CHEMICAL ANALYSIS
IN
ENVIRONMENTAL RESEARCH

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INSTITUTE of TERRESTRIAL ECOLOGY
NATURAL ENVIRONMENT RESEARCH COUNCIL

**Natural
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Chemical analysis in environmental research

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INSTITUTE OF TERRESTRIAL ECOLOGY

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Preface

This symposium was the fourth in a series organized by ITE's Merlewood Research Station on topical subjects in the field of ecology. The symposia were designed to promote the work of the organization and to develop contacts and an interchange of ideas between scientists working in environmental research. We felt it was an appropriate moment to provide a forum on chemical analysis, especially to discuss the latest analytical developments and to review recent applications of more traditional methods. Techniques and instrumental methods for studying the effects of acidic precipitation dominated the meeting, but time was also given to soil and plant methods.

Investigations into the causes and effects of acidic precipitation on the terrestrial environment prompted us to focus on the methods recently developed for analysing samples from these research projects. Measurement of pH in natural solutions is fundamental to such studies, but subject to large errors when applied to solutions of low ionic concentration. Two complementary papers considered in detail the precautions and equipment required. Sulphur methods, in general, have caused problems in the past, and so it was not surprising to have several papers discussing the analysis of sulphate in waters and plant material. Aluminium species and complexation have recently been linked to acidic deposition and fish mortality in streams and lakes. Methods for fractionating the toxic labile fractions which were used to study rivers in the north of England were described. Simulation techniques may sometimes be the only way in which an individual component of the ecosystem may be studied in isolation. A review examining the techniques, advantages and pitfalls illustrated how laboratory simulation has developed for pollution research.

Some of the papers relating to acidic deposition also dealt with modern instrumental techniques. A detailed presentation was given in 3 papers on the development of inductively coupled plasma/optical emission spectrometry for trace element analysis, which also highlighted the problems in pre-concentration techniques. Various applications and techniques in ion chromatography described in 2 papers illustrated the versatility of this rapidly expanding field. Another growth area is the development of chemical sensors, and a review was given of the current status of their applications in environmental research.

Aspects of soil microbial biomass measurement were considered in 2 papers on comparative techniques, one of which assessed the effect of stress on the information obtained. An alternative approach to measuring 'available' phosphorus showed that isotope methods were extremely effective for measuring mineralization rates of phosphorus in soil. Although it is now just over 100 years since Kjeldahl first proposed a sulphuric acid wet oxidation procedure, many developments still appear regularly. Modifications of this procedure enable other elements to be determined from the acidic solution, and 2 papers described such applications. A paper was also given on the use of reference materials in quality control when analysing environmental samples.

The 2-day meeting was attended by 90 delegates who heard 16 papers, and were able to review 14 poster presentations. My thanks go to the speakers who contributed so willingly and to the presenters of the poster papers for their high-quality displays which added so much to the meeting. I especially thank Val Kennedy for her valued ideas and efficiency in organizing the symposium, and to other colleagues at Merlewood who helped. Finally, I would like to acknowledge the contribution of the chairman: Stewart Allen (Head of Station, Merlewood), Bill Davison and John Hilton (Freshwater Biological Association, Ferry House), who co-ordinated the discussion and questions.

Philip Rowland

Aluminium fractionation in fresh waters

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1 Introduction

Aluminium is the most abundant metal and the third most abundant element in the earth's crust. Aluminosilicate minerals are common in igneous and metamorphic rocks, and as clay minerals in well-weathered soils.

Although the original source of aluminium to fresh waters is, most likely, the underlying bedrock, weathering processes are slow, so most available aluminium is present in soils. Even here, aluminium is usually complexed in the lower profiles of soil, and is fairly immobile (Jacks *et al.*1984).The process by which mobilization into fresh waters takes place is complex, and not fully understood, but van Breemen *et al.* (1984) have explained the process in terms of the internal and external proton sources in soils. In acid soils, soil acidification is relatively slow; in consequence, external proton sources of anthropogenic origin may exceed internally generated protons, leading to a release of inorganic aluminium into soil solution, from where it may be flushed into streams and lakes. Here, at low pH levels, when Ca concentrations are very low, the dissolved aluminium may even replace the usual calcium/bicarbonate buffering system.

The importance of this release of aluminium is not just as an indicator of external proton sources, or the creation of an aluminium buffering system in the acid water draining the soil; the importance lies in the toxicity of inorganic aluminium to freshwater organisms, in particular fish.

Aluminium toxicity has been the subject of many investigations, and the results are as complex as the chemistry of aluminium itself. The toxic effects are dependent upon the species and the life stage of the fish, the concentration of calcium in the water, and the pH. The pH may not only affect how a fish responds to dissolved aluminium, but can have a pronounced effect upon the chemistry of aluminium itself. Driscoll *et al.* (1980) have shown that complexation of aluminium can have a marked ameliorating effect on its toxicity. It is important, therefore, to understand something of the chemistry of aluminium, in order to measure the relevant complexes in solution and so predict the consequences of these solutions in streams and lakes.

2 Aluminium chemistry

The complexity of aluminium chemistry lies not only in its ability to form a variety of soluble complexes in aqueous solution, but also in its ability to create hydroxide polymers which may grow until, ultimately, microcrystalline minerals result.

At low and high pHs, there is general agreement on the chemical species of aluminium predominating in solution. At pH$>$7.5, the aluminate anion complex $Al(OH)_4^-$ is the major ion in solution, whilst at pH$<$4 the hexa-aquo-Al(III) ion $Al(H_2O)_6^{3+}$ dominates (Smith 1972). Between these 2 pHs, there is likely to be little agreement on the exact concentrations of chemical species present for any given set of chemical conditions, because there is disagreement on the exact values of the relevant equilibrium constants. Assuming values for equilibrium constants between the different chemical species enables their concentrations to be predicted. Smith (1972) has done the calculation for the Al/hydroxide system over the pH range 3.5–7.0 (Figure 1). It is clear that at pH 5 several species have similar concentrations. Therefore, any uncertainty in the equilibrium constant values may have a marked effect on the relative concentrations of aluminium species. Calculations are further complicated when fluoride and organic chelates, which are found in fresh waters and effectively compete with hydroxide, are included.

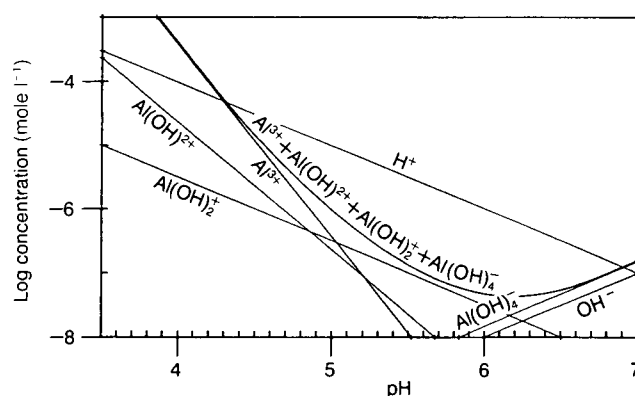*Figure 1. Aluminium concentration against pH (Smith 1972)*

Although we may not be able to define the composition of an aluminium solution in precise terms, this definition may not be necessary. If our aim is to evaluate the biological impact of a given solution, a much more simplified approach may be used.

Driscoll (1980) has defined 3 'types' of aluminium species in solution (Figure 2). These types have

been shown to have biological significance, as well as being defined fairly clearly in chemical terms. *Inorganic monomeric aluminium* is the chemically labile form of aluminium which has been correlated with toxic effects on fish (Driscoll 1980). It does, however, include fluoride complexes which are less toxic. *Organic monomeric aluminium,* the non-labile monomeric aluminium, is far less toxic than the inorganic species. It is complexed, fairly strongly, to organic molecules, often of large molecular weight. *Acid soluble aluminium* is a mixture of polymeric species and strongly bound organic complexes. It is considered non-toxic. The 3 fractions together represent the total (reactive) aluminium in solution.
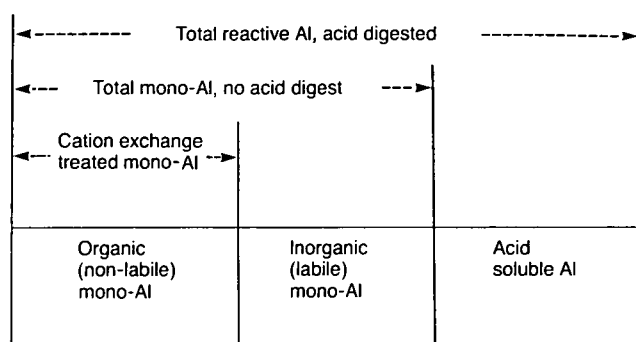
Figure 2. Summary of an aluminium fractionation procedure (Driscoll 1984)

3 Total reactive aluminium analysis

There are 2 major requirements for an analytical method suitable for total aluminium, indeed suitable as an analytical method for any aluminium species in fresh water. First, the method must be sensitive: aluminium is generally found in concentrations of <1 mg l^{-1} in fresh waters. Second, the method must be free from interferences. Unfortunately, conventional atomic absorption spectrometry (AAS) is not very sensitive, and many colorimetric methods are prone to interference.

Driscoll's early work on aluminium speciation (Driscoll 1980) was done using the ferron-ortho-phenanthroline colorimetric method (Rainwater & Thatcher 1960). By acidifying samples to 0.1 N with HCl, a 'cold acid digest', the total reactive aluminium is changed to monomeric form. This form reacts readily with the ferron reagent. The method is not very sensitive, so large cuvettes are needed. The method also suffers from interferences, particularly from dissolved iron and colour. Corrections are, therefore, needed, adding to errors and increasing analysis time.

Improved sensitivity and reduced interference problems are obtained with a method using catechol violet as the complexing agent (Dougan & Wilson 1974). Sensitivity is almost an order of magnitude greater, and iron interference is suppressed with hydroxyl-ammonium chloride and 1,10 phenanthroline. Colour correction is usually minimal as the catechol complex absorbs at 585 nm compared with 270 nm for ferron. The method is applied to acidified samples as above. Its main practical disadvantage is the increase of optical absorption that occurs with the catechol violet complex with time. An initial rapid absorption is complete within the first few minutes, but a further slow increase continues for many hours. The rate of increase is sufficiently slow and predictable, however, so that consistent results are obtained by waiting for a fixed time interval before absorption is measured. Times ranging from 4 to 40 minutes have been used by different workers.

The ferron and catechol methods have been compared by Seip *et al.* (1984), using some Norwegian freshwater samples. The agreement is very good, but they recommend the catechol method because of its sensitivity and lack of interferences. These findings are confirmed by work in our own laboratory.

Flameless atomic absorption spectrometry (FAAS) and inductively coupled plasma (ICP) spectrometry both give greatly improved sensitivity over conventional AAS. ICP equipment is, however, expensive and is still not available to many laboratories. FAAS does offer a reasonable alternative to colorimetry; under suitable graphite furnace conditions, the method is almost as sensitive as the catechol method and is relatively free from matrix effects (Campbell *et al.* 1983; ITE Merlewood, pers. comm.).

It may be expected that not all polymeric aluminium detected by ICP or FAAS would be measured using the 'cold acid digest' of the colorimetric methods. However, Bull and Hall (1986) have found that FAAS and catechol give very similar results for the same samples. Furthermore, Seip *et al.* (1984) observed only a 6% increase in aluminium concentration when acidified solutions were boiled with $K_2S_2O_8$ prior to catechol violet analysis.

Recently, Driscoll (1984) and LaZerte (1984) have used an extraction procedure developed by Barnes (1975), which effectively concentrates the aluminium in solution and may be used to prevent interferences. The aluminium is first coupled with oxine (8-hydroxyquinoline), the pH of the solution is next adjusted to 8.5, and then the oxine complex is extracted with methyl isobutyl ketone. To ensure polymeric aluminium is extracted, a reaction time of 3–6 hours is used (Turner 1969). Alternatively, water samples are acidified to pH 1 for one hour before analysis (Driscoll 1984). The oxine extract may be stored in a freezer until required for analysis. The extract may be analysed colorimetrically (at 390 nm) or, to avoid interferences, FAAS may be used.

4 Monomeric aluminium analysis

We have already described above the 2 types of monomeric aluminium — inorganic and organic, but it is also important to know the concentrations of each, as the toxicity of the organic fraction is much lower (Driscoll 1980). Most laboratories use a 2-step analysis procedure. First, the total monomeric aluminium concentration is measured. Next, either the inorganic or organic fraction is measured and the third fraction found by difference. A different method, used by Campbell et al. (1983), is described later.

Total monomeric aluminium analyses make use of the reactive nature of these species in unacidified solutions. The methods rely upon the speed with which these compounds react with a chelating agent. The ferron analysis (Driscoll 1980) is applied directly to unacidified samples, and the absorption measured soon after adding the agents. If the reaction time is short, polymeric aluminium has no chance to react. Seip et al. (1984) compared the ferron method with a similar approach (unacidified samples and short reaction time), using the catechol violet method. The results were comparable, though again the catechol violet method was recommended because of its sensitivity and lack of interferences. However, using a short reaction time presents some problems because the colour development time usually recommended is 10–30 minutes (Dougan & Wilson 1974). Seip et al. found that a reaction time of 4 minutes was sufficient for colour development but avoided reactions with aluminium polymers. Bull and Hall (1986) have used a reaction time of 10 minutes, which Seip et al. suggest gives an over-estimation of 2–3% in the monomeric aluminium due to depolymerization.

The oxine extraction procedure (Barnes 1975) may also be applied to measuring total monomeric aluminium. Now, however, a rapid extraction is substituted for a short colour development time. Driscoll (1984) has used this technique, whilst LaZerte (1984) has modified the method to give an extraction time of about 15 seconds.

Organic monomeric aluminium concentrations may be measured by remeasuring monomeric aluminium in solutions from which the inorganic fraction has been removed. Driscoll (1980) has shown that the inorganic, labile, fraction binds rapidly to a suitable ion exchange resin, leaving the non-labile, organic, fraction in solution. Passing a freshwater sample containing aluminium through a column of ion exchange resin effectively removes the inorganic monomeric species, leaving only organic monomeric aluminium in the eluate. Driscoll used 9.5 ml of Amberlite IR-120 resin in a 1 cm^2 cross-section column. A flow rate of at least 35 ml min^{-1} was needed. The resin, in the hydrogen form, was pre-treated with dilute NaCl solution so that eluted samples retained their original pH value. The same procedure has been used by other laboratories (Seip et al. 1984; Bull & Hall 1986). The organic monomeric aluminium may be measured using one of the methods described above for total monomeric aluminium, while inorganic monomeric aluminium is calculated by difference. Seip et al. report that direct colorimetric measurements using either ferron or catechol give comparable results. Driscoll originally used the ferron method (Driscoll 1980), but more recently has used the Barnes extraction on eluted samples (Driscoll 1984). Organic monomeric aluminium was measured in a different way by Campbell et al. (1983). They used fractionally loaded Chelex-100 resin in a batch technique (Laxen & Harrison 1981) to remove inorganic monomeric aluminium from solution. However, under the conditions used, low molecular weight polymers are also retained by the resin. This technique has the advantage that the organic fraction can be measured using a method for total aluminium. Campbell et al. have used FAAS. However, without knowing the concentration of total monomeric aluminium, the inorganic, toxic, fraction cannot be estimated. Campbell et al. did not make such measurements directly but used equilibrium calculations (see below).

Inorganic monomeric aluminium may be separated and measured directly using dialysis (LaZerte 1984). The small, mobile, inorganic ions pass readily through a dialysis membrane, whilst the larger, less mobile, organically bound aluminium is retained. Again, any convenient method for total aluminium may be used on the dialyte.

Individual aluminium species have been calculated by several workers using thermodynamic data. Early work by Hem and Roberson (1967) and Smith (1972) on hydroxide complexes in synthetic solutions has been extended by other workers to include other complexes in natural fresh waters.

Equilibrium calculations show that only Al^{3+}, hydroxide and fluoride complexes are present in most natural waters in significant concentrations (Hem 1968). Sulphate complexes are often included in the calculations but are usually of little importance (Seip et al. 1984). Concentrations of fluoride complexes may be found by calculation using fluoride levels measured with a fluoride selective electrode, and the appropriate equilibrium constants (Seip et al. 1984; LaZerte 1984; Driscoll 1984). Fluoride measurements also provide the means of calculating inorganic monomeric aluminium concentrations, as long as AlF^{3-} species dominate the total fluoride (LaZerte 1984; Driscoll 1984). Drummond (1984) reports on the work of Turner who has compared literature values of equilibrium constants. Turner notes that

fluoride constants are better characterized than those for hydroxide. Whilst hydroxide complexes predominate at high pHs, fluoride complexes are more important at low pHs. Although there is no direct evidence, on statistical grounds mixed fluoride/hydroxide complexes should be possible. The limitations of thermodynamic calculations are illustrated by an example by Hunt, also reported by Drummond (1984). Using 2 'alternative' sets of equilibrium constants, Hunt shows that the 2 sets of ion ratios resulting from his calculations give 2 different interpretations to a series of toxicity tests on algae. Further information on aluminium complexes has been obtained by the work of Tipping (Drummond 1984), who has used kinetic studies to look at aluminium species. He has identified a less labile fluoride complex at pH 4–4.3, which he suggests is AlF_2^+.

Individual organic monomeric aluminium species are even more difficult to characterize because of the vast range of humic substances present in fresh waters (Gjessing 1967). Drummond (1984) reports work by Lee who concludes that most complexation in Lake Gardsjon is caused by low molecular weight fulvic acids. Other work (Water Research Centre, pers. comm.) has shown that aluminium complexation with organics extracted from natural waters is very different from that complexed to commercially available humic and fulvic acids. It is perhaps sufficient to conclude that we know organic monomeric aluminium has a much reduced toxicity and is non-labile, and that the best approach for measuring its concentration at present is to use a purely practical means, such as that suggested by Driscoll.

5 Sample collection and pre-treatment
There are risks that concentrations of ions in solution may change between collection of a sample and its analysis, giving a false picture of what was present in the original sample. This is particularly true for aluminium species which are sensitive to pH changes.

One of the first decisions to be made is whether to filter water samples. Some workers have avoided filtration where possible, because many acid waters are low in suspended solids and direct measurements are made of monomeric species (Driscoll 1984; Bull & Hall 1986). Avoidance is not always possible with natural samples. Drummond (1984) reports that Wells has used glass fibre filters which he has tested satisfactorily with spiked samples. He also summarizes work by Salbu, who found it necessary to filter samples through a 0.45 μm filter in order to obtain reproducible results using neutron activation analysis, which presumably measures both dissolved and solid aluminium together. Campbell et al. (1983) have tested 0.4 μm filters in a polycarbonate

apparatus and found no contamination or loss of analyte. Nevertheless, if pH changes are caused by filtration, some shift of species may occur.

Samples for total aluminium analysis samples can be 'fixed' soon after sampling by acidifying them to 0.1 N. These solutions remain stable for many weeks.

Monomeric aluminium is not so easily 'fixed' in aqueous solution. If solutions are being stored for analysis, they should be kept tightly stoppered with minimal air space, at, or near, the temperature when sampled. Solutions should be analysed as soon as possible, though we have found some samples are stable for 2–3 weeks or longer. The rapid extraction of oxine into MIBK offers a method for on-site extraction of monomeric aluminium (LaZerte 1984; Drummond 1984). The organic solution is stable for several months, if stored at low temperature.

Organic monomeric aluminium may also be separated in the field using Driscoll's ion exchange column. If followed by the oxine/MIBK extraction, concentrations can be fixed for future analysis. Otherwise, care is needed to prevent equilibria shifts whilst samples are transported to the laboratory and stored. We have found unacidified samples transported by car from Cumbria to Huntingdon and then passed through the ion exchange column show only small increases in organic complexation, compared with samples columned on-site. Such increases, which are not found with all samples, may be due to pH changes or to equilibrium of a non-equilibrated aluminium/organic mixture collected in the field.

6 Aluminium concentrations in the Rivers Esk and Duddon
In our studies of the Esk and Duddon and their tributaries, we have measured total, and inorganic and organic monomeric aluminium concentrations. We have not done any detailed calculations on fluoride species, though the total fluoride concentrations measured are low.

In the River Esk (Table 1), we find that lower pHs and higher aluminium concentrations are found in the upper reaches of the river. Downstream, pH values are higher and aluminium levels fall. The concentrations of organic monomeric aluminium have generally been low throughout the Esk under all the conditions sampled. Where the total aluminium levels are high, usually the proportion of inorganic monomeric aluminium is also high and the pH low. At higher pHs, the inorganic monomeric aluminium levels are, not surprisingly, lower. The acid soluble aluminium levels also fall, but not as quickly. Some of the aluminium losses may be explained by dilution of the river water by non-acid tributaries with low aluminium levels.

Most of the losses, however, must be due to polymerization of inorganic monomeric aluminium to acid soluble polymers, followed by further polymerization to 'insoluble' aluminium. Absorption, too, is likely to occur during the polymerization process.

(1982) have suggested that such conditions are highly toxic to fish. It may be that similar conditions occur in other parts of the Rivers Esk and Duddon on occasions, with important biological consequences.

Table 1. Al (μg l^{-1}) in River Esk — moderate flow

Distance upstream (km)	pH	'Total' Al	Mono-Al Organic	Inorganic	(%)
0	6.2	70	—	10	(14)
5.6	5.5	220	—	110	(50)
7.4	5.2	260	5	225	(86)
8.6	5.1	310	20	260	(84)
13.7	4.8	370	—	250	(68)

The tributaries of the Esk and Duddon vary considerably in their chemistry, both geographically and with time. Thus, in the Duddon (Table 2), we find that the upper reaches are less acid than the middle reaches of the river. Some tributaries in the upper Duddon are unexpectedly less acid because of the geology of the catchment bedrock (Sutcliffe & Carrick 1973). In contrast, other tributaries, further down the valley, some of which drain afforested catchments, are much more acid and contain higher aluminium concentrations (Bull & Hall 1986), as reflected in the chemistry of the main river.

7 Summary

Aluminium chemistry in fresh waters is complex; the metal may exist in a variety of different species, largely dependent upon the pH of the solution and dissolved anions present. Because different aluminium species have different toxicities to biota, it is important that some estimation of the species present is made. We have discussed some of the procedures used by various workers to determine aluminium concentrations, together with findings of our own. Some results for the Rivers Esk and Duddon, Cumbria, are used to illustrate the concentrations of aluminium which may be found in running waters.

Table 2. Al (μg l^{-1}) in River Duddon — moderate flow

Distance upstream (km)	pH	'Total' Al	Mono-Al Organic	Inorganic	(%)
0	6.7	80	—	—	—
3.5	6.0	150	—	20	(13)
7.9	5.0	360	20	170	(47)
12.0	5.3	190	30	100	(53)
13.8	5.6	90	10	40	(44)

Some tributaries show dramatic changes in their chemistry over quite short distances. Dodknott Gill, in the middle reaches of the Esk, drains from Harter and Birker Fells. Under most conditions, the water running from the fell-side (Figure 3, site A) is fairly acid, pH 4.5—5.2. Between the fell and the confluence with the Esk (Figure 3, site D), the tributary flows through several hundred metres of improved pasture land. It receives water from land drainage and from springs along this stretch; both sources are more alkaline than the gill itself. As a result, pH, calcium levels and alkalinity increase with distance downstream, whilst aluminium concentrations fall (Figure 3). An important consequence of these changes is likely to be a condition of super-saturation of aluminium along some stretches of the gill. Baker and Schofield

Figure 3. Aluminium, calcium and pH levels in water collected from 4 points (A, B, C, D) along Dodknott Gill, Cumbria

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The determination of total sulphur in vegetation

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1 Introduction

The standard method traditionally used in ITE's Merlewood Research Station chemical laboratories for estimating total sulphur in vegetation has been a dry ashing/barium sulphate turbidimetric procedure based on Butters and Chenery (1959). Over the years, this method has proved to be reliable and reproducible. However, it is tedious and time-consuming for routine use when large numbers of samples are involved. Throughput can be increased by replacing the dry ashing stage with a nitric/perchloric acid digestion, but this technique has been found to have limitations with some types of sample. A better alternative is X-ray fluorescence (XRF), which is both rapid and convenient. The sample matrix is largely cellulose and the spectrometer can, therefore, be calibrated by using, as standards, samples which have been carefully analysed by a suitable chemical method, such as the standard one mentioned above.

