case for a laboratory to prepare its own reference material where repeated analyses will in time become the equivalent of certification. Only a small amount of an existing certified material need then be purchased for an occasional check.

In the laboratories at ITE's Merlewood Research Station an internal control sample of heather *(Calluna vulgaris)* has been in use for many years. This paper reviews the factors involved in its selection and analysis, and the suitability of alternative vegetation species. For soil analysis, long-term data have been accumulated from a local drift soil, and these data have proved useful when developing new methods. Natural waters and leachates are inherently unstable and, as such, are unsuitable for control samples, so a synthetic control sample has to be prepared. Development of ion chromatography methodology was aided considerably by the use of a synthetic reference solution, and some of the development work will be discussed briefly.

2 Materials and methods

2.1 Vegetation

2.1.1 Samples were dried at 40°C for 24 hours and ground to <0.7 mm. All results were expressed on a dry weight basis.

2.1.2 Sample solutions were prepared for total nutrient analysis using a modified Kjeldahl digestion (Parkinson & Allen 1975).

2.1.3 N in solution was estimated using an indophenol blue AutoAnalyzer colorimetric method (Rowland 1983).

2.1.4 Other nutrients were determined as in Allen *et al.* (1974), and are as follows:
Na, K — flame photometry
Ca, Mg — atomic absorption including lanthanum addition
Mn, Cu, Zn — atomic absorption
P, Fe — automated colorimetry using AutoAnalyzer methods.
All standard solutions were compensated by the addition of digested reagent blank.

2.2 Soil

2.2.1 Soil samples were dried at 40°C, screened through a 2 mm sieve and then ball-milled to <0.15 mm.

2.2.2 Samples were digested and analysed for N and P by adapting the procedures previously outlined (Rowland & Grimshaw 1985).

2.3 Natural waters

2.3.1 Sample solutions were filtered (<0.45 µm), stored at 4°C, and analysed as soon as possible.

2.3.2 NO_3^-, Cl^- and SO_4^{2-} were estimated using ion chromatography (Dionex 2010i) under standard conditions (Rowland 1986).

2.3.3 Instructions for preparing the synthetic control sample are as follows. Dissolve separately 0.571 g NH_4NO_3, 0.955 g KCl 2.027 g $MgSO_4.7H_2O$, 0.8266 g NaCl, 0.755 g $NaH_2PO_4.2H_20$, 0.6714 g Na_2SiF_6 and 2.1980 g $CaCl_2.6H_20$. Mix together all solutions except $CaCl_2.6H_20$, then add this drop-wise with stirring and finally dilute to 1 litre and store at 1—4°C. Prepare a fresh working control sample weekly by diluting the stock 100-fold. This prescription yields elemental concentrations (mg l^{-1}) of 1.0 NH_4-N, 1.0 NO_3-N, 5.0 K, 2.0 Mg, 5.0 Na, 0.15 PO_4-P, 16.9 Cl, 2.64 SO_4-S, 1.0 Si and 4.0 Ca.

3 Results and discussion

3.1 Vegetation

In ecological research, the macronutrients are of most interest (N, P, K, Ca, Mg), with certain micronutrients (ie Mn Cr, Zn) and other elements (Na) quantified occasionally. Heather was chosen as a control sample because, at the time (early

1970s), many studies included this species and others of similar composition. However, it has become apparent that the digestion conditions for this particular material are more critical than those for most other plant materials, and in some cases the precision has been unsatisfactory. Procedures for preparing and analysing plant material were therefore re-examined to highlight potentially critical stages and sources of error relevant to the use of this control sample.

Precision tests to assess random error had been used to determine warning and action limits for use on control charts (Shewhart). For most species and elements, the coefficient of variation (CV) was better than 2%, but for heather 4 elements (Na, Fe, Cu & Zn) recorded a CV worse than 5%, and up to 30% for Cu. It is difficult to isolate individual components of random error, but the main operations were assessed in an attempt to isolate the likely causes. Subsampling of plant material can be a significant source of random error if the sample is non-homogeneous, but, as most of the elemental analysis showed acceptable precision on the sample solutions, this did not appear to be a significant problem. Dissolution of the vegetation using acid digestion is performed under carefully controlled conditions, and the heather control sample appears more resistant to oxidation than other material. However, variation in nutrient values obtained appears small even under extreme conditions (Table 1), indicating that large random errors are unlikely to occur from the normal temperature variations during oxidation. Precision levels are relatively much larger when the nutrient sample contents are low and close to the detection limit. It is, therefore, these factors which account primarily for the wider precision limits due to random error.