During a calibration exercise of this kind, it was found that X-ray fluorescent intensity of the sulphur Kα line was highly correlated with the total sulphur content of the specimen used. However, for 2 species, dog's mercury *(Mercurialis perennis)* and bracken *(Pteridium aquilinum)*, the relationship did not hold, and further tests revealed errors in the chemical analysis results for these 2 species. Therefore, various chemical procedures were investigated for the analysis of a wide range of vegetation types, with a view to finding a suitable method for XRF calibration.

2 Materials and methods

A selection of air-dry, ground (0.7 mm) samples of vegetation were obtained covering several different species, including dog's mercury and bracken (Table 1).

Four chemical methods for the determination of total sulphur were compared, involving 4 different oxidation procedures and 2 methods for determination of the sulphate-sulphur formed on oxidation.

i. The standard dry ashing/barium sulphate turbidimetric procedure based on Butters and Chenery (1959). The full procedure is described by Allen *et al.* (1974).

ii. Wet oxidation/barium sulphate turbidimetric, in which the sample is oxidized with a mixture of nitric and perchloric acids in the presence of vanadium (Mottershead 1971).

iii. Schoniger flask oxidation followed by ion chromatography (Busman *et al.* 1983). In this study, chemically supressed ion chromatography was carried out on a Dionex (model 2010i) chromatograph using an AS4A column operated under the manufacturer's standard conditions.

iv. Oxygen bomb oxidation, followed by ion chromatography. This procedure was a development from method 3, in which the oxidation was carried out under pressure (20 atmospheres of oxygen). The bomb used was part of a conventional adiabatic bomb calorimeter (Gallenkamp & Co, Model CB-100). The samples were pressed into pellets, 13 mm diameter, and placed in a silica crucible designed

Table 1. Samples used in comparison tests

Sample	Site	Species	Component
1	Merlewood	Dog's mercury	
2	Oxenthwaite	*(Mercurialis perennis)*	Above-ground
3	Merlewood		growth
4	Humphrey Head	Meadowsweet	
5	Oxenthwaite	*(Filipendula ulmaria)*	Leaves
6	Angerham		
7	Merlewood	Rosebay willow-herb	
8	Lindale	*(Chamaenerion augustifolium)*	Leaves
9	Hampsfell	Bracken	
10	Lowick	*(Pteridium aquilinum)*	Fronds
11	Kirkby Moor	Heather	Above-ground
		(Calluna vulgaris)	growth

for use with the apparatus. The crucible was positioned in the bomb, pressurized with oxygen and fired according to the manufacturer's instructions, except that 25 ml water was added to the pressure vessel initially. After firing, 15–20 minutes were allowed before releasing the pressure. The walls and cap of the bomb were washed and the washings added to the 25 ml water already present. After dilution of the solution obtained to 50 ml, sulphate was determined by ion chromatography.

v. The samples were also analysed by XRF using the calibration mentioned earlier, but excluding the results for the samples which gave trouble in the chemical analysis.

3 Results and discussion

The species used in the comparison tests were chosen because low recoveries of total sulphur had been obtained with the dry ashing procedure. With the exception of dog's mercury, all species proved difficult to ash. Nevertheless, the presence of unburnt carbon at the filtration stage did not necessarily result in significantly low recoveries of sulphur. In fact, attempts to ensure complete combustion by extending the time in the furnace often caused more serious losses of sulphur. Possible recovery problems other than incomplete ashing is borne out by the dog's mercury sample, which, although invariably completely ashed, often gave inconsistent values of sulphur content. Reproducible results could only be obtained by controlling closely all stages of the ashing procedure for each individual determination. Such control is tedious but necessary.

The results of the comparison tests are given in Table 2. It was noticeable that the samples which were difficult to ash by dry ashing were also difficult to oxidize by wet digestion. The latter method is known to give low recoveries when

used to oxidize methionine alone (Randall & Spencer 1980; Bethge 1956) and this has been confirmed for the Mottershead procedure (H M Grimshaw, pers. comm.). Two species, meadowsweet (Filipendula ulmaria) and rosebay willowherb (Chamaenerion augustifolium), always gave low and variable results with the wet digestion procedure, despite several attempts and careful attention to the digestion procedure. No attempt was made to determine whether these samples were particularly high in methionine, but the wet digestion procedure is unsuitable in these cases.

The Schoniger oxidation resulted in low recovery of S in most cases. Incomplete combustion was a contributory factor, as there was almost always a residue of unburnt carbon in the platinum basket used to hold the filter paper containing the sample. In addition, the procedure requires some manual skill and practice to obtain good results. The figures given are, therefore, not a true reflection of the potential of the method, and further work would probably have overcome the problems. During the course of the study, however, the Schoniger oxidation was superseded by the oxygen bomb combustion, which was immediately more successful, and work on oxygen flask combustion ceased at that point.

The products of combustion in oxygen tend to remain associated with the water formed during the burning process (Mott & Parker 1958) and, as a result, a large part of the sulphur is condensed on to the walls and lid of the vessel as sulphuric acid, rather than being absorbed by the added water. It is, therefore, important to wash down the walls and lid of the container to ensure that all the sulphate produced is determined by ion chromatography. This was found to be the main precaution necessary in the bomb oxidation procedure. Otherwise, the combustion of the sample pellet proceeded smoothly and the addition of a firing aid was not necessary, as no car-

Table 2. Comparison of results for total S (%) in vegetation by various methods

Sample number	Dry ashing	Wet oxidation	Schoniger oxidation	Bomb oxidation	XRF
1	0.348	0.335	0.312	0.328	0.331
2	0.419	0.407	0.382	0.397	0.400
3	0.298	0.310	0.194	0.309	0.313
4	0.132	0.114	0.122	0.123	0.122
5	0.136	0.088	0.113	0.122	0.119
6	0.157	0.107	0.165	0.145	0.143
7	0.138	0.119	0.164	0.153	0.154
8	0.158	0.106	—	0.145	0.137
9	0.366	0.343	0.211	0.344	0.347
10	0.225	0.220	0.190	0.239	0.217
11	0.130	0.134	0.112	0.129	0.132
Mean	0.228	0.207	0.197	0.221	0.220

Results are means of duplicates, except for sample 11 which are day-to-day running means of heather laboratory reference sample (see text and Table 3)

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bonaceous residue remained in the crucible.

The sample means for each method indicate that lower results were obtained for wet digestion and Schoniger oxidation flask than for the other procedures, for the respective reasons given earlier. Statistical examination of the data from the other 3 procedures showed no significant differences between them (P<0.05). However, the bomb oxidation figures agree more closely with those of XRF than do the data from dry ashing, which suggests either better precision for the bomb oxidation method, or a slight positive bias in this set of dry ashing results.

A reference material of heather *(Calluna vulgaris)* (Rowland 1987) was used to assess the long-term reproducibility of the different procedures and also to check whether there were significant differences between them. The data are summarized in Table 3. The dry ashing and wet digestion data were obtained over a period of several years, the Schoniger and bomb oxidation results over a few weeks, and the XRF values over one year. As might be expected, the XRF procedure is the most precise (coefficient of variation (CV) 1.7%) followed by the bomb oxidation method (CV 4.1%). The other methods all had coefficients between 5% and 10%. Excluding the Schoniger oxidation, the means from the other procedures were not significantly different from each other (P<0.05). The Schoniger mean was significantly different from all the rest, for the reasons given earlier. If a wider range of species were analysed, then it is probable that the wet digestion would also show significant differences, but this procedure appears to be satisfactory for heather.

be confirmed using other certified reference vegetation samples.

Table 4. Analysis of NBS-SRM-1575 pine needles

	% S
Quoted value (Alvarez & Uriano 1985)	0.117
Value obtained by XRF	0.113
Value obtained by bomb oxidation	0.114

4 Conclusions
Both dry ashing/barium sulphate turbidimetry and bomb oxidation/ion chromatography appear to be suitable methods for calibration in X-ray fluorescence, which is rapid and reliable for the determination of total sulphur in vegetation. The bomb oxidation procedure is more precise and more convenient, and is therefore to be evaluated further with a wider range of species. The wet digestion/barium sulphate turbidimetric procedure is not recommended because it is unreliable for some species. Schoniger flask oxidation/ion chromatography requires some manual dexterity, but would probably be satisfactory if the necessary skill was acquired.

5 Acknowledgements
The author would like to thank Liverpool Polytechnic students, A G Geraghty and A O'Shea, for help with this project, during their period of industrial training at ITE's Merlewood Research Station.

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Table 3. Results of multiple analyses of heather reference sample for total S

	Dry ashing	Wet oxidation	Schoniger oxidation	Bomb oxidation	XRF
n	30	35	7	7	12
Mean (%)	0.130	0.134	0.112	0.129	0.132
SD	0.0096	0.0131	0.0111	0.0053	0.0023
(CV)	(7.4)	(9.8)	(9.9)	(4.1)	(1.7)
SE	0.0018	0.0022	0.0042	0.0020	0.0007
95% confidence limit	0.0037	0.0044	0.0103	0.0049	0.0015

The only standard reference material available in the laboratory at the time of writing was National Bureau of Standards (NBS) SRM-1575 pine needles. This sample is not certified for sulphur content but a recommended value of 0.117% has been published by Alvarez and Uriano (1985). This material was analysed by the 2 most precise procedures as a check on accuracy (Table 4). The figures show that both the bomb oxidation and XRF methods are accurate, but this fact needs to

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Rain simulation in acid rain research — techniques, advantages and pitfalls

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1 Introduction

Simulation of precipitation was being used extensively in environmental studies long before acid rain research became popular, particularly in soil erosion studies, in investigations of biogeochemical cycling, and for quantifying the weathering-induced deterioration of construction materials such as plastics. The designs typically employed in the laboratory and under field conditions have been reviewed briefly in the literature (eg Chow & Harbaugh 1965; Chevone *et al.* 1984). Over the past decade, simulators have often been employed in attempts to elucidate mechanisms for interactions of acidic mist, rain and even snow with various components of the soil/plant/water ecosystem, either in isolation or collectively. Aspects investigated include the leaching of nutrients from seedlings exposed to acid mist (Wood & Bormann 1975; Scherbatskoy & Klein 1983), visible foliar injury through acidic rain (Evans & Curry 1979), deterioration of agricultural crop yields and quality (eg Rebbeck & Brennan 1984; Rathier & Frink 1984; Lee *et al.* 1981), and the effects of acid precipitation upon decomposition of organic matter (Abrahamsen *et al.* 1980), microbial activity and microbial respiration (eg Strayer & Alexander 1981). Attempts have been made to simulate acid rain in microcosms (Kelly & Strickland 1984) and, more recently, both rain and snow in complete enclosed ecosystems (Wright 1985).

The aim of this presentation is to summarize the advantages of simulation techniques in acid deposition research and to give an insight into the types of problems which may be usefully solved. The limitations of such techniques, and some of the pitfalls which may be encountered, perhaps unexpectedly, in short-term and long-term experiments, both in the laboratory and in the field, are also discussed. Finally, the design and construction of some simulators are briefly but critically appraised.

2 The advantages and disadvantages of simulated precipitation

In a review of the effects of acid rain on soil, Tabatabai (1985) was critical of the fact that most allegations on the impact of acid rain upon soils and crops were based upon hypothetical or theoretical considerations, or the results of experiments with synthetic acid rain under conditions that were so extreme that they were unlikely to occur in practice, even in grossly polluted areas.

Two criticisms, in particular, are often appropriate:

i. the precipitation may be too acid (eg *c* pH 3 or even more acidic) for meaningful extrapolations to be valid;

ii. the precipitation may be added routinely at a rate far in excess of reality — in extreme cases, for example, a week's rainfall may be added in minutes from a watering can.

It should be stressed that the criticism is not directed at simulation *per se*, but rather at inadequately realistic simulation. Provided sufficient attention is paid to detail at the experimental design stage, artificial rain may be a valuable exploratory tool.

2.1 The provision of suitable controls

Regardless of which component of an ecosystem is being studied, it is imperative to have suitable control materials/situations for reference purposes so that meaningful interpretation of the data obtained is possible. Provision of adequate controls is perhaps the greatest difficulty to be overcome in much acid rain research based upon field observations. For example, although it may be relatively simple to find 2 field sites with similar vegetation type and age, only one of which is subject to serious pollution, variations may still occur in climate, physical and chemical properties of the soils, physiological growth stage, fungal or other attack, and nutrient status of the plant species present.

On the other hand, if simulation is used, virtually all of the other variables, apart from deposition acidity, may be effectively eliminated, or the importance of any one of them may be studied independently of the others. Variability sometimes may be even further reduced, if deemed desirable, by employing clonal plant materials.

2.2 Study of leaching of nutrients from vegetation

The extent to which nutrient cations are leached from vegetation depends upon characteristics of both vegetation and precipitation. Clearly, plant species, physiological growth stage, nutrient status, freedom from disease or other attack, and possibly even a number of associated soil parameters, may be significant (Cresser *et al.* 1986). As far as precipitation characteristics are concerned, solute chemistry (including acidity) and rainfall intensity and duration are certainly important. Rainfall droplet size range may also be rele-

vant, and so may be various climatic factors, because they regulate the time foliage contacts the water. Climate between precipitation events may substantially influence dry deposition-derived contributions. This high number of variables complicates meaningful comparisons of data from diverse field sites, and suggests that simulation may be beneficial, provided that precipitation may be added under realistic conditions.

Such an approach has allowed the authors to clarify the importance of the ion exchange mechanism for acid neutralization by 8-year-old Sitka spruce (Picea sitchensis) trees (Skiba et al. 1986), using a simple and inexpensive rain rig described later. Work with fresh branches from near the top of 30-year-old trees showed similar mechanisms, as did work with small twigs kept in water and subjected to acidic mists. Older spruce tends to have an acidifying effect, however (Miller 1984), suggesting that only limited extrapolation is advisable with respect to plant age. Because of the many variables which may affect throughfall chemistry, extrapolation from laboratory to field conditions may be very difficult on a quantitative basis. Simulation in this instance is best seen as a technique for elucidation of mechanisms. Certainly, however, laboratory simulation results obtained by the authors with heather (Calluna vulgaris) and sphagnum have later been ratified in the field in terms of behaviour patterns. Vegetation freeze/thaw cycles may be quite common in the field, but they are rarely even considered when simulated conditions are employed.

Precipitation composition is generally (but not always, see eg Kelly & Strickland 1984) kept constant when simulators are used, although work by the authors (Edwards et al. 1984) and others has shown that acidity may reach a maximum soon after the onset of a storm as a result of sub-cloud scavenging of acidifying pollutants. Short, light storms may therefore give very acidic water, which is intercepted substantially by the vegetation and may not reach the forest floor. Simulation techniques suggest significant neutralization of such acidity by Sitka spruce (Skiba et al. 1986). Such processes must be borne in mind when the fate of acid inputs is being considered. Changes in acidity with time may be readily simulated. Some typical results showing the corresponding changes in throughfall chemistry with time under young Sitka spruce trees are shown in Figure 1.

2.3 Scope for examination of effects of different acidifying pollutants

Because the solute chemistry of the simulated deposition is controllable and may be constant if required, it is possible to examine the effect of different pollutants, either individually or as mixtures. For example, the authors have compared
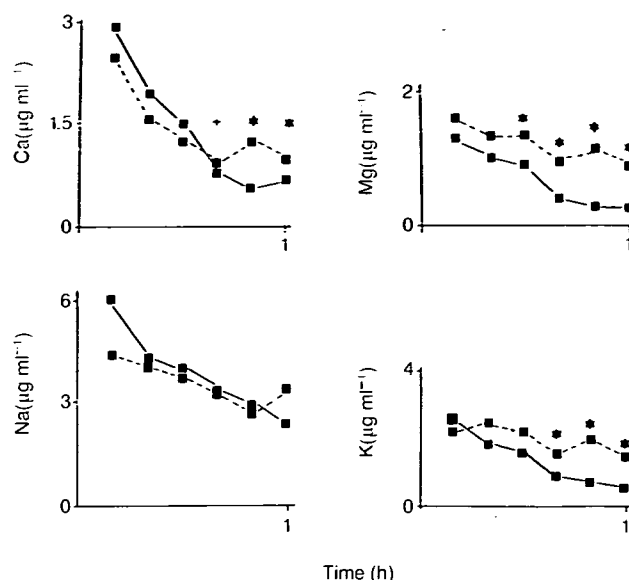
Figure 1. Effect of simulated rain on leaching of Ca, Mg, Na and K from Sitka spruce branches at pH 3 (■-■) or at pH 3, pH 4 and pH 5 for 20 minutes each (■——■). ★ difference significant at P<0.01; + difference significant at P<0.1
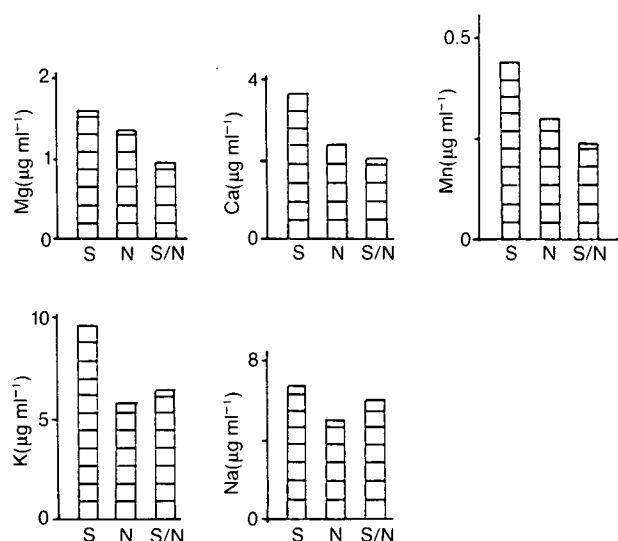
Figure 2. Effect of 'rain' acidified to pH 3.5 with $H_2SO_4(S)$, $HNO_3(N)$ or an equinormal mixture of both (S/N) upon leaching of cations from young Sitka spruce trees

the throughfall chemistry of Sitka spruce trees subjected to simulated rain acidified with H_2SO_4, HNO_3 or an equinormal mixture of both. Calcium, K^+ and Na^+ leaching from trees exposed to H_2SO_4-acidified rain was significantly (P<0.1) higher than from trees exposed to HNO_3-acidified rain (Figure 2). The differences in cation leaching by rain acidified with both HNO_3 and H_2SO_4 or with HNO_3 only were insignificant (Skiba et al. 1986). Compared to non-acid rain (pH 5.3), rain acidified with H_2SO_4 to pH 3.5 increased the leaching of cations from Sitka spruce trees to a significantly greater extent than rain acidified with HNO_3 or H_2SO_4/HNO_3. In a further experiment,

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Sitka spruce twigs were exposed to simulated mist acidified to pH 3.5 with either H_2SO_4 or HNO_3 on 5 consecutive occasions, followed by washing with distilled water. The sulphuric acidified mist caused significantly more leaching of Mg, K and Na from the twigs (Table 1). Calcium leaching was not significantly different when applying H_2SO_4 or HNO_3 acidified mist (Skiba *et al.* 1986).

Table 1. Leaching of cations from Sitka twigs by acid mist

Concentration in eventual leachate	Mist acidified to pH 3.5 with:		
(μeq l^{-1})	H_2SO_4		HNO_3
Ca^{2+}	55		66
Mg^{2+}	10	*	5.8
K^+	3.3	*	0.26
Na^+	129	*	96.8

Means of triplicates, * $P<0.05$

2.4 Scope for examination of effects of pollutant concentration

With precipitation simulators, it is much simpler to examine the effects of pollutant acid concentration upon the parameter under study. Inadequate elimination of effects of other variables may make this examination impossible in conventional field experiments. This fact may be particularly important if an attempt is being made to establish whether there is a threshold acidity below which a particular effect is not observed.

2.5 Geographical convenience and ease of maintenance

Whether in the field or in the laboratory, routine use of simulators may be much more convenient than regular visits to a range of geographically remote field sites. Initial construction/installation costs may soon be recouped, and more frequent sampling programmes may be feasible. Maintenance costs are reduced and vandalism risks may be eliminated.

2.6 Use of simulation in pilot studies

Simple and rapid experiments with simulated precipitation may provide much useful information for planning field experiments, and thus avoid the establishment of long-term field studies in which results will be of limited value through inadequate sampling strategy or replication of sites, or a poor choice of sites.

2.7 Use of artificial rain for confirming or refuting predicted mechanisms

Where statistical analysis of field data reveals correlations between chemical parameters and/or relationships between chemical and physical parameters, simulation techniques may often be employed to show whether the relationship between any pair of variables is causal or coincidental, both depending upon a third parameter.

2.8 Value of simulation in predictive research

Simulation allows experiments to be performed
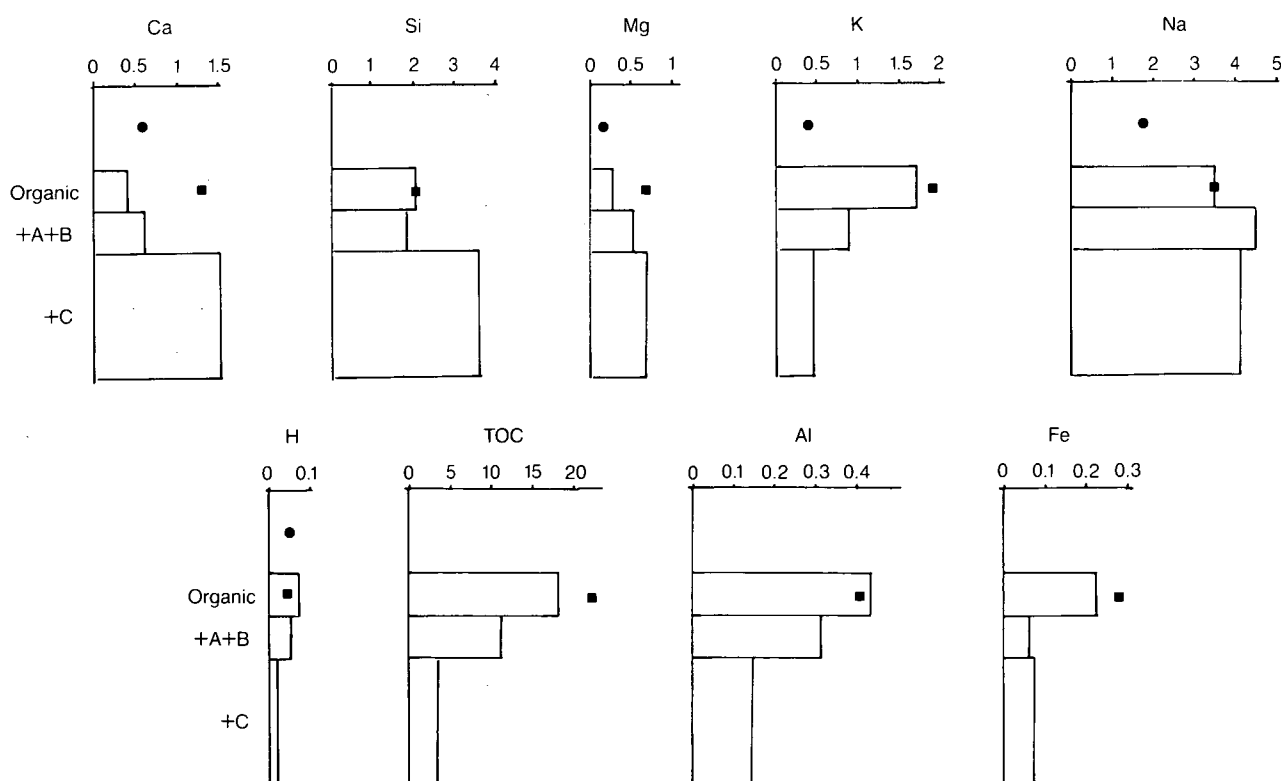
Figure 3. Change in leachate chemistry (μg ml^{-1}) from intact cores with soil depth (organic only, organic A + B, organic + A + B + C horizons). Also shown are the mean annual rain concentrations (●) and the mean annual leachate concentrations from lysimeters in the field at Peatfold (■)

which may demonstrate the effects of deterioration or improvement in acidic pollutant levels. Particularly important is their potential for assessing recovery potential.

2.9 Effects of acid deposition on drainage water chemistry

Chemical information obtained from soil and river water samples collected in the field can be supplemented by laboratory leaching of either intact or repacked soil columns. Both laboratory systems have certain advantages and disadvantages. A major limitation is generally the elimination of the lateral downslope flow component often observed in upland soil profiles. This limitation can be overcome only to a limited extent by using soil cores containing various combinations of soil horizons.

The use of repacked soil columns rather than intact cores poses more of a problem. Repacked columns of fresh, sieved, field-moist soil are easily prepared and have the added advantage of allowing increased replication, and therefore more treatments, which may be difficult using larger, intact cores. The major disadvantages in using repacked cores are related to the reproducibility of packing densities between columns, and the total destruction of the original soil structure, which may be very important in studies of leachate chemistry. A comparison of chloride breakthrough curves has been made for undisturbed cores and repacked columns for a silt loam (Elrick & French 1966) and a swelling clay (Kissel et al. 1973). Chloride appeared earlier from the intact cores in both cases, an observation attributed to the presence of continuous channels and cracks in the undisturbed cores and their absence in the repacked columns. Intact soil cores are, therefore, often more suitable for acid rain studies, whereas repacked cores may be valuable for establishing patterns of behaviour and elucidation of mechanisms.

The results presented in Figure 3 compare the leachate chemistry from leachings of intact cores from Peatfold, Glenbuchat, in the Grampian Region, containing either organic, organic plus B or the whole profile. A total of 70 mm of 'rain' was applied over 20 h, with leachate samples collected every 15 ml. A large increase in the total solute determinants load (excluding TOC) per ml occurs as the rainwater (2.8 μg of solute ml^{-1}) infiltrates the soil and percolates down the profile (8.4, 8.65 and 10.4 μg of solute ml^{-1} respectively for the organic, organic plus B and whole profile). The relative contribution of individual elements to the total output, however, alters with depth of percolation. The authors have found relative element concentrations to be very useful in interpretation of changes in river water chemistry through storm events in terms of changing hydrological path-

ways through the soil as the storm proceeds. They have also found (Cresser et al. 1986) studies of sulphate absorption done with repacked cores to be valuable in demonstrating the importance of soil parent material with respect to the potential fate of sulphate deposited on 2 contrasting upland catchments. Some typical results from the latter study are shown in Figure 4.
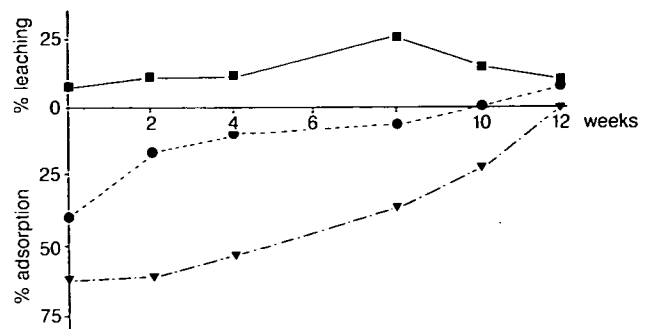
Figure 4. The patterns of SO_4^{2-} adsorption or leaching down reconstituted podzol profiles from Peatfold as a result of simulated rain at pH 3.5: 0—50 mm (■), 0—150 mm (●) and 0—250 mm (▼)

2.10 Effects of acid deposition on soil chemical and microbiological properties

Simulation experiments with soils and acid rain may be conducted to assess short-term or longer-term changes in soil chemical or microbiological properties and, if intact cores are used, changes in soil structure, as well as changes in drainage water chemistry. It then becomes particularly important to add water at an appropriate rate and as a spray. Addition of water at an excessive rate may sometimes dramatically alter the path water follows through a lysimeter, to the extent that much of the soil may be scarcely contacted by the water, and drainage may be too rapid to allow weathering to proceed to a realistic extent. The environment thus produced in the soil in a medium- to long-term experiment may bear little relation to that which would occur naturally. Furthermore, as for drainage water chemistry, the interpretation of the results for related field sites is again complicated by the lack of modifications resulting from lateral flow, which may be substantial on medium to lower hill slopes.

Relatively few lysimeter studies have been performed with vegetation in situ, but again this may be an important factor which should not be ignored. Our results have shown, for example, that dissolved organic matter from foliage or litter tends to stimulate microbial activity by providing a highly suitable substrate. There is also now much evidence for increased nutrient cation leaching from foliage in relation to rainfall acidity. The cations returned to the soil surface may be absorbed, thus helping to ameliorate the cation leaching losses to some extent by the redistri-

bution of proton inputs to rooting depth in the soil. Vegetation, particularly heather and sphagnum on low nutrient status acid moorland soils, may absorb nitrate from acid deposition to a very substantial extent (Edwards *et al.* 1985). Furthermore, plant roots may play an important role in stabilizing soil structure. Long-term (4 months) experiments with sieved peat soils without vegetation (Peirson-Smith & Cresser, unpubl.) suffered from substantial shrinkage to relatively impermeable, structureless, blocks. The effect was most pronounced initially for more acidic (pH 3.5) 'rain' on very acidic soils. Structure collapse problems have also been encountered with repacked columns after 12 weeks.

The general conclusions which may be drawn from the above observations are that relatively large, intact cores, or at least repacked systems with well re-established plant communities, are most appropriate for long-term studies, and that repacked systems generally should be confined to shorter-term, mechanistic, investigations.

The authors have studied the importance of changes in rainwater chemistry with time through storms. No different response of microbial respiration to fluctuating acidity of the precipitation was observed when soil from Peatfold, Glenbuchat, was subjected to 'rain' acidified with sulphuric acid to pH 2.75, 4.25, 4.5, 5.0 and 5.5, added in the above or a reversed order. A constant pH (pH 3.4) rain with the same total H^+ loading was also applied. Four hours after applying the 'rain', the rates of microbial respiration were measured, but they were not significantly different in the soils that received different treatments (Table 2).

Table 2. Effect of acid rain treatments on soil respiration rates

Treatment	Rate of respiration (μg CO_2 g^{-1} soil h^{-1})
Constant H^+ loading (pH 3.4)	60.3 ± 3.3
pH 5.50–2.75	62.7 ± 2.1
pH 2.75–5.50	61.6 ± 1.6

Means of triplicates ±SD, P<0.1

3 The chemistry of artificial rain
In a number of studies, the 'rain' used has been dilute sulphuric acid (eg Baath *et al.* 1980; Scherbatskoy & Klein 1983; Fritz-Sheridan 1985; Hovland *et al.* 1980; Morrison 1983). Rain, however, also contains substantial amounts of sodium, magnesium, calcium, potassium, ammonium, chloride, nitrate and organic matter, as well as minor trace constituents, apart from sulphate. The precise composition may vary considerably with both time and space (Martin & Barber 1978). The chemical composition of 'rain' used in our studies

has been based upon the mean rainfall composition of weekly samples collected at Peatfold (Grampian Region) during 1979–80 (Table 3). The major mechanism of cation leaching from foliage and soil is by cation exchange. The relative proportion of individual base cations leached depends on their concentrations in the incoming rain or throughfall. Tukey (1970) reported that K^+ and Na^+ ions increased the quantities of nutrients leached from foliage, whereas Ca^{2+} ions tended to inhibit leaching losses. Studies with simulated precipitation by the authors showed that Ca^{2+} may, in fact, be retained from rain or throughfall by surface soils of low base status (Table 4). The Glendye soil has a lower base status and therefore retains Ca^{2+} more effectively than Peatfold soil.

Table 3. Mean composition of Peatfold rain (pH 4.6)

Cations (μeq ml^{-1})		Anions (μeq ml^{-1})	
Na^+	0.079	Cl^-	0.060
K^+	0.012	SO_4^{2-}	0.105
Ca^{2+}	0.031	NO_3^-	0.033
Mg^{2+}	0.014	PO_4^{3-}	0.003
NH_4^+	0.025		

Table 4. Calcium* (μg ml^{-1}) in leachates from soils exposed to 'rain' at pH 3.5 or pH 5.3

Soil	pH 3.5 rain	pH 5.3 rain
Peatfold	+ 0.196	− 0.201
Glendye	− 0.086	− 0.267

* Concentrations are means of triplicates over 12 weeks, expressed relative to the concentration in the rain

Throughfall studies with young Sitka spruce trees exposed to simulated artificial rain acidified to pH 3.5 with HNO_3 showed a net uptake of N by the trees after several weeks of treatment (Skiba *et al.* 1986). The above results all point clearly to the importance of using 'proper rain' and not just dilute sulphuric acid in simulation studies.

4 The acidity of simulated acid rain
4.1 The acidity of natural rain
The average acidity of rainfall in western Europe measured from 1972–81 lay between 4.3 and 4.5 (Environmental Resources Ltd 1983). Unpolluted rain may have a pH range of 4.5–7.4. At Banchory, in the Grampian Region, 50% of yearly rainfall samples collected daily had a pH of less than 4.4 (Nicholson *et al.* 1980). At Peatfold in Grampian, the mean rain pH for weekly samples was 4.6, and the range from 3.8 to 6.0. In very polluted areas, for example near the Sudbury smelter (Ontario, Canada), the pH of water collected

during most rain events was below pH 4.0 (Freedman & Hutchinson 1980).

4.2 The acidity of precipitation in simulation studies

In acid rain simulation studies, precipitation generally has been acidified to pH 3, 3.5 or 4 (eg Shriner & Cowling 1974; Lee & Weber 1979; Abrahamsen et al. 1980). One has to remember that such studies only simulate conditions occurring in highly polluted areas, or during isolated showers in less polluted areas. Such studies may sometimes be useful tools in predicting long-term effects of acid precipitation. Intensifying the acid inputs can be done either by increasing the acidity of the precipitation (eg Firestone et al. 1984) or by increasing the volume of rain added (eg Strayer and Alexander 1981). Both approaches have their limitations. Interactions of acidity with the soil physical and microbial components, for example, are time-dependent and therefore a too high H^+ ion loading could give seriously misleading results (Bache 1980). Furthermore, response may not vary linearly with H^+ ion concentration. On the other hand, too large amounts of precipitation added to soil cores may cause waterlogging. In throughfall studies, excessively large volumes of simulated precipitation would not significantly increase nutrient leaching from foliage.

4.3 H_2SO_4 and HNO_3 in acid rain

Sulphuric and nitric acids generally make the major contributions to the rain's activity. The annual average contribution of H_2SO_4 and HNO_3 to rain acidity in northern Britain was 70% and 30% respectively (Fowler et al. 1982). It is important to include HNO_3 in simulation studies, especially when looking at effects of rain acidity on plant growth and microbial activity. From acid rain, 20 kg nitrogen $ha^{-1}yr^{-1}$ enters the ecosystem in central Europe and increased forest growth occurs at many sites (Ulrich 1980).

5 Simulator designs

In our initial work, artificial rainwater was applied using a simple nebulizer system (Edwards 1984) to produce an even and reproducible 'rainfall' distribution. Continual dripping of water or even the use of a saturated constant head of liquid may well have been easier to set up. The latter technique does not nearly reproduce natural field conditions and, if cores with little or no organic layers are to be used, problems from soil structural deterioration may develop very rapidly. A saturated constant liquid head also bears little resemblance to the field situation (flow being controlled by the soil's infiltration capacity and hydraulic conductivity) and may also suffer from problems caused by preferential flow down the core sides. Wilson et al. (1961) and Nielsen and Biggar (1962) showed that salts can be leached more efficiently by rainwater than by continuous ponding of water on the soil surface. They concluded that with rain-

fall much more of the water was transmitted through the soil than through cracks, so permitting more efficient salt removal. Using the nebulizer system, edge effects are thought to be negligible during leaching of intact soil cores (Brunstad & Njös 1980).
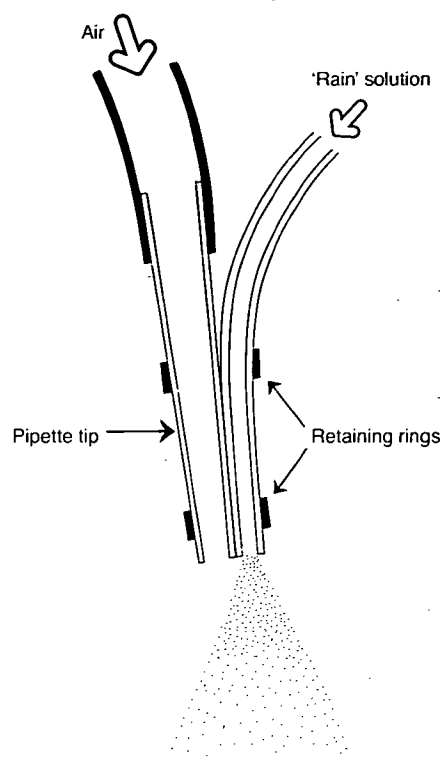
Figure 5a. The simple pneumatic nebulizer design used in the authors' rain simulator

The authors make extensive use of simple, laboratory-made pneumatic nebulizers to produce rain. Initially, glass melting point tubes were fixed in grooves in a rigid perspex support at an angle of 45° and solution gravity fed to one tube and air at c 10 psi to the other, to produce a cross-flow nebulizer. Solution flow rate may be controlled by the solution head and the use of a suitable length of capillary tubing. The tube positions and air pressure are adjusted to give a suitable spray. More recently, pairs of plastic pipette tips have been used, held together with plastic rings (Figure 5a). A Dexion rig containing up to 14 such nebulizers was used in the foliar leaching experiments described earlier. Whenever information is required on changes with distance, eg along a branch, parallel gutter channels have been made by looping polythene sheet over parallel support bars (Figure 5b).

With a little care, systems such as that described above may give a reasonably uniform distribution of fine rain. Alternatively, 'rain' may be made to drip from arrays of suitable capillaries (eg Chevone et al. 1984; Chow & Harbaugh 1965). In the former study, uniformity of wetting was ensured by placing plants on a rotating table under a series

of 'rain' arms coming radially from a central hub. In the latter work, a very large number of tubes at 1″ spacing was used. Perforated discs may also be used, but tend to give excessively large droplets over a short period. The most elaborate systems to date are undoubtedly the enclosed mini-catchment systems at Risdalsheia in southern Norway (Wright 1985). Here, sprinklers are used for 'rain'. Snow, too, has been simulated using snow generators of the type used in winter sports activities, but only with modest success. Air temperatures too close to 0°C lead to slush production and tree damage. In this and other field-based simulation studies, incident rain is often collected and deionized, and used to produce the simulated rain. To economize on apparatus, it is possible to use mobile rain rigs which may be moved over different plots to apply the appropriate treatment.

6 Conclusions

Rain simulation is a valuable tool in acid rain research, provided the limitations of simulation techniques are not overlooked. It is particularly useful for the elucidation of mechanisms, although quantitative transfer of results from the simulation system to a field situation is often difficult. Generally, the 'rain' produced by the simulator should be chemically and physically as close as possible to that encountered in the field, and even then the results should be complemented by field studies. When simulation is useful, valuable results may often be obtained with simple and inexpensive apparatus. Use of artificial rain may provide otherwise unobtainable controls to facilitate data interpretation.

Multi-gutter system for studying changes in throughfall along a branch
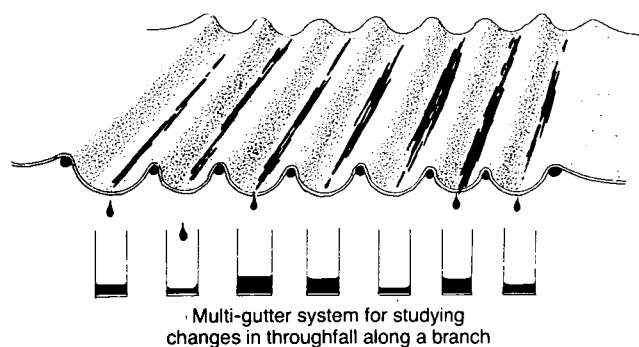
Figure 5b. Guttering system used to fractionate throughfall along a branch

7 Summary

The advantages of employing simulated precipitation in laboratory- and field-based acid rain research are summarized with respect to techniques for the study of effects upon plants, soils and drainage water chemistry. The practical limitations of such an empirical approach are also critically discussed. The different types of simulator design which have been used, from the simplest to the most complex, are described briefly, and the advantages and disadvantages of the various systems summarized. Applications of simulation techniques from the authors' laboratories and elsewhere are considered for a range of specific acid deposition-related problems. The results obtained in each case are outlined, and the possibility of extrapolation from laboratory results to explanations of behaviour in the field situation is critically appraised. The problems associated with making predictions of long-term trends in the field from shorter-term field and/or laboratory experiments are considered briefly.

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Non-suppressed ion chromatography in 'acid rain' analysis

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1 Introduction

The ITE Edinburgh and Banchory Research Stations have been collecting and analysing rain samples since late 1977. Recently, interest has turned to more intensive sampling of rainfall events in addition to bulk monthly samples, and to the study of cloudwater chemistry. Until 1984, chemical analyses were performed at ITE Edinburgh using a variety of methods, including continuous flow colorimetry, ion-selective electrodes and atomic absorption/emission. A non-suppressed ion chromatograph was commissioned during 1984 and has been performing all the chemical analyses, with the exception of pH, for about a year. It has allowed a greater number of samples to be analysed within the constraints of staff time.

2 Theory

The use of a single chromatographic column to separate anions and their subsequent direct detection by conductivity were described by Gjerde *et al.* (1979) and developed by the same group to include cation analysis (Gjerde *et al.* 1980; Fritz *et al.* 1980). Their approach differed from the earlier ion chromatographic techniques of Small *et al.* (1975), developed commercially by Dionex, in that the background conductivity of the eluant was subtracted electronically, rather than chemically, thereby eliminating the need for a chemical 'suppressor' column to reduce eluant conductivity after the chromatographic column. However, the separation method is similar, using a low-capacity ion exchange column with an aqueous eluant at a controlled pH. Initially, anion separations were performed using phthalic acid (normally *ortho-*, but the 2 other isomers have been used (Glatz & Girard 1982)) at a pH less than 6.0, the highest pH which may be used with a silica-based column. More recent column technology using resin-based columns has allowed the use of pH range 1—12, and a wider range of buffer eluants such as p-hydroxy benzoic acid at pH 8.5. Monovalent cations are generally separated using dilute nitric acid (2×10^{-3} M), and separation of divalent cations is achieved by using a complexing agent such as ethylenediamine. Detection of the ions after separation can be achieved by measuring conductivity changes. For an eluant such as phthalic acid with low background conductivity, the presence of an inorganic anion, eg chloride, in the detector cell increases the conductivity, and a positive peak is produced. In cation analysis, where the nitric acid

eluant has a larger conductivity, the presence of an ion such as sodium produces a decrease in the conductivity and a negative peak. In either case, both peak heights and areas are linearly related to the amount of each ion injected on to the column. Alternative detection methods have been employed, and for anions these may be significantly more sensitive than straightforward conductivity detection. Small and Miller (1982) in the USA, and Cochrane and Hillman (1982) in the UK reported the use of indirect ultra-violet detection with phthalate as eluant. The UV absorption of the phthalate eluant is decreased as non-absorbing anions pass through the detector. This method has the added advantage of allowing the estimation of an unknown ion's valency from the variation of retention time with eluant concentration. The response (expressed as the area of the negative peak) also depends only on the concentration of the ion present in a sample, when measured in equivalents per litre, so that the response is independent of the ion and allows quantitative estimation of an unknown (Small & Miller 1982).

A similar technique, but using changes in refractive index, has been applied by Haddad and Heckenberg (1982) to the analysis of both anions and cations.
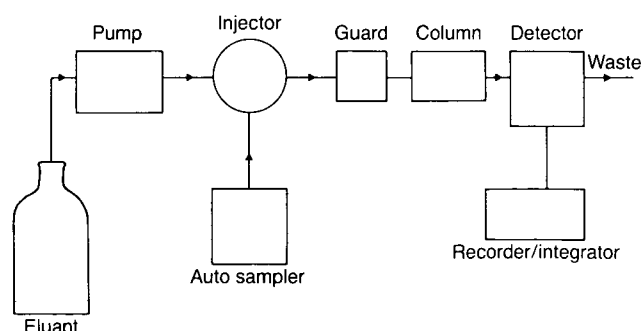
Figure 1. Schematic ion chromatography system based on standard HPLC equipment

3 Practice

The great practical advantage of non-suppressed ion chromatography over suppressed (ie using a chemical suppressor column) is that a system can be built from readily available standard components for high-performance liquid chromatography (HPLC). A schematic diagram of such a system is shown in Figure 1, and the equipment used at ITE Edinburgh is listed in Table 1. Other detectors (eg UV detector) could be used

in place of the conductivity detector. For a laboratory with existing HPLC equipment, only a suitable column needs to be purchased to achieve a capability for ion chromatography.

Table 1. Ion chromatography equipment used at ITE Edinburgh

Pump: Kontron 414 single-piston pump with pulse dampener
Autosampler: Kontron MSI660 with pneumatic injector (Rheodyne) capacity 60 × 2 ml vials
Column holder and detector: Wescan ion chromatography module (conductivity)
Recorder/integrator: Shimadzu C-R1A single channel printer/plotter

The operation of the system requires the same care as any HPLC equipment. All samples are filtered through a disposable 0.2 μm filter, and eluants are also filtered through an 0.2 μm 'candle' filter before use. Initially, problems were found with the quality of the laboratory supply of deionized water which, although 'pure' in that its conductivity was around 0.1 μS, had sufficient colloidal material present to accumulate on the guard column and create a high back-pressure. Analytical columns are not cheap (£200—£600), but the use of a guard column and care in sample preparation permits several thousand injections without serious loss of column efficiency. The size of the injection loop can be varied to suit the samples: for normal rain samples, we use a 200 μl loop, but for cloudwater samples, which generally have much larger concentrations, a 20 μl loop has been used successfully.