Table 1. 'Recovery' of macronutrients from heather using different digestion treatments

	% recovery (compared to mean)			
Low temperature	96	104	93	98
Routine method	100	106	95	100
Excess heat	99	106	95	100

Several of the sources contributing to random error can also lead to systematic error or bias. Over the years, the main sources of bias have been eliminated through improved laboratory practices and routine servicing/checking of the vital equipment. The quality control sample is included to determine when bias has occurred in a batch, although, if this control is subject to a gross random error not affecting the samples, it can provide false information. Bias arising from, say, faulty calibration or from correction for reagent blank contamination can be greater when solution concentrations are close to the detection limit or at the lower end of the analytical range. In the light of our conclusions, the use of other natural species as control samples was investigated in order to work at nutrient levels more optimal for the equipment used and for the range of materials now analysed.

Nutrient data from leaves, current year's growth or aerial tissue of 172 species from the UK sample in surveys from late July to early August over several years (H M Grimshaw & S E Allen, pers. comm.) are summarized (Figures 1, 2 & 3). For Ca 16 species and for Na 23 species have been excluded because levels were extreme. Leaves of 3 species: alder *(Alnus glutinosa)*, hazel *(Corylus avellana)* and meadowsweet *(Filipendula ulmaria)*, and leaflets of rosebay willow-herb *(Chamaenerion angustifolium)* appear to be optimal for nearly all the 10 elements, because the nutrient value of the species fell within 25–85% of the analytical range selected for the equipment. These plants are widespread in Europe and therefore potentially suitable as European reference materials. In addition, leaves of holly *(Ilex aquifolium)* and hawthorn *(Crataegus* spp.)

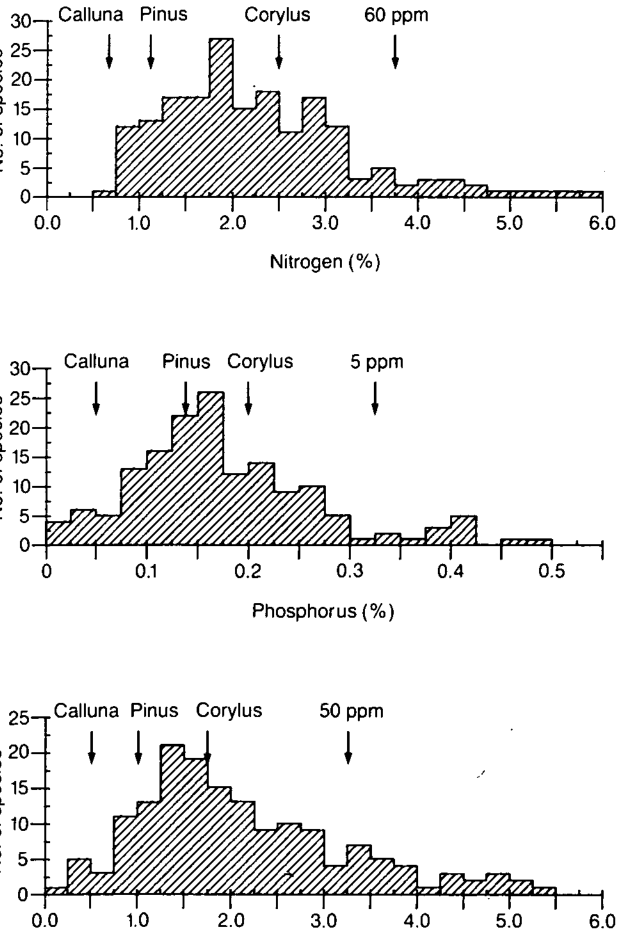

Figure 1. Frequency distributions for N, P and K in vegetation (the ppm value is the concentration in solution after dilution for analysis)