The limits of detection are around 2×10^{-6} M for direct injection. Other workers (eg Roberts *et al.* 1981) have pre-concentrated samples on a short column in place of the sample loop. Up to 10 ml of sample may be passed through the concentrating column; the ions are retained at the head of the concentrating column and are then flushed on to the analytical column by pumping eluant backwards through the concentrating column. In this way, concentrations down to 10^{-8} M may be detected, but at these levels the likelihood of contamination is very great, and precautions such as using pre-soaked polycarbonate labware and a dust-free environment must be observed to obtain reproducible results.

4 Analysis of anions

The 3 major anions in rainwater (chloride, nitrate and sulphate) are readily separated and detected, and, by choice of a suitable eluant, other inorganic ions such as fluoride and phosphate may be separated and measured. The order of elution is
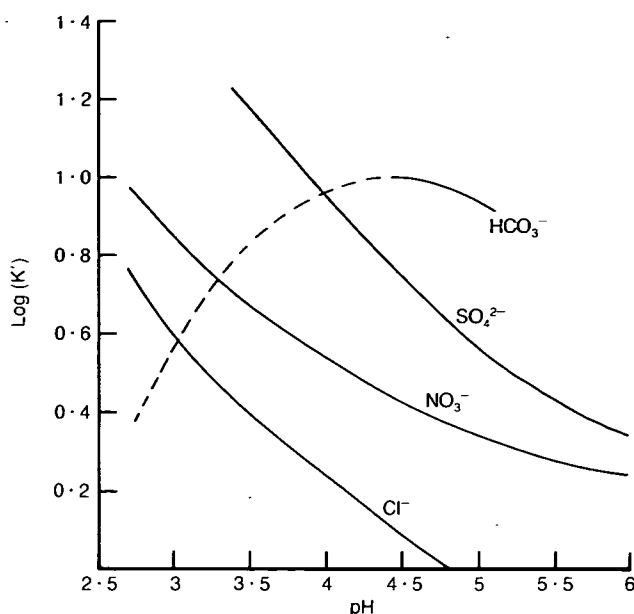
Figure 2. Retention of major inorganic ions in rainwater as a function of pH, using 4×10^{-3} M phthalate eluant (after The Wescan Ion Analyser, **4,** *3 (1982), Wescan Inc., Santa Clara, California)*

determined by the concentration of the eluant and its pH. For a phthalate eluant, the optimum pH is between 4 and 5 to achieve elution of the sulphate ion in reasonable time. The sensitivity of the method increases as eluant concentration is decreased, but a compromise must be sought between elution time (which increases with decreasing concentrations) and sensitivity. For a 4×10^{-3} M solution of potassium hydrogen phthalate, Figure 2 shows the effect of changing pH on retention of the common anions. For rainwater analysis, a pH must be chosen sufficiently high to elute sulphate quickly without 'losing' the chloride peak in the initial 'solvent' peak. After some trial and error, we have adopted as standard an eluant for anion anlaysis of 0.332 g o-phthalic acid, 24.0 ml 0.1 M NaOH and 10 ml methanol per litre of deionized water. This is equivalent to a phthalate concentration of 2×10^{-3} M at pH 4.74. Addition of a small quantity (1%) of methanol alters the retention characteristics to some extent, but has been found useful for avoiding bubble formation in the detector, which can occur even with vacuum-degassed eluant. Methanol (5%) is also added to the wash-water of the autosampler to prevent droplet formation in the PTFE (polytetrafluoroethylene) sample tubing. This eluant allows the separation of the 3 major ions in less than 15 minutes at a flow rate of 3 ml min^{-1} using a 25 cm × 4 mm column. A typical analysis is shown in Figure 3 for a rainwater sample. Calibration is achieved by inserting a single standard solution (usually 200×10^{-6} M Cl$^-$, 50×10^{-6} M, NO$_3^-$, 50×10^{-6} M SO$_4^{2-}$) after every 5 samples. Response factors are calculated automatically by the integrator and used for

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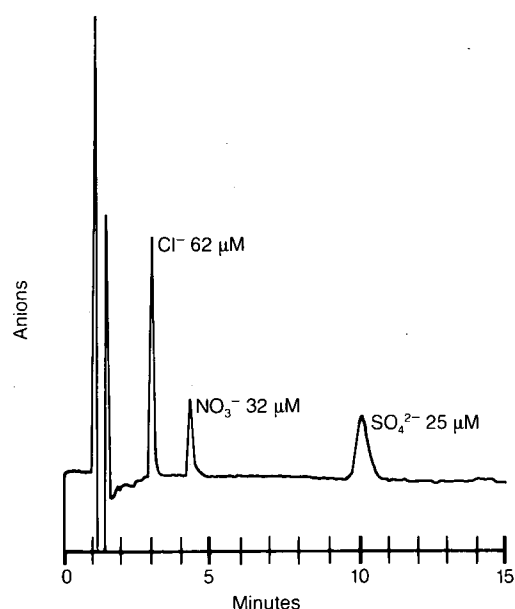
Figure 3. Anion analyser of rainwater sample from Great Dun Fell, Cumbria (17 April 1985). Flow rate: 3 ml min^{-1}, 100 µl injection; eluant: 2 × 10^{-3} M phthalate at pH 4.74 in 1% methanol; column: Vydac 302IC 25 cm × 4 mm

subsequent samples. Analysis of a range of standard solutions has demonstrated a rectilinear response through the origin (Figure 4), which
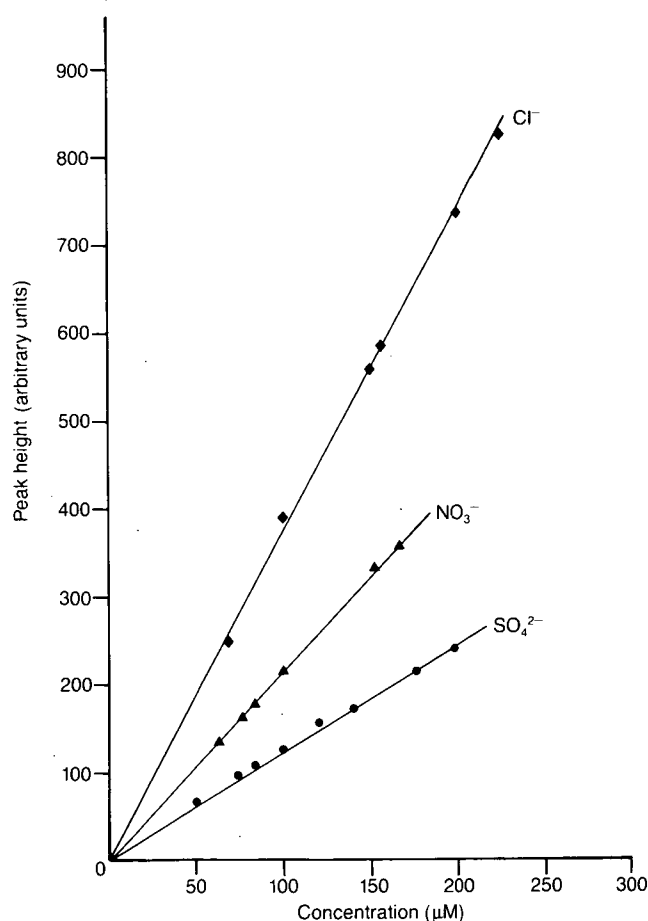
permits the routine use of a single-point standard. Most samples are analysed in duplicate, with agreement usually to within 2%. The limits of detection vary, and are best for chloride (<1 × 10^{-6} M) and worst for sulphate (1−2 × 10^{-6} M), but are adequate for rainwater samples, where systematic errors introduced in the process of sampling may be up to 30% (Fowler & Cape 1984).

5 Analysis of cations

With the exception of hydrogen ions, the most important cations in rainwater analysis are sodium, potassium, ammonium, calcium and magnesium. Although potassium concentrations are usually rather small, they are a good indicator of contamination of a sample from bird droppings, etc. Large concentrations of ammonium and high pH are not sufficient in themselves to designate a sample as 'contaminated', as they could arise from dissolution of atmospheric ammonia (Cape et al. 1984). As yet, no simple and reliable method is available for simultaneous analysis of monovalent and divalent cations by non-suppressed ion chromatography, so samples must be analysed using 2 different eluants.
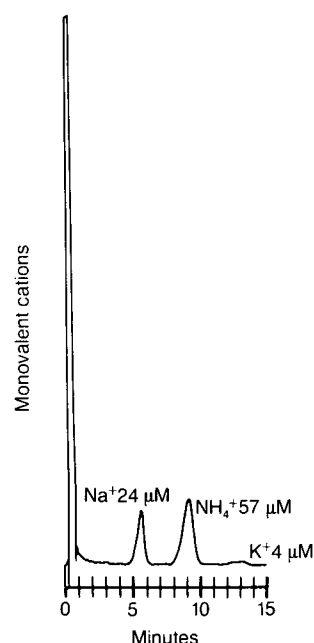
Figure 5. Monovalent cation analysis of rainwater sample from Castlelaw, Midlothian (24−27 March 1985). Flow rate: 1.7 ml min^{-1}, 200 µl injection; eluant: nitric acid at pH 2.50 in 1% methanol; column: Wescan cation 25 cm × 4 mm

Monovalent cations are separated (with a suitable low-capacity cation exchange column) using an eluant containing high-purity nitric acid at pH 2.50 in 1% methanol at a flow rate of 1.7 ml min^{-1}. An analysis takes less than 15 minutes, but the length of the column (25 cm) and consequent pressure drop precludes larger flow rates. Again, a balance must be found between speed of analysis (larger

Figure 4. Calibration of anion analyser using a range of standard solutions. Conditions as for Figure 3

eluant concentrations) and sensitivity (smaller eluant concentrations). A typical analysis is shown in Figure 5 for a rain sample. The calibrations are again linear, permitting the routine use of a single standard (usually 200×10^{-6} M Na^+, 100×10^{-6} M NH_4^+, 50×10^{-6} M K^+). However, peak areas must be used, as divalent cations accumulate on the column and retention times decrease systematically as ion exchange sites are filled, giving rise to higher peaks and shorter retention times. The original column performance can be restored using a 'sample' of 1% nitric acid injected routinely every 6 samples prior to the calibration standard. Sensitivity is better than 1×10^{-6} M for sodium and $1-2 \times 10^{-6}$ M for potassium, with reproducibility similar to the anion analysis.

chromatography over other methods (eg colorimetry, ion-selective electrodes) is that all anions or cations present in a sample are analysed simultaneously. This means that interferences from components which are not expected can be identified, where separate analysis for individual ions would perhaps not show the presence of a 'contaminating' ion. This advantage is best illustrated by means of an example: a monthly bulk rainwater sample (from Waterhead, SW Scotland, July 1984) had been contaminated by (presumably) bird droppings, given the large NH_4^+ and K^+ concentrations and high pH. Re-analysis of this sample for anions after one year gave the trace shown in Figure 7, in which the additional peak (cf Figure 3) was identified as nitrite ion. In the previous

Figure 6. Divalent cation analysis of rainwater sample from Achnagoichan, Speyside (January 1983). Flow rate: 1.5 ml min^{-1}, 200 µl injection; eluant: 0.84 × 10^{-3} M ethylenediamine at pH 6.0 adjusted with conc. nitric acid in 1% methanol; column: Wescan cation 25 cm × 4 mm
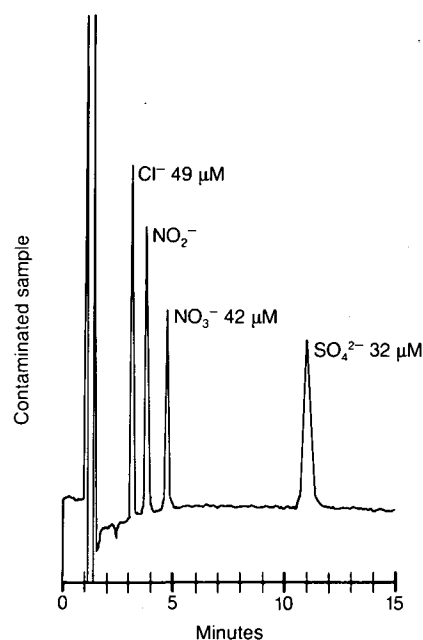
Figure 7. Anion analysis of contaminated rainwater sample after one year's storage, showing the presence of nitrite in solution. Conditions as for Figure 3

Divalent cations are separated on the same column but using an eluant comprising 56 µl ethylenediamine per litre of 1% methanol, adjusted to pH 6.0 with concentrated nitric acid (0.84×10^{-3} M in ethylenediamine). A typical analysis (Figure 6) takes 10 minutes at a flow rate of 1.5 ml min^{-1}. Calibrations using peak heights or areas are linear, and standard solutions (usually 50×10^{-6} M Ca^{2+}, 50×10^{-6} M Mg^{2+}) are used to recalibrate automatically every 6 samples. Detection limits are not quite as good as for the other analyses, being around 2×10^{-6} M under optimum conditions.

colorimetric technique, it would have appeared as nitrate, as the method did not distinguish between nitrite and nitrate. In practice, this is not usually a problem in rainwater analysis as nitrite ions are rarely, if ever, present. It appears, in this case, that the nitrite ions were produced by oxidation of ammonium ions in the sample over the year, even though the sample was kept at 4°C in an unlit coldroom. However, the sample was grossly contaminated, and there has been no evidence of similar oxidation in uncontaminated samples with a lower pH.

6 Discussion

The main advantage of any form of ion

A secondary, but very important, advantage of ion chromatography is its ease of automation, and non-suppressed ion chromatography has the added advantage that there is no need to regenerate a suppressor column (although in

practice this problem is not insuperable). The system used at ITE Edinburgh routinely runs 24 hours per day for 5 days a week. Samples are analysed in batches for anions, monovalent cations and divalent cations over a period of about one month using a single analyser. In this way, about 100 samples per month can be analysed in duplicate for 9 ions (including H^+ from pH) by one technician. This figure allows for re-analysis using appropriate standards and/or sample injection loops where one ion (eg Cl^-) may be present in very large concentrations.

Planned improvements to the present system include active control of the column and detector temperatures, which is expected to reduce baseline noise and drift and to improve sensitivity and reproducibility. There has been an occasional problem when the system has been left running unattended overnight in an unheated laboratory.

7 Summary

The technique of ion chromatography may be employed using conventional HPLC hardware and low-capacity ion exchange columns. For conductivity detection, eluants must have conductivities very different (greater or smaller) than the ions to be measured. Modern detectors have the capacity to suppress the background conductivity electronically, in a manner similar to the chemical background suppression used in 'suppressed' ion chromatography (eg Dionex).

The routine use of non-suppressed ion chromatography for the analysis of rainwater and cloudwater, both for anions and cations, is described. Care must be taken in sample preparation to remove particles in order to prolong column life, but this is not usually a problem for this type of sample. The major anions of interest (Cl^-, NO_3^-, SO_4^{2-}) are separated using a 2×10^{-3} M solution of phthalic acid in 1% methanol, adjusted to pH 4.74 with NaOH. The monovalent cations (Na^+, NH_4^+, K^+) are separated using pH 2.5 nitric acid, while divalent cations (Mg^{2+}, Ca^{2+}) are separated using 0.84×10^{-3} M ethylene-diamine solution adjusted to pH 6.0 with HNO_3. Each analysis takes about 15 minutes.

Peak heights (recorded on a dedicated integrator) are linearly related to concentration over at least 3 orders of magnitude. Using a 200 µl injection loop, sensitivity is better than 2×10^{-6} M and reproducibility is usually within 1% for multiple analyses.

An autosampler, autoinjector and integrator allow for continuous operation, with up to 40 duplicate analyses per day. There is no suppressor column to be regenerated, but for rain samples column 'clean-up' is required daily for about 30 minutes.

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Anion chromatography in agricultural analysis

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1 Introduction

The majority of routine methods for the analysis of anions in digest or extracts of agricultural materials rely on specific chemical reactions with quantification by colorimetry or titration (MAFF 1981). For a number of anions, in particular nitrate, bromide and sulphate, these methods have serious limitations for agricultural analysis. The extensive use of the commercial ion chromatography system (Small *et al.* 1975) has demonstrated the advantages that a chromatographic approach can provide. In addition, recent publications, most notably those by Small and Miller (1982) and Wheals (1983), have shown that excellent separation and quantification of anions can be obtained using conventional high-performance liquid chromatography (HPLC) equipment.

We have developed methods for the analysis of a number of anions based on a system fundamentally the same as that described by Wheals (1983). Separations are performed on a conventional HPLC reversed-phase column, modified by the introduction of an ion-pairing agent into the eluant. An eluant containing citric acid is used, with detection being by direct or indirect ultra-violet (UV) absorbance or electrochemical oxidation (ECD). An ion chromatograph using chemical suppression (Dionex) has recently been introduced, in which separations are carried out on a conventional ion exchange resin column using a sodium carbonate/bicarbonate eluant with conductivity detection. The intention of this paper is to describe briefly the methods developed for the analysis of some anions of agricultural interest, and to compare the use of the 2 alternative approaches. Wherever possible, the sample extraction and digestion methods used are standard procedures. This fact imposes some restrictions on the chromatographic system, but helps to ensure that the results obtained are comparable with standard methods.

2 Results

2.1 Nitrate

The determination of nitrate in soils and plant materials is of agricultural importance for both crop and animal nutrition. In addition, considerable interest has been shown recently in nitrate pollution in watercourses, caused by agricultural runoff. Nitrate is extracted from plant materials by shaking the dried ground sample with water; soil samples are extracted with saturated calcium sulphate solution.

Either chromatographic system can be used for the direct quantification of nitrate in waters or sample extracts; however, the strong UV absorbance of the nitrate ion at 220 nm means that UV detection is the most sensitive and specific. Practical detection limits on the HPLC system correspond to 1 ppm in soil and 10 ppm in plant material. The suppressed conductivity method is approximately 5 times less sensitive for this ion, and the analysis time for soil samples is considerably extended by the long retention of the sulphate in the extractant. Similar problems on the HPLC are avoided by replacing the citric acid counter ion with the perchloric ion. This prevents the indirect detection of sulphate and reduces the analysis time per sample from 12 to 4 minutes.

2.2 Bromide

Methyl bromide is used extensively for the sterilization of glasshouse soils. Residual bromide produced by the breakdown of this compound can impair the growth of certain types of crops. In addition, there is concern in Europe over possible human health risks associated with elevated bromide levels in salad crops. It is important, therefore, to be able to monitor bromide levels in both soils and plant materials. Soil samples are extracted with saturated calcium sulphate solution and plants are dry ashed after treatment with ethanolic sodium hydroxide, the residue being taken up in dilute sulphuric acid (Roughan *et al.* 1983).

The bromide ion shows UV absorbance at 205 nm and is electrochemically oxidizable at 1.6 V applied potential. It is therefore particularly amenable to analysis on the HPLC anion system. However, the citric acid eluant is unsuitable because it gives a high UV and electrochemical background under the conditions optimum for bromide detection. An eluant containing perchloric acid allows both low wavelength UV and high-potential electrochemical to be used. Detection limits for UV are approximately 1 ppm for soil samples and 10 ppm for plant materials. Some problems are encountered with interference from nitrate in soil extracts, which restricts the effective sensitivity for soils to 10 ppm, but the additional selectivity of the ECD for bromide eliminates this problem because nitrate does not give any response. The sensitivity of the Dionex system is approximately 5-fold less than for the HPLC with UV detection, and similar problems with interference from high levels of nitrate in soil extracts are also observed.

2.3 Sulphate

The renewal of interest in the possibility of crop growth being restricted by sulphur deficiency has led to an increased demand for sulphur analysis both in plant material and as extractable sulphate in soils. Threshold levels for crop sulphur deficiency are thought to be in the range 2—7 ppm extractable sulphur in the soil. It is therefore important to be able to reliably quantify sulphate down to at least this level. Soil samples are extracted with 0.016 M potassium dihydrogen phosphate. Plant samples are dry ashed with magnesium nitrate and the residue taken up in 10% hydrochloric acid.

On the HPLC system, sulphate, which is neither UV nor electrochemically active, has to be detected indirectly as a negative UV peak (Small & Miller 1982). Sensitivity is, therefore, seriously limited. With sufficient sample pre-treatment and concentration, it is possible to quantify sulphate at levels of agricultural interest in both soils and plants; the procedure, however, is very laborious and does not form the basis of a practical method. Suppressed conductivity detection gives approximately a 50-fold increase in sensitivity and allows easy quantification of sulphate in our samples. Soil extracts are injected directly, whilst plant digests require dilution 100-fold before injection to eliminate the effect of the high acid strength on the chromatography. Initial results indicate that this method will be reliable for total sulphur estimation. Problems have, however, been encountered with column contamination, possibly from the high concentration of magnesium ions in the digest, and we have not yet acquired sufficient data to justify its routine use.

3 Discussion

Both of the described approaches to anion chromatography are effective in the quantification of anions in agricultural samples. Reproducibility tests for replicate injections of the same extracts show that coefficients of variation range from 1.1% to 2.5%, and a wide linear range is obtained for both systems for the anions described. Significant savings in staff time are made over traditional agricultural analysis methods. The results obtained from the chromatographic methods have been verified for a range of sample types by standard addition, comparison with standard methods, and analysis of standard materials, where these are available. Where problems did occur with comparison with established methods, further work invariably showed that the chromatographic system was providing the more accurate result.

Effective and rapid separation of the common anions is obtained on both systems. Column contamination is a serious problem on the commercial ion exchange resin column in that, despite precautions to exclude the majority of possible contaminants, a limited lifetime is obtained for the guard columns incorporated into the system. Because these represent a significant expense, this is a major limitation in the use of the method. A wide range of sample types were analysed in the development period, and we are not yet certain of the origin of the contamination. However, it appears likely that the major problem arises from organic co-extractives of the soil and plant extracts. We are currently investigating methods of extending the lifetime of the columns for routine agricultural analyses. The HPLC system is subject to fewer problems in this respect. Columns are initially relatively inexpensive and are not subject to contamination, despite the injection of a very wide range of sample types.

All the detection methods described were easy to use and reliable in routine operation. For nitrate and bromide, the HPLC system provides the most effective method of analysis because of the additional sensitivity and selectivity of UV and electrochemical detection. For sulphate, the additional sensitivity obtained from the use of suppressed conductivity detection provides significant practical advantages.

4 Summary

Anion chromatographic methods have been developed for a number of agricultural analyses: sulphate in soil extracts; nitrate in plant and soil extracts; and bromide in plant and soil extracts and digests. Chromatographic separations were carried out both on a conventional reversed-phase HPLC column converted to an ion exchange system by incorporating an ion-pairing agent into the eluant, and on a commercial ion exchange resin column. Both systems provided excellent resolution of all the commonly encountered anions; the HPLC system proved to be less subject to contamination with sample co-extractives. The eluted ions on the HPLC system were monitored using direct or indirect UV absorbance and oxidative electrochemical detection. This system provided the optimum sensitivity and selectivity for nitrate and bromide. For other ions, in particular sulphate, the ion exchange column with the chemically suppressed conductivity detector gave a much improved performance as compared to indirect UV detection.

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Measuring the pH of fresh waters

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1 Introduction

pH is probably the most frequently determined analytical quantity. It is easy to measure with simple, inexpensive equipment. Meters, electrodes, and buffers for use as calibration solutions are all readily available, and electrodes usually respond quickly to provide a stable answer within minutes.

Rarely are problems encountered when measurements are made on high ionic strength solutions, but at low ionic strengths it is often difficult to obtain reliable measurements. Only recently, particularly with the impetus of research into acid rain, has the problem become documented and generally accepted. Different results may be obtained, depending on whether the solution is stirred or quiescent, and this stirring shift may be typically 0.1–0.2 pH (Davison & Gardner 1986). Measurements on quiescent solution have been advocated (Galloway *et al.* 1979), but even with this procedure there can be considerable disagreement between laboratories, and results may be no better than ±0.2 pH (Davison & Gardner 1986).