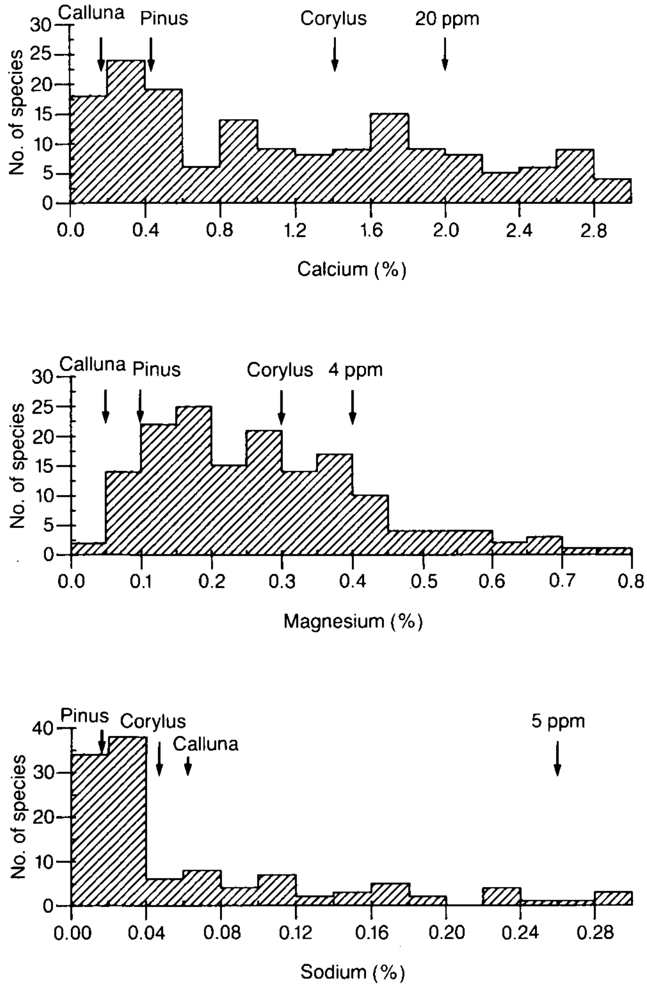

Figure 2. Frequency distributions for Ca, Mg and Na in vegetation (see Figure 1)

are suitable for N, P, K in particular, as their values for these 3 elements coincide with the modes of distribution. In practice, hazel was selected as a reference material to supplement the heather, along with pine *(Pinus sylvestris),* which is a species commonly encountered in many studies. The relative positions of heather, hazel and pine are given on the distribution diagrams (Figures 1, 2 & 3). To obtain a better indicator of analytical bias, one or both of these control samples are included with each analytical batch.

As suggested earlier, the certified plant materials currently available do not meet all the requirements of ecological work, although they are of value for specific tests. The National Bureau of Standards pine needles or citrus leaves, or the Commision of European Communities olive leaves would be the most suitable, but are low in potassium. Bowen's kale and the CEC aquatic plant are much higher in nitrogen and phosphorus than in natural vegetation in the UK, and the NBS tomato leaves are much higher in nitrogen and potassium.

3.2 Soil

The natural variation of soil nutrient data is much greater than for plant material. Because extraction values are required for most soil nutrients and totals only for N and P, and because the material is more heterogeneous, the tests are less easy to reproduce. Accuracy checks have to be through recovery and comparison tests, as there are no reference materials with certified 'available' nutrient values. The range of nutrient values in soil is so variable that it is impossible to select one ideal quality control sample to cover a wide range of soil types. At Merlewood, a local glacial drift control soil sample has been used for some years. It has a low nutrient status, eg 0.3% N, and therefore the precision for this element is poorer than for most British soils. The working control limits for operation are around ±10% for extractable nutrients and ±5% for totals.

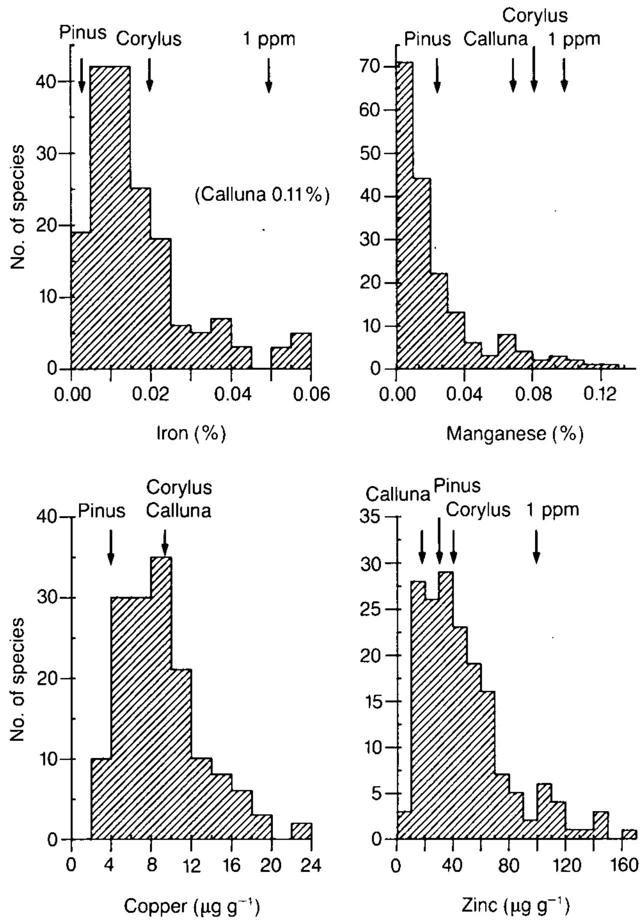

Figure 3. Frequency distributions for Fe, Mn, Cu and Zn in vegetation (see Figure 1)

Control data over a long time period supplement development work, and may be used to illustrate changes in accuracy and precision. The introduction of a modified soil digestion procedure (Rowland & Grimshaw 1985; Grimshaw 1987), together with improved sample handling techniques, have reduced the working limits from ±12% to ±5%.

3.3 Waters

A wide variety of natural waters generated by research on acid rain, geochemical and nutrient cycling, and clear-felling of forests has to be analysed chemically in the author's laboratory. Previous attempts to use control samples with natural waters were over-shadowed by storage deterioration.

Least changes occurred in samples which were filtered (<0.45 µm), stored at 1–4°C rather than frozen, and analysed without delay. However, a synthetic sample prepared from laboratory reagents (Section 2.3), and stored in concentrated form, was found to be stable if stored at 1–4°C. This control sample provides a check on bias because (unlike heather) its exact composition is known. The composition of the synthetic control solution can be adjusted to match the sample composition so that it is subject to the same systematic errors.

The synthetic control sample has been crucial in the development of ion chromatography methods. This non-specific technique has been automated to determine Cl^-, NO_3^-, and SO_4^{2-} in approximately 8000 samples yr^{-1}. The margin for error in a multi-ion technique is much smaller than that in single-ion analysis because each ion provides an independent check.

As the control sample has proven theoretical values, development problems such as verification of non-linear calibration, instrumental drift and sample carry-over are minimized. The overall error over a one-month period for each element was found to be about 1% CV.

4 Conclusion

Inter-laboratory studies sometimes produce disturbing results (Sherlock *et al.* 1985), and these authors point out that inaccurate and unreliable data rarely meet the customers' needs. Once a reliable method has been selected and tested, quality control procedures using selected materials, replication and blank determination should become accepted practice. Careful selection of the control sample and the continuity of long-term routine analysis are important to assess laboratory bias, and the data may be useful to assess method development.

5 Summary

Ecological research often involves the chemical analysis of samples over long time periods, when analytical techniques may improve or even be superseded. Therefore, it is of the utmost importance to maintain stocks of internal control materials to monitor such changes. For historical reasons, a heather *(Calluna vulgaris)* vegetation reference has been used for 20 years and, in comparison with other vegetation samples, it has proved difficult to handle. Data are presented to demonstrate the suitability of this and alternative materials for use as vegetation control samples. The importance of soil and synthetic water references are also illustrated by examples from recent nutrient and pollution studies.

6 References

Allen, S.E., Grimshaw, H.M., Parkinson, J.A. & Quarmby, C. 1974. *Chemical analysis of ecological materials.* Oxford: Blackwell Scientific.

Grimshaw, H.M. 1987. Total phosphorus estimation in soils. In: *Chemical analysis in environmental research* edited by A.P. Rowland, 92–95. (ITE symposium no. 18.) Abbots Ripton: Institute of Terrestrial Ecology.

Parkinson, J.A. & Allen, S.E. 1975. A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological material. *Commun. Soil Sci. Plant Anal.,* **6,** 1–11.

Rowland, A.P. 1983. An automated method for the determination of ammonium-N in ecological materials. *Commun. Soil Sci. Plant Anal.,* **14,** 49–63.

Rowland, A.P. 1986. Automated suppressed ion chromatography as applied to acid rain research. *Analyt. Proc.,* **23,** 308–309.

Rowland, A.P. & Grimshaw, H.M. 1985. A wet oxidation procedure suitable for total nitrogen and phosphorus in soil. *Commun. Soil Sci. Plant Anal.,* **16,** 551–560.

Sherlock, J.C., Evans, W.H., Hislop, J., Kay, J.J., Law, R., McWeeney, D.J., Smart, G.A., Topping, G. & Wood, R. 1985. Analysis — accuracy and precision. *Chem. Br.,* **11,** 1019–1021.