Several recent studies have investigated the nature of the problem (Brezinski 1983; Covington *et al.* 1985; Davison & Woof 1985). Although errors due to the glass electrode, particularly the possibility of interference by organics, have been reported (Herczeg & Hesslein 1984), there is little doubt that the problem is chiefly associated with the liquid junction of the reference electrode.

2 Liquid junctions and the definition of pH

To appreciate problems associated with the liquid junction, it is necessary to understand the pH scale. pH is defined operationally in terms of the operation or method used to measure it. Cell A represents a reference electrode and glass electrode inserted into the solution, X, of unknown pH. In cell B, the unknown solution is replaced by a standard buffer solution, S.

| Reference electrode | KCl (aq. conc.) | Solution X | glass electrode | (A) |

| Reference electrode | KCl (aq. conc.) | Solution S | glass electrode | (B) |

To determine pH, the potentials of cells A and B, E(X) and E(S), are measured and compared. The unknown pH of solution X, denoted by pH (X), is related to the pH of a standard reference solution, S, denoted by pH (S), by the definition:

$$pH(X) = pH(S) + \frac{E(S) - E(X)}{(RT/F)\ln 10}$$

where R is the gas constant, T the thermodynamic temperature and F the Faraday constant.

The definition of pH is completed by assigning a value of pH at each temperature to one or more chosen solutions, designated as standard reference solutions (Covington *et al.* 1983a), and used as calibration buffers.

Liquid junctions, which are an integral part of cells A and B, have to be considered in most practical measurements of pH. The concentrated KCl solution which forms part of the reference electrode is in contact with either the unknown solution, X, or the buffer solution, S. Junctions between 2 different solutions give rise to a potential. In any measurement, this is included as part of the potential of the cell. pH is defined by taking the difference between the potentials of 2 cells, so, if the liquid junction potential is the same in cells A and B, it will not contribute to the measured pH. If the individual junction potentials differ, there will be a residual liquid junction potential which will constitute an error in the measured pH of the unknown solution.

In practice, liquid junction potentials depend on the construction and geometry of the junction. pH scales, such as the NBS scale, have been defined in such a way that, if the geometry is ideal, so that there is no restraining element and ions can diffuse freely into one another, the residual liquid junction potential should be negligibly small for all solutions with an ionic strength less than 0.1 M (Bates 1981). However, most practical junctions are formed at a restraining frit which is found to give rise to different junction potentials, depending on the solution in which the electrode is immersed. Precise reasons for the non-ideal behaviour of restrained junctions are unknown, but some possible causes have been suggested (Brezinski 1983). Potentials may arise due to perturbation of the diffusion profiles which extend from the junction into solution, or to large surface charges within the pores of a partially clogged junction. Inadequate flushing of the junction between measurements may also cause errors. Whatever the cause of the finite residual liquid junction potential, it depends on the construction of the junction and introduces errors in the

determination of pH.

3 Overcoming liquid junction problems

3.1 Alternative measurements

One proposed solution to the measurement of pH is to calculate its value from alternative analytical procedures. Total acidity can be determined by titration (Marinenko & Koch 1984). It is a measure of all the hydrogen ions which may be titrated, including those associated with weak acids. Therefore, in natural waters of unknown composition, it cannot be equated to pH which approximates to the activity of free hydrogen ions.

If the pH of a water is exclusively determined by carbonate equilibria, it can be calculated from the concentrations of particular carbonate components. Herczeg et al. (1985) have proposed determining total dissolved inorganic carbon and the partial pressure of CO_2 by gas chromatography and then calculating pH. This procedure suffers because it only works for waters which contain appreciable bicarbonate ions and so have a pH greater than 5.5.

Neither of the above procedures give an estimate of hydrogen ion activity suitable for use in speciation calculations and kinetic or equilibrium studies. Although these alternative measurements do not determine pH, they may be useful as indicators of environmental change in their own right.

3.2 Calibration with low ionic strength solutions

Calibration using dilute acids or buffers has been considered (Galloway et al. 1979; Covington et al. 1983b). In using such a procedure, there is a presumption that the liquid junction potential in the dilute calibrating solution is the same as in the natural water. The implicit assumption does not appear to hold because electrode pairs can perform well in dilute acid and still produce errors in natural waters (Davison & Gardner 1986), and pH errors are not generally constant for different low ionic strength solutions (Davison & Woof 1985). There is little intrinsic justification for using dilute standards because the problem is not due to the buffers, which, as already stated, will contribute negligible residual liquid junction potential if the junction is ideally formed.

3.3 Raising the ionic strength

One obvious procedure would be to add neutral salt, such as KCl, to raise the ionic strength of fresh water to a level where the liquid junction problems are inappreciable. However, there could be little guarantee that the pH would remain unaffected. Even in a simple homogeneous solution, changes in activity coefficients would change stability constants and solution equilibria, and there is always the possibility of adding contaminants with the KCl.

3.4 Free-flowing junctions

pH errors can be reduced by ensuring a good flow of bridge solution ($10-100$ µl h^{-1}) through the frit. With free-flowing frits, the pH shifts observed in stirred solutions are usually small and can be less than 0.01 pH. There is also good evidence (Brezinski 1983; Davison & Woof 1985) that the value obtained is close to the true pH.

Such junctions suffer from being poorly defined, because they are still dependent upon a frit whose manufacture can be highly variable. Moreover, the high leakage rate of bridge solution can contaminate the unknown solution, if the measurement takes more than a few minutes.

3.5 Free-diffusion junctions

Frits are employed in constrained junctions to provide electrical contact between the bridge solution and solution being measured, while preventing the solutions from mixing. Removing the frit alleviates errors due to residual liquid junction potentials but introduces practical problems. Consequently, free-diffusion junctions have rarely been used for routine measurements.

Ideally, a free-diffusion junction should have cylindrical geometry and so should be formed in a tube (Bates 1964). Brezinski (1983) formed such a junction by withdrawing the sample solution into a tube initially filled with bridge solution. Covington et al. (1983b, 1985) also withdrew the sample solution into a tube, but they formed the junction at a 'T' intersection. Their junction, which could be readily renewed for fresh measurements, was tested by using it in the determination of the second dissociation constant of phosphoric acid in dilute solutions. This test verified that the pH could be measured accurately, to ±0.02 pH, in a 10^{-3} M solution. These free-diffusion junctions have proved useful as reference systems for assessing the performance of other junctions (Davison & Woof 1985), but they are impractical for routine use.

One manufacturer, Hach, Iowa, USA, has produced a commercial reference electrode using a free-diffusion junction based on the design of Illingworth (1981). It consists of a $\frac{1}{2}$ mm bore plastic tube into which is inserted a silver wire. The end of the wire nearest the opening is chloridized. A syringe piston supplies a 2.4 M solution of KCl saturated with AgCl and containing 40% glycerol as an antifreeze. The liquid junction is formed at the end of the plastic tube which is positioned upward in the solution being measured. New junctions can be formed by dispensing more filling solution from the syringe. Such an electrode has an ill-defined and non-cylindrical junction geometry, and, as the bridge solution is expelled to form each new junction, contamination of the sample being measured is inevitable.

Table 1 compares the performance of the Hach electrode with a Beckman calomel, quartz-fibre reference electrode. The latter uses a free-flowing junction and has been found to work well (Davison & Woof 1985). Both reference electrodes gave good results, showing very little potential shift on stirring even in very dilute solutions. Results for the Hach electrode were slightly more scattered, probably reflecting irreproducibility due to renewing the junction for each measurement. There was a systematic difference of 0.01—0.06 pH between the electrode assemblies, the results being closer to the values calculated from concentrations when the Beckman reference electrode was used. As the Beckman electrode was easier to use, there appears to be little gain in using the Hach system.

fundamental issues as the use of inappropriate liquid junctions. There are 2 main approaches to overcome this latter problem and so provide accurate pH measurements for fresh waters: use of free-diffusion junctions, or of free-flowing restrained junctions.

Generally, it is difficult to assign a true value to an analytical determinand in an unknown solution such as a natural water. pH is no exception. Because it is operationally defined, there is a temptation to say that a measurement made using the prescribed cell (Covington et al. 1983a) must give the correct answer. However, clearly the measurement depends on how the liquid junction is formed, the criteria for which have not been explicitly defined. Free-diffusion junctions are

Table 1. Performance of Beckman 39416 quartz fibre sat. KCl-filled, and Hach injection, reference electrodes at $20\pm0.1°C$. The same Corning 003 11 101J triple-purpose glass electrode was used with both reference electrodes for all measurements. Daily duplicate measurements were made on 5 different solutions using each electrode pair. The solution was stirred for 2 minutes, a reading taken and then the stirrer switched off for 2 minutes when another reading was taken. The procedure was repeated on 10 different days. A new liquid junction was formed for each measurement made with the Hach electrode. Electrode pairs were calibrated freshly each day using the same pH 4.001 and pH 6.881 buffer solutions which were prepared from standard reagents and used throughout the tests. The 'unknown' buffer was prepared freshly each day from commercial buffer tablets nominally pH 4.00. Dilute acids were prepared freshly each day. Natural water samples were subsampled each day from bulk stock solutions collected from streams. Analysis of variances provided means and total standard deviations

Reference electrode	Solution	Mean pH	Standard deviation
Beckman	pH 4.00 buffer	3.973 (3.970)[1]	0.003 (0.001)[1]
Hach	pH 4.00 buffer	3.951 (3.951)	0.014 (0.009)
Beckman	5×10^{-5} M H_2SO_4[2]	4.003 (4.009)	0.004 (0.002)
Hach	5×10^{-5} M H_2SO_4	3.964 (3.980)	0.018 (0.011)
Beckman	1.5×10^{-5} M H_2SO_4[3]	4.525 (4.535)	0.008 (0.005)
Hach	1.5×10^{-5} M H_2SO_4[3]	4.496 (4.500)	0.017 (0.013)
Beckman	Natural water A	5.754 (5.747)	0.104 (0.122)
Hach	Natural water A	5.700 (5.690)	0.147 (0.151)
Beckman	Natural water B	4.453 (4.453)	0.006 (0.004)
Hach	Natural water B	4.438 (4.440)	0.036 (0.035)

[1] Numbers in parentheses are for quiescent solutions, other numbers are for stirred solutions
[2] Calculated pH from concentration using activity correction is 4.006
[3] Calculated pH from concentration using activity correction is 4.524

4 Conclusions and recommendations

The preceding discussion is by no means exhaustive. It has concentrated on problems in the measurement of the pH of low-ionic strength solutions, which are associated with the liquid junction. Other causes for concern, such as possible errors due to the glass electrode and sampling and handling procedures, have not been discussed. Although they should not be neglected, they do not raise the same

implicitly incorporated into pH scales. The NBS scale uses such a junction for determining the pH of different standard buffers and the self-consistency of the NBS scale has been verified by showing that the residual liquid junction potential between different buffers is minimal. Thus, a free-diffusion junction with cylindrical symmetry is an obvious choice as a standard junction for precise measurements. Unfortunately, the mechanics of producing such a junction are complicated and

procedures reported to date have not been suitable for routine measurements. There is a need to develop a standard pH measuring system, based on a free-diffusion junction, which is easy to use and readily available. Such a system could, by agreed definition, be used to assign the value of the 'true' pH of any dilute solution including natural waters. It could be used to calibrate more conventional and more convenient electrodes which use constrained junctions. The widespread use of such a system as a standard reference procedure would ensure the comparability, between laboratories and with respect to time, of future pH measurements.

The preceding paragraph addresses the future. What of the present? We have to make the best possible measurements with the electrodes which are currently available. Where possible, use of a free-flowing junction will help, but equally important is the strict adherence to a well-defined analytical procedure. The measurement of pH is apparently so simple that it has encouraged poor analytical technique. All measurements must be made under identical conditions, paying attention to such apparently trivial details as a fixed geometry for electrodes, stirrer and beaker, a defined washing procedure between all solutions, and a minimum time before the meter reading is accepted. Few laboratories apply any quality control in their pH determinations. The appendix gives some recommended procedures for measuring the pH of fresh water. Simple methods of quality control are suggested, including tests which verify that the liquid junction of the reference electrode is suitable for waters of low ionic strength.

5 Summary
Errors associated with measuring the pH of low ionic strength solutions are usually due to non-ideal behaviour of the liquid junction formed between the reference electrode and the solution. Selection of appropriately designed junctions can result in the accurate determination of the pH of fresh waters, using existing procedures and standard buffer solutions. Conventional frit junctions which allow a high flow rate of filling solution from the reference compartment, although not ideal, can provide high-quality data, especially if quality control is strictly applied. This pragmatic approach is used to formulate practical guidelines for measuring pH using commercially available equipment. Free-diffusion junctions with no restraining element such as a frit provide a means of measuring pH which, by definition, will be correct. At present, they are impractical for routine use, but they could form the basis of a new, standard reference procedure for measuring pH.

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Appendix
Recommended procedures for measuring the pH of fresh waters — a practical guide

Introduction
If these recommendations are followed, the measured pH should be a good estimate of the true value. No procedure can guarantee the correct answer, and other equipment and procedures may produce equally good results. Selection of an appropriate reference electrode and adoption of a well-defined analytical procedure with built-in quality control are the 2 most important considerations. Combination electrodes are usually more prone to error and often deteriorate more quickly than separate glass and reference electrodes. No account is taken here of sample collection and storage which may contribute significant errors.

Equipment
Reference Electrode. Use a Beckman 39416 calomel with quartz fibre junction, and filled with 3.5 M KCl.

Glass Electrode. Most manufacturers' research grade electrodes are adequate. A Corning 003 11 101 and a Radiometer G202C have been found to work well.

Meter. Most modern meters with a facility to read in mV to <1 mV should be satisfactory. Beware of dampness affecting battery-operated instruments.

Calibration and storage
Use buffers, based on phthalate (pH 4) and phosphates (pH 6.8—7.6), purchased from a reputable manufacturer. If they require preparation from a powder or tablet, take great care and avoid contamination. Buffers and unknowns should be at the same temperature ($\pm 2°C$) and the pH of the buffer, appropriate to that temperature, should be used. Calibrate each day and recheck at the end of each series of measurements; with good electrodes, there should be no change, to within 0.02 pH. Store the reference electrode in 3.5 M KCl, and the glass electrode in pH 4 buffer.

Quality control
Adopt a well-defined routine for performing the measurements and do not deviate from it.

Electrode performance tests
These should be applied to newly purchased electrodes, to electrodes which have not been used for more than a week, and to regularly used electrodes at monthly intervals.

1. Electrode sensitivity. Using the standard calibration buffers and the pH meter in mV mode, verify that the electrodes give Nernstian response: at 20°C, 58 ± 1 mV per pH unit and at 25°C, 59 ± 1 mV per pH unit.

2. Accuracy in dilute solutions. Prepare accurately by weight, from a commercial volumetric stock, a solution of 10^{-4} M hydrochloric acid, or 5×10^{-5} M sulphuric acid. The pH should be 4.00 ± 0.02, irrespective of whether the solution is stirred or quiescent. If the pH of the acid is outside these limits, dilute accurately, by 5-fold, a solution of pH 4.00 buffer. At 20°C, the pH should be 4.11 ± 0.01, at 25°C 4.12 ± 0.01 (Covington *et al.* 1983b). If these values are obtained on stirred and unstirred solutions, the preparation of the acid is probably in error.

3. Stirring shift. The shift in pH upon stirring a formerly quiescent solution of the acid should be <0.02 pH.

4. Response time. The reading obtained using dilute acid should be stable, to within 0.01 pH units within 2 min of immersion of the electrodes, irrespective of the solution used prior to washing.

If the electrodes fail these tests, establish, by substitution of alternative electrodes, whether the glass or reference components are responsible. Failure of tests (2) and (3) are usually associated with the reference electrode. (1) and (4) could also be due to the glass electrode. Either try to rejuvenate the defective electrode and retest it, or discard it.

The Beckman reference electrode should have a flow rate >0.05 ml per hour (Davison & Woof 1985). To verify this, weigh the tissue-dried electrode, immerse to 1 cm in distilled water for 1 hour, dry with a tissue and reweigh. The weight should decrease by >50 mg. If the junction is clogged, discard the electrode.

Daily checks
To ensure fast response and homogeneous solutions, measurements should usually be performed whilst stirring. Occasionally switch off the stirrer and verify that the pH of a low ionic strength sample does not change by more than 0.02. Wash between measurements with distilled water which, when it has been air-equilibrated, should have a pH of 5.64 ± 0.1. Plot the daily pH of the distilled (not deionized) water, from a given laboratory source on a quality control chart. It will probably be fairly constant. If it suddenly changes, retest the electrodes.

General
Try to understand how electrodes work (see Westcott 1978) and follow manufacturers' recommendations. The *Corning guide to electrode troubleshooting* is excellent. Because of the high flow rate of KCl, the Beckman

reference electrode is unsuitable for continuous measurements or determinations of small volumes of solution. Ideally, it should be used for rapid pH measurements, <4 min on >50 ml of solution.

Measurement of nitric acid and particulate nitrate in air

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1 Introduction

As evidence has increased that inorganic nitrogen species play an important role in atmospheric chemistry and the acid deposition phenomenon, interest has developed in the sampling and measurement of these species, which are generally present only at very low concentrations.

A variety of methods has been used to measure nitric acid/particulate nitrate. Dual filter techniques, which rely on the collection of particles on an inert pre-filter and HNO_3 on a second, reactive filter, are simple to use but subject to interferences. High particulate values can result from retention of HNO_3 gas by reaction with particles collected on the first filter, whilst high nitric acid values can occur due to volatilization of particles (Appel & Tokiwa 1981; Appel *et al.* 1980; Forest *et al.* 1982).

Diffusion denuder techniques, based on the more rapid diffusion of gases than particles to the walls of a tube through which the sample stream is passed, avoid these problems. The tube walls are coated with a base and act as a perfect sorbent for HNO_3. Particles are carried through and are collected by downstream filter packs.

At Warren Spring Laboratory, filter and denuder techniques are currently being evaluated with a view to measuring HNO_3 and particulate nitrate at rural sites. At these sites, Palmes' diffusion tubes (Palmes *et al.* 1975) are being exposed to measure average NO_2 levels. These tubes collect NO_2 by passive diffusion to a triethanolamine coated gauze at the end of a sealed tube, which is then extracted and determined colorimetrically (Atkins *et al.* 1978).

2 Filter tests

Tests were carried out to compare the efficiency of different filters for collecting HNO_3. Nylon (Appel *et al.* 1980; Shaw *et al.* 1982) and cellulose impregnated with Na_2CO_3 (Ferm 1982) or NaCl (Forest *et al.* 1980, 1982) were used behind PTFE filters. The results of these tests were variable but showed no significant systematic differences between filter types, except for showing that nylon filters had to be extracted with ion chromatographic buffer solution, because aqueous extraction, used for the other filters, was inefficient. Cellulose filters impregnated with Na_2CO_3, however, gave the most reproducible results. Results of experiments where ambient air was sampled, at the edge of an
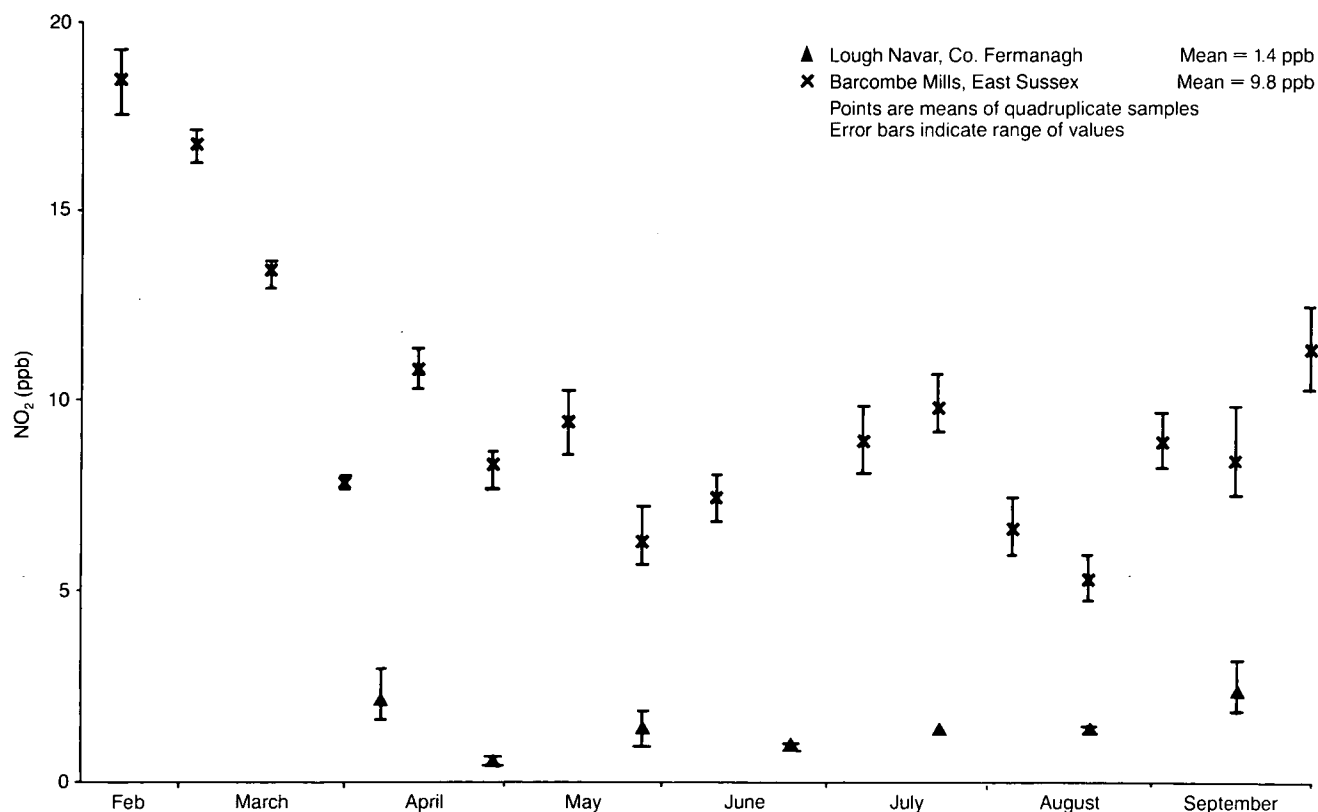
Figure 1. NO_2 concentrations during 1985

urban area, gave values of <2 ppb particulate nitrate and <3 ppb total nitrate. These results are similar to those found for a comparable site in Michigan (Mulawa & Cadle 1985).

3 Denuder experiments

MgO (Shaw et al. 1982) and NaF (Slanina et al. 1981) posed difficulties for ion chromatographic analysis and so were rejected as possible coatings. Limited results using Na_2CO_3 coatings, which were prepared after the method of Ferm (1982) using NaOH in methanol, and leached with deionized water, showed great variability. In many cases, however, the division between particulate and gaseous nitrate was similar for each denuder assembly, which suggests that this variation may have been due to difficulties with flow regulation. For example, in one experiment where ambient air was sampled, total nitrate varied between 0.58 ppb and 2.7 ppb but the fraction of the total which appeared as particulate nitrate varied only from 69% to 75%. The corresponding fraction for filter packs operated in parallel was 56%, indicating possible losses due to volatilization of about one quarter of the total particulate nitrate.

4 Results from NO_2 tubes

Results from 2 sites are shown in Figure 1. Tubes were exposed for fortnightly periods and no results exceeded 20 ppb. At Lough Navar, Northern Ireland, where concentrations were below 3 ppb, 4 weeks' exposure was necessary to collect measurable amounts. At these low levels, high blank values were a problem and conditions for preparing and analysing tubes are being modified to reduce these. It appears that there may be a seasonal variation, but a longer study is required to confirm this.

5 Acknowledgements

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The application of inductively coupled plasmas to the analysis of natural waters and acidic deposition

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1 Introduction

High-power, electrodeless, plasma discharges were first sustained in air at atmospheric pressure by Babat (1947), working at the Svetlana plant in Leningrad during World War II. His research ceased when the entire power system of that blockaded city failed and several of his colleagues perished in the hostilities. Almost 20 years later, Reed (1961) described the formation and stabilization of an inductively coupled argon plasma which he used as a very high temperature torch in research on the growth of single crystals of refractory oxides.

The analytical potential of Reed's inductively coupled plasma (ICP) as a spectroscopic source was recognized independently in the UK by Greenfield *et al.* (1964) and in the USA by Wendt and Fassel (1965). Since the mid-1960s, and particularly during the last 10 years, when various types of commercial spectrometer using ICP sources have been available, there has been an enormous increase in the use of ICP spectrometric techniques in many fields of analysis. This paper seeks to review briefly those techniques relevant specifically to the analysis of natural waters.

2 The ICP source

A conventional ICP torch typically consists of 3 accurately aligned, concentric silica tubes (Figure 1). The longest, outermost tube is surrounded at its tip by a 2- or 3-turn, water-cooled induction coil, connected to a radio-frequency generator operating usually at 27.12 or 40—50 MHz. Argon flows at carefully controlled rates through the 2 annular spaces formed between the tubes. The normally non-conducting argon is partially ionized initially by a spark from a Tesla coil and a toroidal plasma is sustained by inductive coupling between the coil and the ionized gas. Temperatures of 6000—9000°K result from Ohmic heating, and one of the functions of the high argon flow rate (10—20 l min^{-1}) between the outer and middle tubes is to cool and protect the torch tip from the intense heat. Sample aerosol is injected into the axial channel of the plasma via a stream of argon flowing up the central quartz tube.

3 Inductively coupled plasma-optical emission spectrometry (ICP-OES)

3.1 Instrumentation

By far the largest number of ICP spectrometric systems in use at the present time involve the
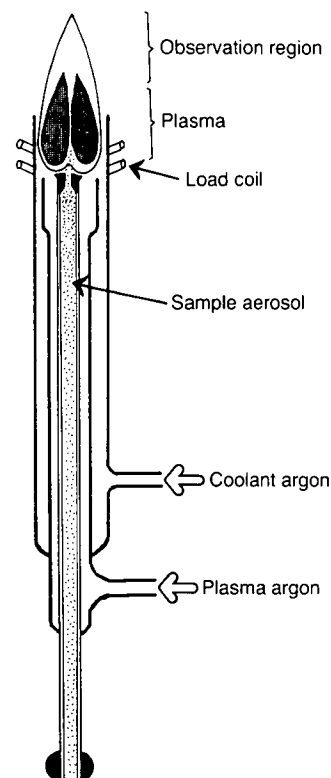
Figure 1. ICP torch

detection of the photon emission from the ICP by means of some form of spectrometer having conventional optics consisting of lenses, primary entrance slit, diffraction grating, and one or more exit slits and detectors. Two types of spectrometer are commonly employed: poly-chromators having the Paschen-Runge mounting with a fixed concave grating and typically 20—60 fixed exit slits on the Rowland circle, each with its own detector (Figure 2); and monochromators with a movable flat grating in a Czerny-Turner mounting (Figure 3). Polychromators permit the simultaneous determination of many elements at pre-determined wavelengths, whereas in monochromators the grating is turned, usually under computer control, so that spectral lines chosen at the start of each analytical programme are monitored sequentially. One manufacturer has recently produced a novel sequential system based on a Paschen-Runge mounting, in which small displacements of the primary slit are used to image the chosen spectral line on one of 200 exit slits equally spaced around the Rowland circle, where it is monitored with a movable detector. Simultaneous and sequential systems each have their strengths and weaknesses. An ideal system
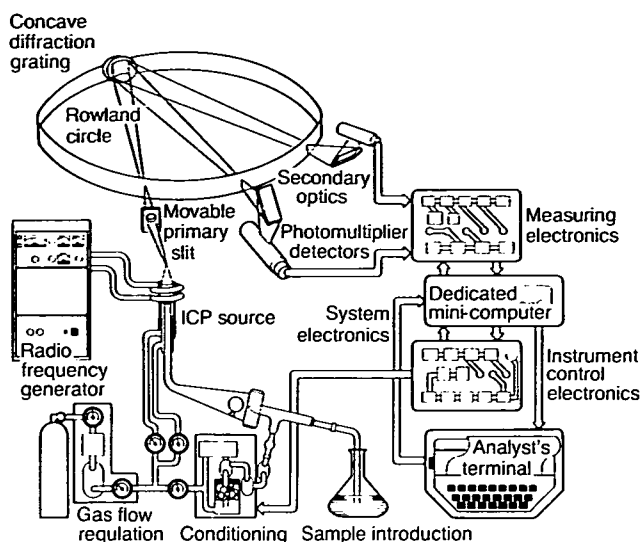
Figure 2. Schematic diagram of an ICP polychromator

Figure 3. Schematic diagram of a scanning ICP monochromator

would combine the high data rate and good stability of a polychromator with the flexibility of a monochromator. This combination can be achieved by viewing the plasma with both types of spectrometer at the same time, as is done in some systems.

3.2 Sample introduction
The most commonly used method of sample introduction in ICP-OES is as an aerosol generated by pneumatic nebulization of a liquid into a spray chamber, and thence to the torch. Natural waters are thus in an ideal form for direct analysis, requiring the minimum of pre-treatment. Concentric glass Meinhard nebulizers (Figure 4) have proved very popular and will run consistently for long periods, provided that no suspended matter is present in the test solutions (Baginski & Meinhard 1984); membrane filtration of water samples is recommended if blockage is to be avoided.

Figure 4. Concentric glass Meinhard nebulizer

Sample uptake rate is usually 1−2 ml min^{-1} which, combined with the simultaneous, multi-element capability of ICP-OES, enables large amounts of analytical data to be acquired from relatively small volumes of solution. In studies of acidic deposition, this feature is particularly helpful when sample volume is very limited, for example during investigations of solute mobilization and transport in soil and unsaturated zone pore waters extracted by centrifugation (Kinniburgh & Miles 1983).

Despite the relatively low sample consumption rate, the transport efficiency of the combination of aerosol-producing nebulizers such as the Meinhard and a conventional spray chamber is very poor, with only about 2% of the aspirated sample reaching the plasma and the remainder draining to waste. A novel approach to improving the overall efficiency of the sample nebulization and transport system, by ensuring that a much higher proportion of the liquid aspirated is ultimately injected into the ICP, has been described by Hulmston (1983) in the form of a recirculating nebulizer (Figure 5). A normal Meinhard nebulizer is mounted vertically in a specially designed spray chamber such that aerosol droplets impacting on the chamber walls drain down to the inlet of the nebulizer and the solution is recirculated. Approximately 2 ml of sample are required for 20 minutes of continuous operation, and sensitivity, precision and stability are reported to be comparable to those obtained with a conventional nebulizer system (Hulmston & McKillop 1985). However, wash-out times could be expected to be long with such a system, if sample carry-over is to be avoided. Alternative pneumatic nebulizers include the cross-flow (Kniseley et al. 1974) and Babington types, of which the latter is particularly suitable for nebulizing solutions containing high concentrations (>5%) of dissolved salts or those containing suspended particles (Ebdon & Cave 1982). The conespray nebulizer, recently developed by Sharp and co-workers (British Technology Group 1984), was designed to combine the merits of both the cross-flow and Babington V-groove nebulizers.

Ultrasonic nebulization followed by desolvation has been used to improve detection limits significantly by increasing the amount of solute entering the ICP in unit time (Taylor & Floyd 1981). Although, in principle, it is an inherently attractive technique, it has not been widely used in routine analysis, possibly because of the operational reliability of such nebulizers in terms of stability and memory effects. A commercial ultrasonic nebulizer and desolvation system has recently been produced by the Baird Corporation, and it will be interesting to see if it gains acceptance.

Direct nebulization of aqueous samples does not provide sufficient sensitivity to enable several important trace elements to be determined by ICP-OES at the concentrations at which they typically occur in natural waters. For those elements such as germanium, tin, lead, arsenic, antimony, bismuth, selenium and tellurium, which form gaseous hydrides at ambient temperatures, vapour phase sample introduction may be used to obtain considerably enhanced sensitivity and lower limits of detection. Thompson and co-workers (1978a, b, 1979, 1981) described a continuous-flow hydride generation system which they used for the simultaneous determination of As, Sb, Bi, Se and Te in waters; pre-concentration by collection on freshly precipitated lanthanum nitrate lowered detection limits further to about 50 ng l^{-1}.
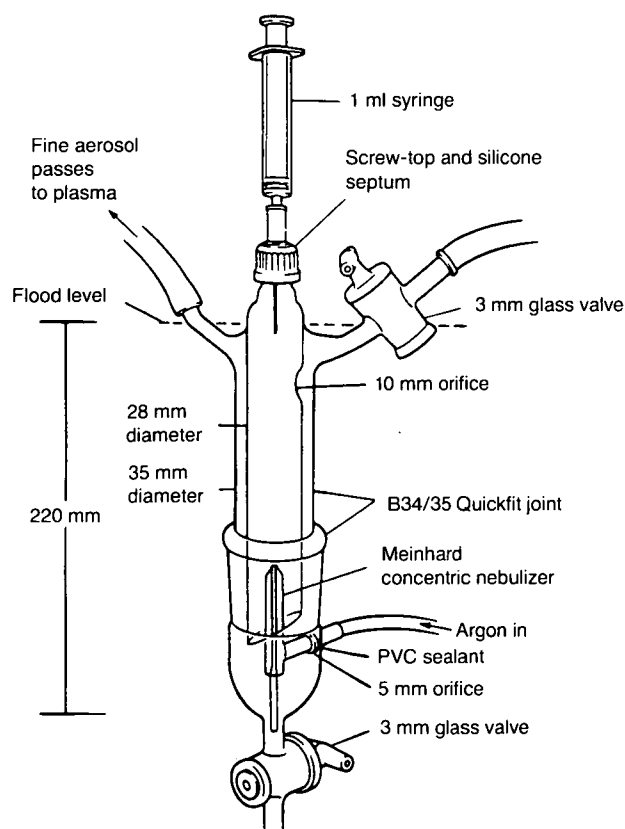
Figure 5. Recirculating nebulizer. (Redrawn from Hulmston 1983. Copyright: Royal Society of Chemistry)

3.3 Wavelength selection

The wide calibration ranges spanning 4–6 orders of magnitude, which are characteristic of ICP-OES, mean that most elements can be determined in natural water samples by choosing a single, appropriate wavelength. Occasionally, to maintain good precision, and to avoid saturation of the detector or the need to dilute samples, it may be helpful to use an alternative spectral line of considerably different intensity for those few elements which may be encountered at extreme concentrations, eg calcium in rainwater or geothermal brines.

Four main factors govern wavelength selection in ICP-OES: analyte concentration, overall sample composition, potential spectral interferences, and the spectral range of the instrument. In the case of natural waters, the major analytes are Na, K, Ca, Mg, Si and total S, and their concentrations typically range from 0.1 to 10^4 mg l^{-1}. With the possible exception of the determination of potassium in very dilute waters, appropriate spectral lines can be selected which will allow all these constituents to be determined directly in most samples, without the need for dilution or pre-concentration. In the case of Ca and Mg, it is necessary to avoid their most sensitive lines in order to achieve calibration ranges which can be extended to high enough concentrations. For minor and trace elements, present at concentrations ranging from <1 to 10^3 µg l^{-1}, the most sensitive emission lines are usually chosen, subject always to any constraints imposed by spectral interferences from major components of the sample. Several elements, including Sr, Ba, Li, B, Fe and Mn, can be determined directly in natural waters at typical environmental concentrations. However, the limits of detection attainable in ICP-OES are insufficient to allow many important transition and rare earth elements to be determined without pre-concentration. Nevertheless, direct measurement may still be used to screen large numbers of samples rapidly for anomalously high concentrations of a wide range of geochemically significant or potentially toxic elements. Numerous pre-concentration procedures have been reported in the literature (Sturgeon et al. 1980, 1982; Thompson et al. 1982; Long & Snook 1983; Vos et al. 1984), and an example of this application of ICP-OES to the analysis of natural waters is discussed elsewhere in this volume by Cook (1987).

Several compilations of ICP spectral lines have been published recently (Parsons et al. 1980; Michaud & Mermet 1982; Anderson et al. 1982; Boumans 1984; Brenner & Eldad 1984; Anderson & Parsons 1984; Winge et al. 1985; Forster et al. 1982; Wohlers 1985). These compilations may be used in the initial selection of analytical wavelengths, but it is vital that the suitability of

individual lines be fully evaluated in relation to the spectroscopic characteristics of the instrumental system employed and the composition of the waters under investigation. Modern computer-controlled data acquisition and display facilities make this a relatively rapid and straightforward operation.

The most intense and sometimes the only analytically useful ICP emission lines of several elements lie in the ultra-violet region of the spectrum below 200 nm and may be utilized with the vacuum spectrometer systems which are now widely available. Sulphur emission at 180.7 nm has been used as the basis of a rapid means of determining sulphate simultaneously with major and trace metals in oxygenated waters (Miles & Cook 1982). Total phosphorus may be determined down to c 0.1 mg l^{-1} using the 178.29 nm line (Cook & Miles 1985). Of particular relevance to the application of ICP-OES to studies of acidic deposition is the recent investigation of the vacuum ultra-violet region by Uehiro *et al.* (1984), which revealed that the aluminium 167.1 nm line provides much greater sensitivity than the 308.2 nm line commonly used (Figure 6). A 3σ detection limit of 0.5 μg l^{-1} was reported, enabling Al to be directly determined in lake waters; slight interference from a nearby iron line was recorded.

A typical wavelength programme that is routinely used for the determination of major, minor and

Figure 6. Spectra in the vicinity of the 167.1 nm and 308.2 nm lines of aluminium showing the enhanced sensitivity obtained with the vacuum ultra-violet line. (Redrawn from Uehiro et al. 1984. Copyright: American Chemical Society)

Figure 7. Typical ICP-OES calibration graphs for Na, Ca, Cu, Mn and Ni

Table 1. Typical ICP-OES wavelength programme for the determination of major, minor and trace elements in natural waters

Element	Wavelength (nm)	3σ Detection limits (mg l^{-1})
Na	589.0	0.02
K	766.5	0.15
Ca	315.9	0.006
Mg	279.1	0.03
Si	288.2	0.015
S	180.7	0.1
B	249.7	0.007
Li	670.8	0.002
Sr	407.8	0.0005
Ba	455.4	0.0005
Fe	259.9	0.002
Mn	257.6	0.0006
Cu	324.8	0.002
Zn	213.9	0.002
Cr	267.7	0.006
Ni	231.6	0.02
Al	308.2	0.02
Cd	226.5	0.005
Sc	361.4	0.0005
Y	371.0	0.0005
Be	313.0	0.0005

trace elements in rain, surface and groundwaters is given in Table 1. Examples of calibration graphs are shown in Figure 7.

3.4 Spectral interferences

Two types of spectral interference are recognized in ICP-OES: spectral line coincidence or overlap, and enhanced spectral background caused by recombination continuum radiation or stray light from intense emission lines, typically those of alkaline earth elements. In addition, instrument-specific stray light interferences may arise because of, for example, incomplete optical isolation of adjacent detectors in a multi-channel spectrometer. These interferences must be avoided or quantified and compensated for, if accurate data are to be obtained during the analysis of natural waters.

Figure 8. Spectra in the vicinity of 180.73 nm during the aspiration of (A) 8 mg l^{-1} sulphur, (B) 1000 mg l^{-1} calcium, and (C) deionized water, showing almost complete line overlap interference

An example of interference caused by almost complete spectral line overlap is shown in Figure 8. A weak calcium line virtually coincides with the most sensitive sulphur line at 180.73 nm. However, the difference in intensity of the 2 lines is such that a 1000 mg l^{-1} Ca solution produces a signal corresponding to an apparent sulphur concentration of only c 8 mg l^{-1}. Because of the very different relative intensities of the 2 lines, and the fact that these 2 elements occur at similar order of magnitude concentrations in water, correction for this slight interference effect is easily achieved by establishing the relationship between calcium concentration and apparent sulphur signal, and inserting this information in the controlling software. The effect of calcium is thereby automatically subtracted during the analysis of samples.

Interference caused by the partial overlap of the wing of one spectral line on another is illustrated in Figure 9. The wing of the 202.58 nm Mg line overlaps the nearby 202.55 nm Zn line to an extent that, although automatic correction is possible at low Mg concentrations, above c 500 mg l^{-1} Mg it would be wise to choose an alternative Zn line, such as the slightly less intense 213.86 nm line.

Small but significant shifts in spectral background intensity are observed when high concentrations

of certain elements are nebulized into the ICP. Radiation is emitted between 190 nm and 220 nm when large amounts of aluminium are present, because of the recombination in the plasma of aluminium ions with free electrons having a continuous energy distribution. This interference can be a serious problem in the analysis of many geological samples, where aluminium is a major constituent, but is insignificant as far as most natural waters are concerned because Al is present only at trace concentrations. Much more important are the enhanced background signals which are observed at certain wavelengths in the presence of calcium and magnesium at concentrations typical of some moderately saline waters. An example of the interference caused by calcium at the 371.03 nm yttrium line is given by Cook (1987).

The magnitude of this type of spectral interference is both analyte and interferent specific (Table 2), and probably also varies between instruments because of differences in optical configuration. While the so-called 'on-peak' mode of correction outlined for the Ca/S interference may be used to minimize the errors caused by background shifts, an alternative 'off-peak' correction procedure may also be used, particularly where the background in the vicinity of the analyte line is relatively flat and unstructured. In addition to making the usual intensity measurement at the correct wavelength (ie on-peak), intensity measurements are made at pre-determined positions on either side of the line by displacing the entrance slit or grating under computer control. A mean background level is then interpolated for each sample and subtracted from the total intensity.
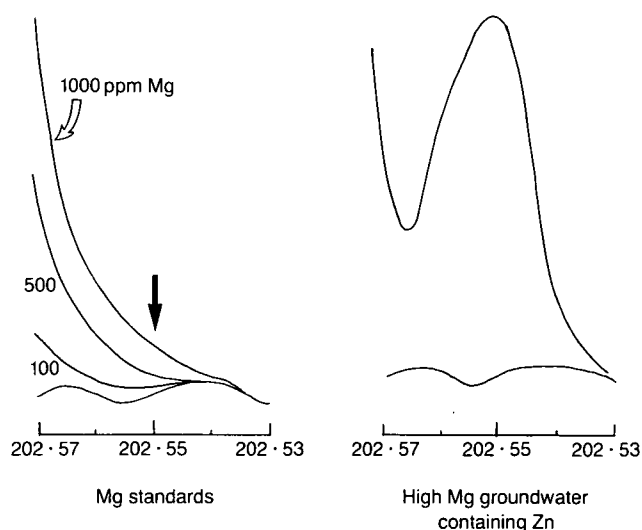
Figure 9. Spectra in the vicinity of the 202.55 nm line of zinc showing interference from the wing of the 202.58 nm line of magnesium at high concentrations

Table 2. Apparent analyte concentrations, in μg l⁻¹, arising from background shifts in ICP-OES when calcium and magnesium solutions are nebulized

Interferent	Analyte element and wavelength (nm)						
	Cr 267.7	Fe 259.9	La 398.8	Mn 257.6	Si 288.16	Ti 337.3	Y 371.0
1000 mg l⁻¹ Ca	—	—	200	—	●	130	4
1000 mg l⁻¹ Mg	48	25	—	5	350	—	—

● Line overlap interference from a weak Ca line at approximately 288.17 mm

Compared with some types of sample, eg geological and biological materials, natural waters tend to present fewer spectral interference problems. Elements such as aluminium with its recombination continuum and iron with its very line-rich spectrum are typically present as trace constituents rather than major components, and so exert little influence. Precipitation samples and upland surface waters of the type frequently examined in studies of acidic deposition are usually so dilute that interferences from major cations on minor and trace element measurements are minimal. However, all spectral interferences become proportionately more important as the true analyte signal decreases and the detection limit is approached, and thus must be carefully evaluated when reliable trace element data are being sought. Trafford (1987) has described how additional problems of reagent purity have to be taken into account when examining spectral interferences.

3.5 Advantages and limitations

Within the specific context of the analysis of natural waters, as opposed to, say, geological materials or metals, some of the main advantages and limitations of ICP-OES may be summarized as follows.

3.5.1 Advantages
i. Samples are in an ideal form for presentation.

ii. Calibration ranges covering 4—5 orders of magnitude allow samples of widely varying composition, eg rainwater and moderately saline groundwater, to be analysed without further treatment using a single spectral line and calibration function.

iii. Good sensitivity for some minor and trace elements, eg Sr, Fe and Mn, permits their direct determination at environmental concentrations, simultaneously with the major constituents. Samples may be screened for several other trace metals, eg Co, Cu and Zn, at potentially toxic or legislatively undesirable levels.

iv. Elements such as Fe and Al, which can give rise to spectroscopic problems if present at high concentrations, as for example in metallurgical analysis, occur mainly at trace levels in natural waters.

v. The high temperature of the ICP minimizes chemical interferences.

vi. The multi-element capability of the technique, whether simultaneous or rapid sequential, considerably reduces sample handling and the attendant risk of contamination.

vii. Elements such as B and Ba, which for various reasons are difficult or tedious to determine by other techniques, can be determined rapidly by ICP-OES with adequate sensitivity.

3.5.2 Limitations
i. Suspended material may block certain nebulizers.

ii. High concentrations of certain major constituents, particularly calcium and magnesium, give rise to spectral interferences in the determination of trace elements.

iii. The sensitivity of ICP-OES is inadequate to permit the direct determination of many trace elements at the very low concentrations at which they typically occur in natural waters.

iv. Emission intensities relate to total elemental concentration; information on the chemical speciation of the analyte cannot be obtained without additional treatment, eg by HPLC. Data on isotopic composition are not recoverable.

4 *Inductively coupled plasma-fourier transform spectrometry (ICP-FTS)*

Fourier transform spectrometry (FTS) is a very widely used technique in infra-red spectroscopy, where it has virtually replaced conventional dispersive-based measurement, but its application to the visible and UV regions has been much more limited, partly because of the more demanding mechanical and optical tolerance dictated by the shorter wavelengths. However, in recent years, various workers have been actively exploring the coupling of Fourier transform spectrometers to ICP sources, both for plasma diagnostic studies and as the basis of potentially powerful analytical systems (Faires 1986).

In FTS, a Michelson interferometer replaces the conventional grating spectrometer. Collimated light from the ICP is divided by a beam splitter and reflected from one fixed and one moving mirror

before being recombined and reflected to the detector (Figure 10). Differences in path length caused by the moving mirror result in an interference pattern which is related to wavelength and the position of the mirror. The original spectrum is recovered from the interferogram by Fourier transformation of the digitized wave form.
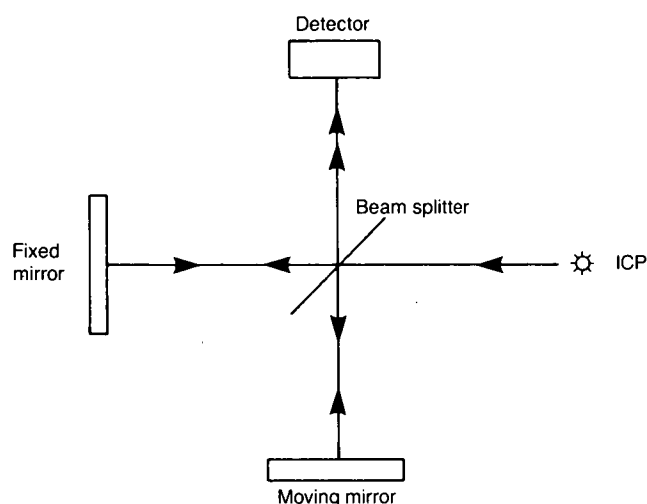
Figure 10. Schematic diagram of an ICP Fourier transform spectrometer

The inherent attractions of FTS are considerable. Spectral information is gathered simultaneously over the entire region of interest, rather than at specific wavelengths corresponding to pre-selected lines. High resolution is attainable and excellent wavelength accuracy is provided by the laser-referenced interferometer. Execution of the Fourier transformation requires significant computing capacity, but the power and speed of modern, small computers allows this to be accomplished relatively rapidly.

In contrast to IR-FTS, where noise is detector-limited, noise in ICP-FTS is source-limited. Consequently, because of the multiplex nature of the technique, noise from the more intense spectral lines is distributed into the spectral baseline. In addition to this so-called multiplex disadvantage, intense emission at certain wavelengths also poses problems through the dynamic range limitations of the detector. Comparisons conducted so far (Stubley & Horlick 1985a) indicate that ICP-FTS detection limits are 1—2 orders of magnitude poorer than those attainable by ICP-OES using a modern polychromator. Stubley and Horlick (1985b) have recently demonstrated that the interposition of a medium resolution monochromator between the ICP and the interferometer, to provide an adjustable optical filter with a bandpass of c 4 nm, can very largely overcome both dynamic range and signal-to-noise limitations. Improvements in detection limits of about one order of magnitude

were reported. As a novel departure from the use of the fast Fourier transform algorithm to recover the original spectrum, Ng and Horlick (1985a) have also used cross-correlation techniques to process the interferogram directly. Square-wave, digital, cross-correlation masks of any number of spectral lines of interest can be stored compactly in the computer, and the advent of powerful electronic multiplier chips opens up the possibility of real-time correlation-based data processing and rapid, automatic qualitative analysis by ICP-FTS (Ng & Horlick 1985b).

At present, no complete ICP-FTS system is commercially available and studies of the technique are confined to a small number of research laboratories. However, it is understood that at least one major ICP equipment manufacturer will shortly be marketing an ICP-FTS system.

5 Inductively coupled plasma-mass spectrometry (ICP-MS)

The principle of using an atmospheric pressure plasma as an ion source for atomic mass spectrometry was first demonstrated in the early 1970s by Gray (1974, 1975a, b), initially using a DC capillary arc plasma. Because of the severe inter-element and matrix effects experienced with this type of plasma, an ICP source was later substituted (Houk *et al.* 1980; Date & Gray 1981).

A schematic diagram of a typical ICP-MS system is shown in Figure 11. A conventional ICP source is positioned horizontally so that the plasma tail flame plays on a water-cooled, metal cone having a small (0.5—1.1 mm diameter) hole at its tip through which a portion of the ionized gas passes into the vacuum system of a quadrupole mass spectrometer. Considerable research effort continues to be concentrated on elucidating the processes controlling ion transport and recombination which occur in and around this interface between the very hot, atmospheric pressure plasma and the ambient temperature, high-vacuum environment of the mass spectrometer. A major step forward in the development of ICP-MS was the achievement of continuum flow sampling of ions from the bulk plasma, rather than from the cool boundary layer (Gray & Date 1983), and this form of ion extraction is embodied in the 2 commercial ICP-MS systems which have recently become available.

Liquid sample introduction in ICP-MS is fundamentally identical to that in optical ICP spectrometry, and is thus considerably more direct and rapid than that involved in thermal ionization mass spectrometry. Even for complex natural samples, the mass spectra obtained are relatively simple compared with corresponding optical emission spectra, and inherent in the
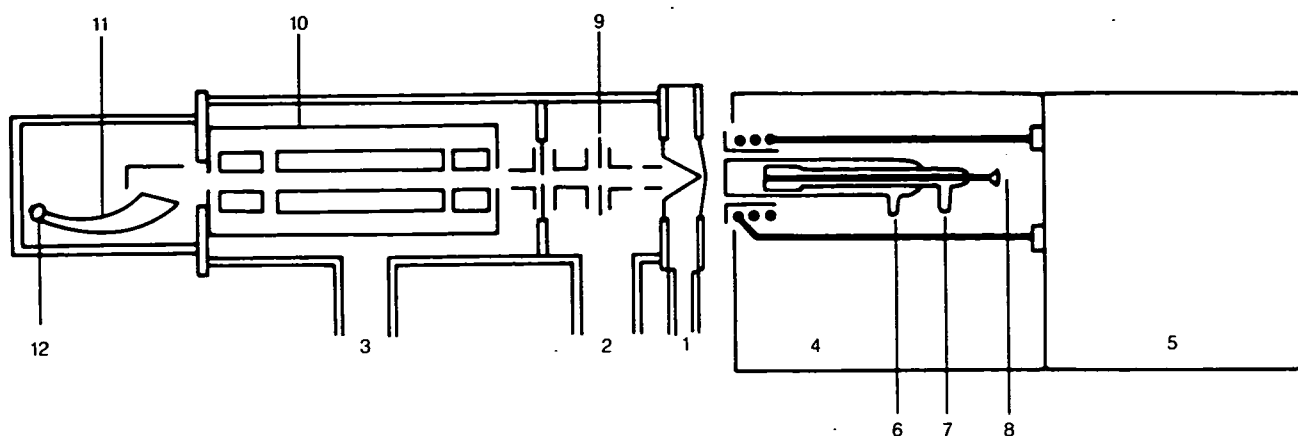
Arrangement of plasma and mass analyser. 1, 2, 3, Successive vacuum stages; 4, torch housing; 5, radiofrequency matching unit; 6, 7, coolant and plasma gas inlet; 8, sample (injector) gas flow; 9, ion lens; 10, quadrupole analyser; 11, ion detector; and 12, signal output

Figure 11. Schematic diagram of an ICP-MS system. (Redrawn from Gray & Date 1983. Copyright: Royal Society of Chemistry)

technique is the provision of information on the isotopic as well as total elemental composition of samples. This latter feature opens up the possibilities of the wider use of stable isotopic tracers in studies of hydrologic systems and of the use of stable isotope dilution techniques in accurate and precise, low-level, analytical methods.

Spectral interferences in ICP-MS arise from a variety of sources, including plasma gases, solvent, isobaric singly charged analyte ions, doubly charged ions, oxide and other molecular ions. Thus, background spectra show strong peaks at 40 and 41 m/z corresponding to $^{40}Ar^+$ and $^{40}ArH^+$ respectively, which preclude the detection of $^{40}Ca^+$ and $^{41}K^+$. Similarly, peaks corresponding to ions of H, C, N and O, singly or in combination, occur at various m/z values below 81. The significance of these interferences depends upon the relative intensity of the corresponding analyte peak. For example, $^{40}Ar^{14}N^+$ and $^{40}Ar^{16}O^+$ peaks degrade the detection limits for $^{54}Fe^+$ and $^{56}Fe^+$, but do not totally preclude the determination of these isotopes. Interference from ions of even a minor isotope of a major constituent can swamp the signal derived from a trace element in the same sample, eg $^{48}Ca^+/^{48}Ti^+$. In such cases, it is often possible to choose an alternative, interference-free isotope by which to determine the trace element, but some sensitivity may be sacrificed because of its lower abundance.

Careful choice of sample pre-treatment can substantially reduce some molecular ion interferences. McLaren *et al.* (1985) have shown that acidification with HNO_3 rather than HCl is to be preferred in order to avoid interference from chlorine-containing molecular species, such as that of $^{35}Cl^{16}O^+$ on $^{51}V^+$ and $^{40}Ar^{35}Cl^+$ on $^{75}As^+$. Interferences from metal-oxide species (MO^+) have been widely reported, but their origin,

magnitude and control are disputed. However, available evidence points to their being produced in the region of the ion extraction zone. In addition, Olivares and Houk (1985) have recently demonstrated that MO^+/M^+ ratios can be related to plasma operating conditions, such as nebulizer gas flow rate and forward power.

Current ICP-MS detection limits under realistic multi-element operating conditions are typically of the order of 0.1 µg l^{-1} for a wide range of elements. They are characteristically more uniform than those attainable by ICP-OES, to which they are generally superior by 1–2 orders of magnitude.

6 Conclusion

In the 20 years since its initial recognition as a potential spectroscopic source, the ICP has proved to be a stable and powerful vaporization, atomization, excitation and ionization cell. It has been successfully combined with several different modes of spectrometric detection, including direct and Fourier transform optical emission spectrometry, atomic fluorescence spectrometry and mass spectrometry. Used in appropriate combination, these techniques provide the bases of analytical methods by which natural waters may be characterized almost completely for their total elemental and isotopic composition. However, much remains to be achieved in extending the sensitivity of the overall analytical procedures to enable many trace elements to be detected reliably at the sub-ppb concentrations at which they typically occur in the environment.

7 Summary

Traditionally, molecular and atomic absorption spectrometry has been widely used in the analysis of natural waters, with the latter being especially popular for the determination of alkali, alkaline earth and some transition metals. In recent years, the development of the inductively

coupled plasma (ICP) as a spectroscopic source has provided the basis of sensitive atomic emission spectrometric techniques utilizing both optical and mass spectrometric detection. The advantages and disadvantages of plasma emission spectrometry for the analysis of natural waters and acidic deposition are reviewed, including instrumentation, sensitivity, sample treatment and interference effects. Utilization of emission in the UV region for the determination of non-metals such as sulphur and phosphorus are discussed.

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Trace element measurements of environmental water samples using inductively coupled plasma optical emission spectrometry

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1 Introduction

One of the advantages of inductively coupled plasma optical emission spectrometry (ICP-OES) as an analytical technique is that major, minor and trace element determinations can be performed simultaneously, thus minimizing sample handling and reducing sample contamination. However, for dilute waters, detection limits obtained by ICP-OES are not always sufficiently sensitive to allow the direct determination of some important trace elements. Sample pre-concentration is often required and any procedure which is used must therefore be applicable to a wide range of elements, in order to utilize the multi-element capability of the ICP. Thus, a simple method of evaporation under ultra-clean conditions was developed at BGS Wallingford, initially for the purpose of determining trace element concentrations in groundwaters, where many determinands were found to be present in unpolluted sources at very low levels.

Subsequently, the procedure has proved to be very robust when applied to rain and surface waters in studies of acidic deposition.

Underlying the methodology described here is the broader philosophy of trace element measurement. This paper reviews some of the precautions which need to be taken at each stage, from sample collection to data interpretation, and discusses them in relation to the determination of trace elements in natural waters by ICP-OES.

2 Sample handling strategy

To obtain reliable environmental data it is important to make a critical assessment of the procedures adopted at each of the 4 interdependent stages of sample collection, sample storage, laboratory analysis and data interpretation. Each stage will influence the accuracy and precision of the final result. Miles and Cook (1981) have outlined the problems of collecting data on the inorganic chemical composition of groundwaters.

At all stages of handling the samples, the overall objective should be to minimize:

i.   contamination of the sample,
ii.   loss of analyte by adsorption on to surfaces, and
iii. modification of the chemical form of the analyte.

For example, during sample collection and storage, attention should be focused on the materials used in the construction of the collection apparatus and the sample containers themselves. Pre-treatment of apparatus and containers is often necessary, particularly to reduce contamination.

The overall aims of a project may well dictate whether filtered or unfiltered samples are collected. A common practice is to use a filter with a nominal pore size of 0.45 μm as an arbitrary division between 'dissolved' and 'particulate' material. This distinction may be inadequate, especially for samples containing colloidal material (Danielsson 1982). A study of the particle size distribution of iron and manganese species in fresh waters by Laxen and Chandler (1983) showed that it was essential to filter on-site, as changes in the size distribution occurred within several hours. Filtration introduces the possibility of contamination from the apparatus and loss of analyte by adsorption (Gardner & Hunt 1981).

Consideration should be given to the stability of the analyte during storage. Sorption losses on to container surfaces have been demonstrated by Massee *et al.* (1981) for a number of trace elements, and Batley and Gardner (1977) have reviewed published data on storage experiments for various analytes.

Altogether there is a wealth of literature which addresses the problems associated with the sampling and storage of natural waters. In the end, whatever the procedure adopted, the essential prerequisite is that it be thoroughly evaluated, prior to its application to a particular project.

Once a sample reaches the laboratory, anything that comes in contact with it is a potential source of contamination. A direct consequence of this is a requirement for (i) a very clean working environment, and (ii) a source of high-purity water.

At BGS Wallingford this requirement is achieved in the following manner.

i.   Air is filtered before it enters the trace element laboratory, and a positive pressure is maintained in the room, relative to the external atmosphere. Laminar flow hoods are used to

provide an extra clean working area within the laboratory. The effectiveness of these measures can be judged from Figure 1, which compares the levels of iron, lead and zinc in the ambient air outside with those of the filtered air inside the laboratory and that produced by the laminar flow hoods. Even coarse filtration reduces the levels significantly, and the metal concentrations in the air in the laminar flow hoods are a fraction of those of the ambient air.
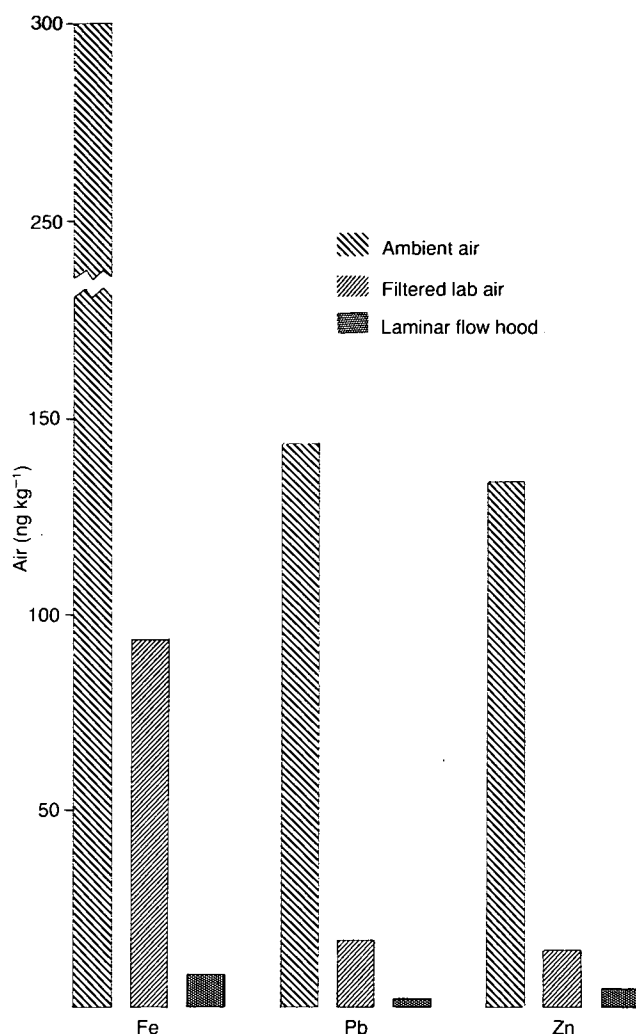
Figure 1. Concentrations of particulate iron, lead and zinc in the filtered laboratory air and laminar flow hoods, compared with those of the unfiltered atmosphere

ii. Water from a reverse osmosis plant is supplied to a *Millipore* system containing a pre-filter, 2 mixed-bed ion exchange columns, one column of activated carbon and a final filter of pore size 0.22 μm. The final water produced has a resistivity of up to 18 Megohms cm^{-1} and will be referred to as MilliQ water in this paper.

3 Collection and storage of trace element samples
One litre polyethylene bottles were soaked in 50% v/v Analar HCl for 24 hours, rinsed with high-purity water (MilliQ), soaked in 10% v/v high-purity Aristar HCl for another 24 hours, and copiously rinsed and stored full of MilliQ water. Just before transport to the field, the bottles were rinsed a further 3 times in MilliQ water and packed into plastic bags.

All plastic tubing and filtration equipment with which the water comes in contact was also acid-washed, rinsed with MilliQ water and rinsed thoroughly with the sample before its final collection.

High-purity Aristar HCl was added to the samples to give a final concentration of 1% v/v, lowering the pH to approximately pH 1—2 to stabilize the trace elements in solution and discourage adsorption on to the container walls. A blank of MilliQ water was also acidified in an identical manner in the field, to check on contamination from the bottles and the acid. Samples were stored at 4°C in the dark until required.

4 Pre-concentration by evaporation
PTFE beakers, of 500 ml capacity, were thoroughly soaked in 50% v/v Aristar HCl overnight and copiously rinsed with MilliQ water. Four hundred millilitres of sample were poured into each beaker and placed under an infra-red lamp in a laminar flow hood. After 6—7 hours, the approximately 5 ml of solution that remained was decanted into an acid-washed 20 ml volumetric flask. The PTFE beaker was rinsed with 0.2 ml Aristar HCl and several aliquots of MilliQ water, and all the washings were decanted into the flask and made up to volume; this procedure resulted in a 20-fold concentration of the sample.

Blanks of 1% v/v Aristar HCl in MilliQ water were also carried through the whole analytical procedure with each set of evaporations to monitor contamination levels.

5 Analysis by ICP-OES
All measurements were carried out using an Applied Research Laboratories (ARL) 34000C inductively coupled plasma optical emission spectrometer, with a 44-channel vacuum polychromator, operated under the compromise conditions given in Trafford (1987). A typical analytical programme determines 28 elements simultaneously, using conventional pneumatic nebulization, with an uptake rate of just over 2 ml min^{-1}.

The ARL 34000C is fitted with a scanning accessory for multi-element analysis (SAMI), which is linked to the primary slit of the polychromator. Under computer control, the SAMI displaces the primary slit in precise increments, causing the spectrum to move across the secondary slits. In this way, detailed information

on the spectral structure in the region of each analyte wavelength can be obtained.

In trace element analysis, particular care has to be taken in the interpretation of small signals, and scans obtained with the SAMI are an invaluable aid in this process. The power of the scanning facility is demonstrated for an evaporated acidic surface water which has been scanned around the Be 313.04 nm line (Figures 2a & 2b). At this wavelength, the background has some structure (Figure 2a) and it is not easy to quantify the amount of Be in the sample. However, if the computer is used to subtract the background signal from the sample signal, the difference scan in Figure 2b is produced, showing a clean beryllium peak of about 2 µg l^{-1}, which represents a concentration of 0.1 µg l^{-1} in the original sample.

Scans in the neighbourhood of analyte lines are also useful when investigating possible spectral interferences (Miles 1987). Stray light from a strong emitter such as calcium can cause a shift in the background signal, as shown in the scan around the yttrium 371.03 nm line in Figure 3. The sample is a shallow groundwater which contained about 20 mg l^{-1} calcium before pre-concentration. The evaporated sample does contain a measurable amount of yttrium, but about one third of the total signal is due to the background shift produced by the 400 mg l^{-1} calcium present in solution after pre-concentration. The background can be subtracted manually or by computer, leaving a signal of 8 µg l^{-1} yttrium, which represents a concentration of 0.4 µg l^{-1} yttrium in the original solution.
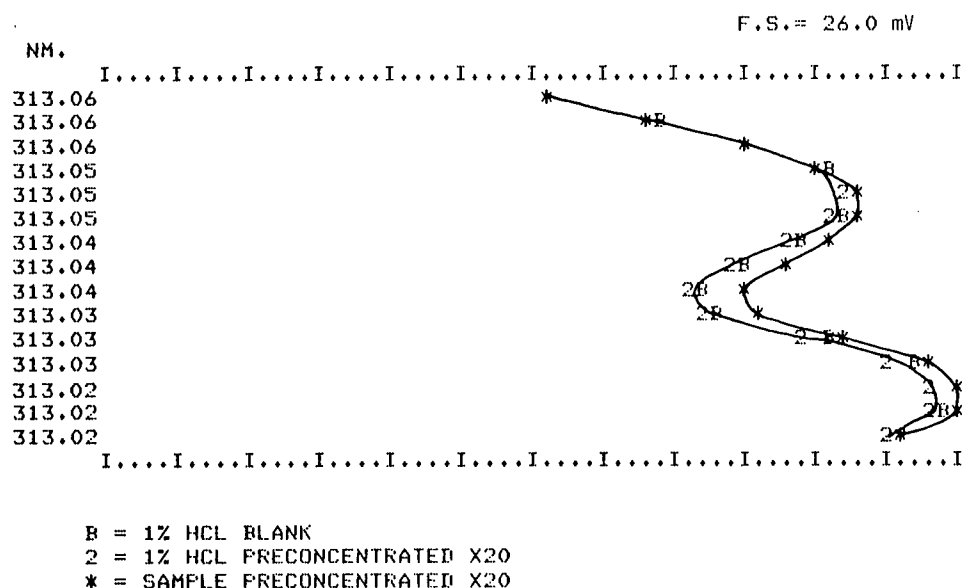
Figure 2a. Scans around the 313.04 nm beryllium line obtained while aspirating a pre-concentrated acidic surface water and blanks, indicating a small quantity of beryllium present in the pre-concentrated sample
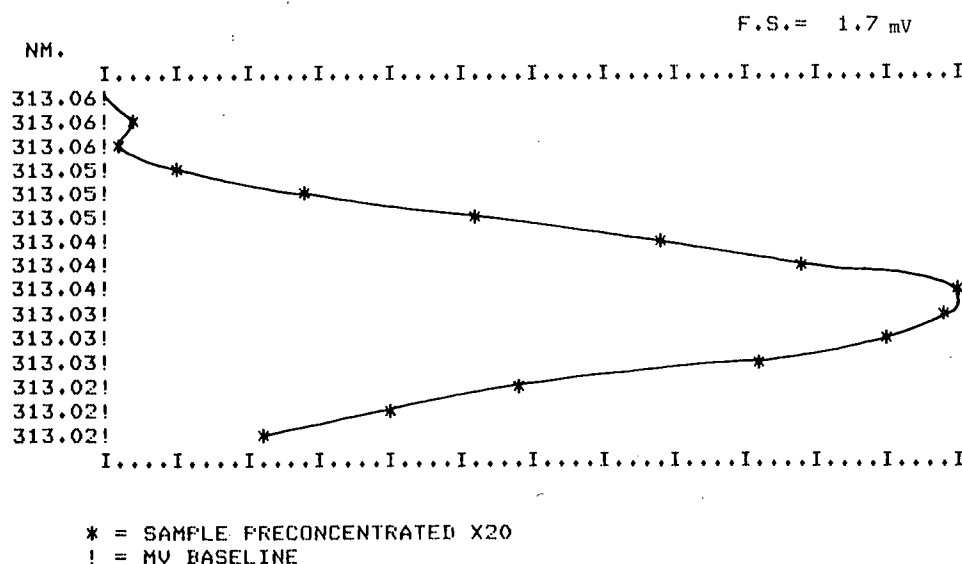
Figure 2b. Scan data in Figure 2a displayed in an alternative format by subtracting the blank from the pre-concentrated sample signal to reveal a sharp beryllium peak of about 2 µg l^{-1}

6 Data evaluation

The blanks which are carried through the pre-concentration step are normally low and reproducible but, in spite of all the precautions taken, they always contain small amounts of boron, barium, iron and zinc. A typical iron blank is about 30 µg l^{-1} Fe and is significant when compared with the iron signal from the pre-concentrated sample of rainwater (Figure 4). Such a blank will degrade the ultimate detection limit obtainable, but in practice this is very seldom a limiting factor for most of the waters under investigation.

To assess the recovery of the pre-concentration procedure, a standard solution was analysed before and after pre-concentration. Table 1 shows that the recovery is better than 95% for a wide range of elements. Typical recoveries are also illustrated by results from surface waters from Loch Fleet, which is situated in a granitic upland catchment in SW Scotland, and is the subject of a multidisciplinary project on acidic deposition and the effect of liming a catchment. Data from this project are shown in Table 2 for elements sensitive enough to be detected both before and after evaporation. The agreement is good and, after pre-concentration, extra confidence can be placed in the second significant figure.

For those elements which cannot be determined directly by ICP-OES, pre-concentration by a factor of 20 permits measurements of several elements well below 1 µg l^{-1} (Table 3). The figures for lead


```
                                           F.S.= 13.6 mV
   NM.
        I....I....I....I....I....I....I....I....I....I....I....I....I....I
371.06
371.05
371.05
371.05
371.04
371.04
371.04
371.03
371.03
371.03
371.02
371.02
371.02
371.01
371.01
371.01
        I....I....I....I....I....I....I....I....I....I....I....I....I....I

        B = 1% HCL BLANK
        2 = 1% HCL BLANK PRECONCENTRATED X20
        * = SAMPLE PRECONCENTRATED X20
```
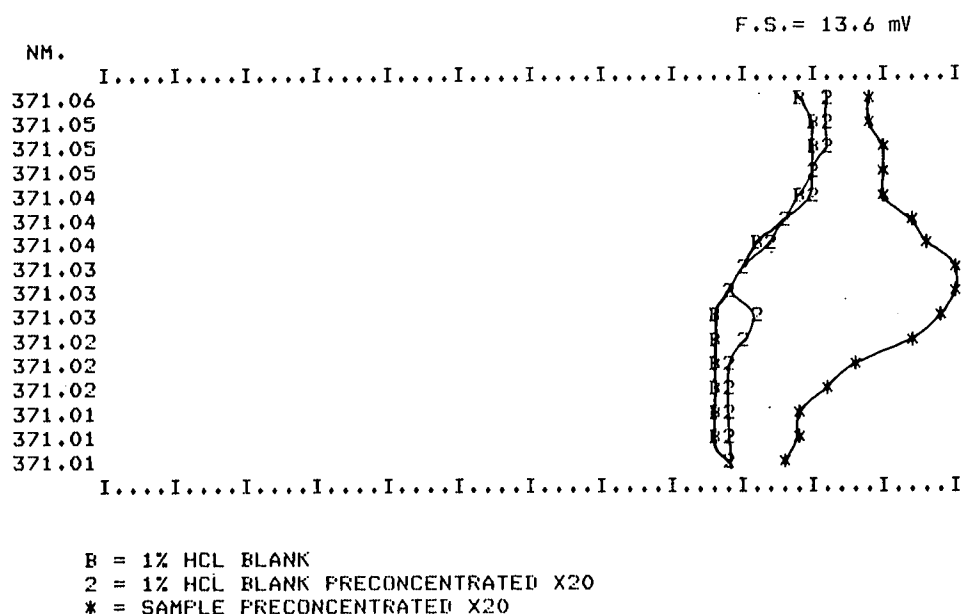
Figure 3. Scan around the 371.03 nm yttrium line obtained by aspirating a pre-concentrated shallow groundwater, showing the background shift caused by 400 mg l^{-1} Ca present in the pre-concentrated sample


```
                                        F.S.= 29.3 mV
   NM.
        I....I....I....I....I....I....I....I....I....I....I....I....I....I
259.96
259.96
259.96
259.95
259.95
259.95
259.94
259.94
259.94
259.93
259.93
259.93
259.92
259.92
259.92
        I....I....I....I....I....I....I....I....I....I....I....I....I....I

        B = 1% HCL BLANK
        2 = 1% HCL BLANK PRECONCENTRATED X20
        * = SAMPLE PRECONCENTRATED X20
```
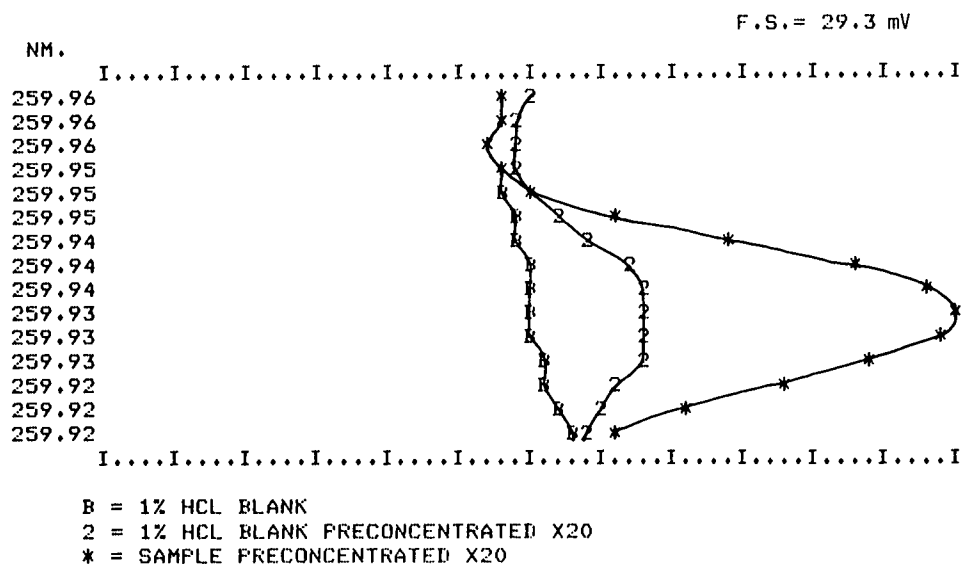
Figure 4. Scan around 259.94 nm iron line illustrating that a typical pre-concentrated iron blank contains a significant amount of iron compared with a pre-concentrated sample of rainwater

indicate that the improvement in detection limit is greater than a factor of 20; this is a result of the extra work involved in interpreting the small signals above background, using the scans, which would not be done normally in routine analysis.

Table 1. Recoveries obtained by evaporation of a standard solution. Concentrations in µg l⁻¹

Element	Before evaporation	After evaporation ×20	% recovery*
Y	44	846	98
Ba	48	942·	98
Mn	44	863	98
Cu	46	873	94
Fe	44	896	100
V	47	900	96
Cd	20	397	99
Co ·	40	811	101
Zn	38	765	99
Ni	154	3060	99
Mo	154	3010	97
Al	178	3380	93
Pb	163	3330	102

*All recoveries calculated after blank correction

Table 2. Comparison of major and minor element determinations on samples from Loch Fleet outflow (30.8.85) before and after pre-concentration

Element	Original measurement (mg l⁻¹)	After pre-concentration (mg l⁻¹)
Na	4.0 ·	4.0
K	<0.5	0.22
Ca	0.82	0.86
Mg	0.52	0.56
SO₄	4.4	4.5
Sr	0.005	0.0052
Ba	0.039	0.042
Mn	0.17	0.17
·Fe	0.13	0.13
Zn	0.032	0.035
Al	0.18	0.18

Table 3. Comparison of trace element determinations on samples from Loch Fleet outflow (30.8.85) before and after pre-concentration

Element	Original measurement (µg l⁻¹)	After pre-concentration (µg l⁻¹)
B	<35	12
Li	<10	0.9
Be	<1	0.10
Y	<2	0.08
V	<20	0.3
Cu	<10	1.6
Ni	<100	4
Pb	<200	13

Table 4. Chemical data for Loch Fleet inflow and outflow (30.8.85) compared with bulked rain for August 1985

Element	August rain	Inflow	Outflow
	mg l⁻¹		
Na	1.3	2.5	4.0
Mg	0.13	0.32	0.56
SO₄	1.4	2.6	4.5
	µg l⁻¹		
Al	<5	300	180
Fe	2.6	120	130
Mn	1.3	23	170
Sr	1.6	3.2	5.2
Cu	1.5	1.2	1.6
Y	<0.05	0.21	0.08
Be	<0.05	0.06	0.10
Ni	<1	<1	4

7 Results

Representative trace element data obtained using the technique of pre-concentration by evaporation and measurement by ICP-OES from the Loch Fleet catchment are shown in Table 4, as an example of the precision and sensitivity of the method. A selection of the data for the outflow and major inflow to Loch Fleet are compared with those for the bulked rainfall for the same month. Some of the main features of the data generally are the following.

i. Sodium, magnesium and sulphate show a similar proportional increase in concentration between the rain, the major inflow and the outflow.

ii. The aluminium data represent the total fraction less than 0.45 µm. From several months' data it has been observed that these values for the inflow are variable and usually greater than the concentration in the outflow, whereas the aluminium level in the outflow is normally between 0.18 and 0.19 mg l⁻¹.

iii. For iron, the inflow and outflow concentrations are about the same and there is no apparent trend. In contrast, manganese displays an order of magnitude increase in the outflow over the inflow.

iv. Reliable low level data for strontium have been obtained which show that strontium behaves in a similar manner to many of the major elements.

v. There are similar levels of copper in the rain, inflow and outflow waters indicating that rain is probably the major source of copper in the catchment.

vi. Traces of yttrium and beryllium are detected, which are probably derived from the granite; there is constantly more yttrium in the inflow than the outflow.

vii. Nickel is always detected in the outflow.

8 Conclusions

To obtain reliable trace element data, it is necessary to appreciate the problems which may arise and the precautions which need to be taken all the way through sample collection, sample storage, laboratory analysis and data interpretation. A method of pre-concentrating water samples by simple evaporation prior to analysis by ICP-OES was originally developed for trace element analysis of groundwaters and has been successfully applied to rain- and surface waters.

9 Summary

Sample collection, sample storage, laboratory analysis and data interpretation are 4 interdependent stages in the measurement of any environmental sample. Each stage will influence the accuracy and precision of the final result, and this is particularly true when handling natural waters where the elements of interest may be present at well below 1 mg l^{-1}. The problems which arise and the precautions which need to be taken are discussed.

Detection limits obtained by ICP-OES are not sufficiently sensitive to determine some important trace elements directly, and pre-concentration of the sample may be necessary. However, any pre-concentration technique used must be applicable to many elements, in order to utilize the multi-element capability of the ICP. A method of pre-concentrating water samples by simple evaporation under ultra-clean conditions is discussed.

Primary spectroscopic data from a sophisticated computer-controlled instrument such as an ICP require careful interpretation, especially when deciphering small signals close to the detection limit. Typical data on trace element levels in groundwaters and dilute surface waters subject to acidic deposition are presented.

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Investigation of spectral interferences in the determination of trace elements in water by ICP-OES

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1 Introduction

Inductively coupled plasma optical emission spectrometry (ICP-OES) is an established technique for the inorganic analysis of waters for major, minor and trace elements. At the British Geological. Survey's (BGS) laboratories at Wallingford, an Applied Research Laboratories 34000C ICP is at present calibrated for the simultaneous determination of 28 elements in groundwaters; it operates under compromise conditions given in Table 1.

Table 1. Compromise ICP operating parameters for the ARL 34000C

Spectrometer	ARL 34000C Quantovac; 1-m Paschen-Runge mounting; grating ruled 1080 lines mm^{-1} Primary slit width 20 µm Secondary slit width range 37.5—75 µm
Frequency	27.12 MHz
Forward power	1080 W
Reflected power	0—5 W
Observation height	17 mm above load coil
Argon flow rates	Coolant 11 l min^{-1}, plasma 1.2 l min^{-1}, carrier 1 l min^{-1}
Sample uptake rate	2.5 ml min^{-1}
Sample acidity	1% v/v HCl
Nebulizer	Concentric glass, J E Meinhard model TR-30-A3
Read-out	Digital read-out of mean signal from 3 10-second integrations

The Hydrogeochemistry Section of BGS Wallingford has a particular interest in the occurrence and behaviour of trace elements in groundwaters and surface waters vulnerable to acidic deposition, funded by a major commissioned research contract from the Department of the Environment (DoE) and various science budget projects. Because the natural levels of many elements in these waters are extremely low, a pre-concentration procedure, based on evaporation under infra-red lamps in ultra-clean conditions, has been developed. However, this method of pre-concentration is non-selective and the resulting high concentrations of alkaline-earth elements give rise to spectral interferences. These have been thoroughly investigated and appropriate inter-element corrections entered into the instrument's data processing software to minimize their effect.

Normally, the levels of iron in natural waters are low and do not cause any problems. Recently, some iron-rich groundwaters were collected and pre-concentrated. The enhanced iron concentration in this particular set of samples, combined with the fact that iron has a very line-rich emission spectrum, made it necessary to investigate its potential spectral interference in the determination of other trace elements.

2 Investigations into spectral interferences caused by high concentrations of iron

Iron solutions of 200 mg l^{-1} Fe were prepared by dilution from 2 different stock solutions:

i. 1000 mg l^{-1} Fe AAS standard (Spectrosol, BDH Chemicals)

ii. 1000 mg l^{-1} Fe, prepared by dissolution of Fe powder (Specpure, Johnson Matthey Chemicals) in Aristar grade HCl.

Scans over small wavelength regions around the spectral lines of interest were obtained by displacing the primary slit in precise increments under computer control.

Wavelength scans of the 2 different Fe solutions were obtained for each of the trace element wavelengths listed in Table 2, and plotted against an appropriate blank. With the BDH solution, peaks were observed at the Sr, Cr and Co lines, whereas the corresponding scans of the Specpure solution were indistinguishable from those of the blank. Figure 1 illustrates the response obtained at the 407.77 nm Sr line for both Fe solutions and shows that significant amounts of Sr are present as impurities in the BDH AAS Fe solution. Similar results were

Table 2. Trace elements routinely determined by ICP-OES at Wallingford

Element	Wavelength (nm)	Element	Wavelength (nm)
Sc	361.38	Cr	267.72
Sr	407.77	Ni	231.60
Y	371.03	Mo	202.03
Ba	455.40	Al	308.21
Mn	257.61	Pb	220.35
Be	313.04	Co	228.62
Cu	324.75	Zn	202.55
V	311.07	Ce	380.15
Cd	226.50	La	398.85
Zr	343.82	B	249.68
		Li	670.78

obtained for Co and Cr.

However, for both Fe solutions, peaks of similar intensity were observed near the Zn, Zr, V and Cd wavelengths, as illustrated by a comparison of the 2 scans at the 266.50 nm Cd line in Figure 2. A search of various line tables (Brenner & Eldad 1984; Parsons *et al.* 1980; Michaud & Mermet 1982) revealed Fe lines close to, or coincident with, the Zn, Zr, V and Cd lines, as shown in Table 3.

Once the existence of spectral interferences from weak Fe lines at these analyte wavelengths had been established, it was relatively straightforward to calculate apparent concentrations for Zn, Zr, V and Cd caused by the iron present in the samples (Table 4) and to use these to automatically correct any trace element signals recorded.

Table 3. Wavelengths of interfering Fe lines listed in spectral line atlases

Element	Wavelength (nm)	Fe line (nm)	Reference
Zr	343.82	343.83	1,2
Cd	226.50	226.50	1
V	311.07	311.07	1
Zn	202.55	202.55	3

1 Brenner & Eldad (1984)
2 Parsons *et al.* (1980)
3 Michaud & Mermet (1982)

```
SCAN OF SPECTROSOL FERRIC NITRATE STANDARD AROUND SR 407.77nm

                              INC.=   0.20     F.S.= 11.9mV
    NM.
        I....I....I....I....I....I....I....I....I....I....I....I....I
    407.82                       B*
    407.81                       B *
    407.81                       B    *
    407.80                       B      *
    407.79                       B          *
    407.79                       B             *
    407.78                       B                *
    407.77                       B                   *
    407.76                       B                       *
    407.76                       B                       *
    407.75                       B                    *
    407.74                        B              *
    407.74                        B         *
    407.73                         B    *
    407.72                         B *
    407.72                         B *
        I....I....I....I....I....I....I....I....I....I....I....I....I

        B = 1% HCL BLANK
        * = 200 PPM FE


SCAN OF STANDARD PREPARED FROM SPECPURE IRON POWDER
AROUND SR 407.77nm

                              INC.=   0.10     F.S.=  6.0mV
    NM.
        I....I....I....I....I....I....I....I....I....I....I....I....I
    407.82                                              *
    407.81                                              B*
    407.80                                              *
    407.80                                             *B
    407.79                                             *B
    407.78                                             *B
    407.78                                             *B
    407.77                                             *
    407.76                                             *B
    407.76                                             *
    407.75                                             *B
    407.74                                              *
    407.74                                               *B
    407.73                                              *
    407.72                                               *B
    407.72                                              *
        I....I....I....I....I....I....I....I....I....I....I....I....I

        B = 1% HCL BLANK
        * = 200 PPM FE
```
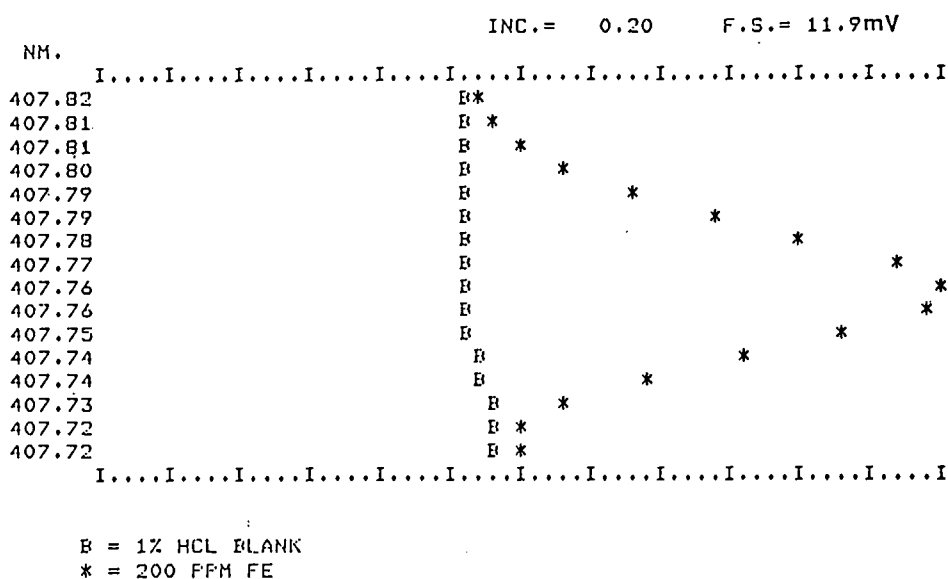
Figure 1. Scans around the 407.77 nm Sr line of a BDH iron solution and a standard prepared from Specpure iron powder reveal that there is a significant strontium impurity in the BDH solution

Table 4. Apparent analyte concentration caused by the presence of 200 mg l⁻¹ Fe

Element	Apparent concentration mg l⁻¹
Zr	0.025
Cd	0.054
V	0.023
Zn	0.023

3 Conclusions

3.1 Comparison of 2 iron standards showed the need for careful selection of contamination-free stock solutions in multi-element analysis.

3.2 Good line atlases are invaluable as an aid to identifying interfering lines when augmented by spectral scans.

3.3 The effect of sample pre-treatment must be taken into account. Spectral interferences not normally encountered in water analysis by ICP-OES become significant following sample concentration by evaporation.

4 References

Brenner, I.B. & Eldad, H. 1984. A spectral line atlas for multitrace and minor element analysis of geological and ore mineral samples by ICP-AES. *ICP Inf. Newsl.,* **10,** 451—508.

Michaud, E. & Mermet, J.M. 1982. Iron spectrum in the 200-300 nm range emitted by an inductively coupled argon plasma. *Spectrochim. Acta,* **37B,** 145—164.

Parsons, M.L., Forster, A. & Anderson, D. 1980. *An atlas of spectral interferences in ICP spectroscopy.* New York: Plenum.

```
SCAN OF SPECTROSOL FERRIC NITRATE STANDARD AROUND CD 226.5nm

                          INC.=    0.26     F.S.= 15.4mV
NM.
       I....I....I....I....I....I....I....I....I....I....I....I
226.52                                        B *
226.52                                          *
226.52                                        B*
226.51                                         B   *
226.51                                        B       *
226.51                                      B         *
226.50                                      B         *
226.50                                    B       *
226.50                                E          *
226.49                                B    *
226.49                                B      *
226.49                                  B *
226.48                                      B*
226.48                                          B *
226.48                                             B*
226.47                                                B *
       I....I....I....I....I....I....I....I....I....I....I....I

       B = 1% HCL BLANK
       * = 200 PPM FE


SCAN OF STANDARD PREPARED FROM SPECPURE IRON POWDER
AROUND CD 226.5nm
                          INC.=    0.27     F.S.= 16.1mV
  NM.
       I....I....I....I....I....I....I....I....I....I....I....I
226.52                                        B*
226.52                                        B*
226.52                                        B*
226.51                                         B  *
226.51                                         B    *
226.51                                         B      *
226.50                                        B         *
226.50                                       B         *
226.50                                      B        *
226.49                                      B    *
226.49                                     B    *
226.49                                      B *
226.48                                        B *
226.48                                            B*
226.48                                               *
226.47                                                B *
       I....I....I....I....I....I....I....I....I....I....I....I

       B = 1% HCL BLANK
       * = 200 PPM FE
```
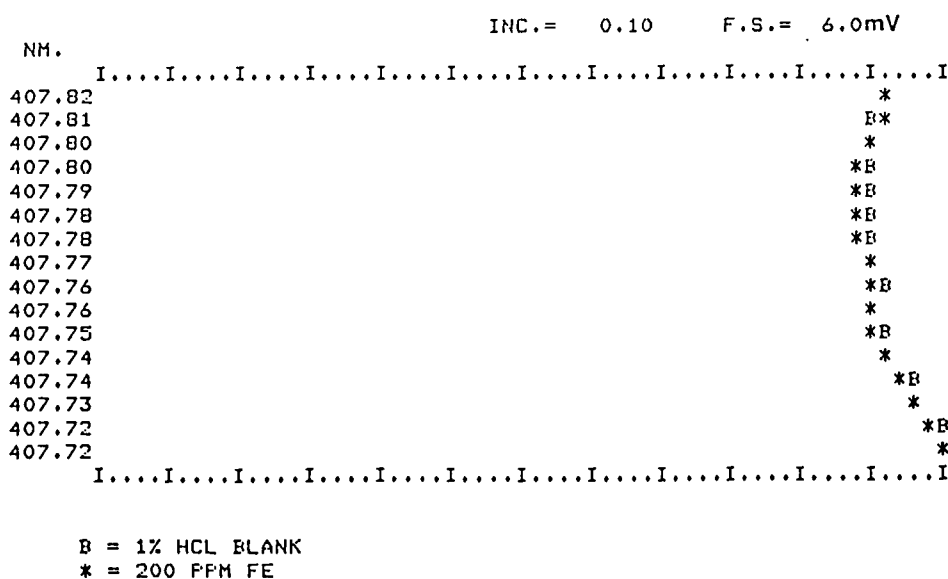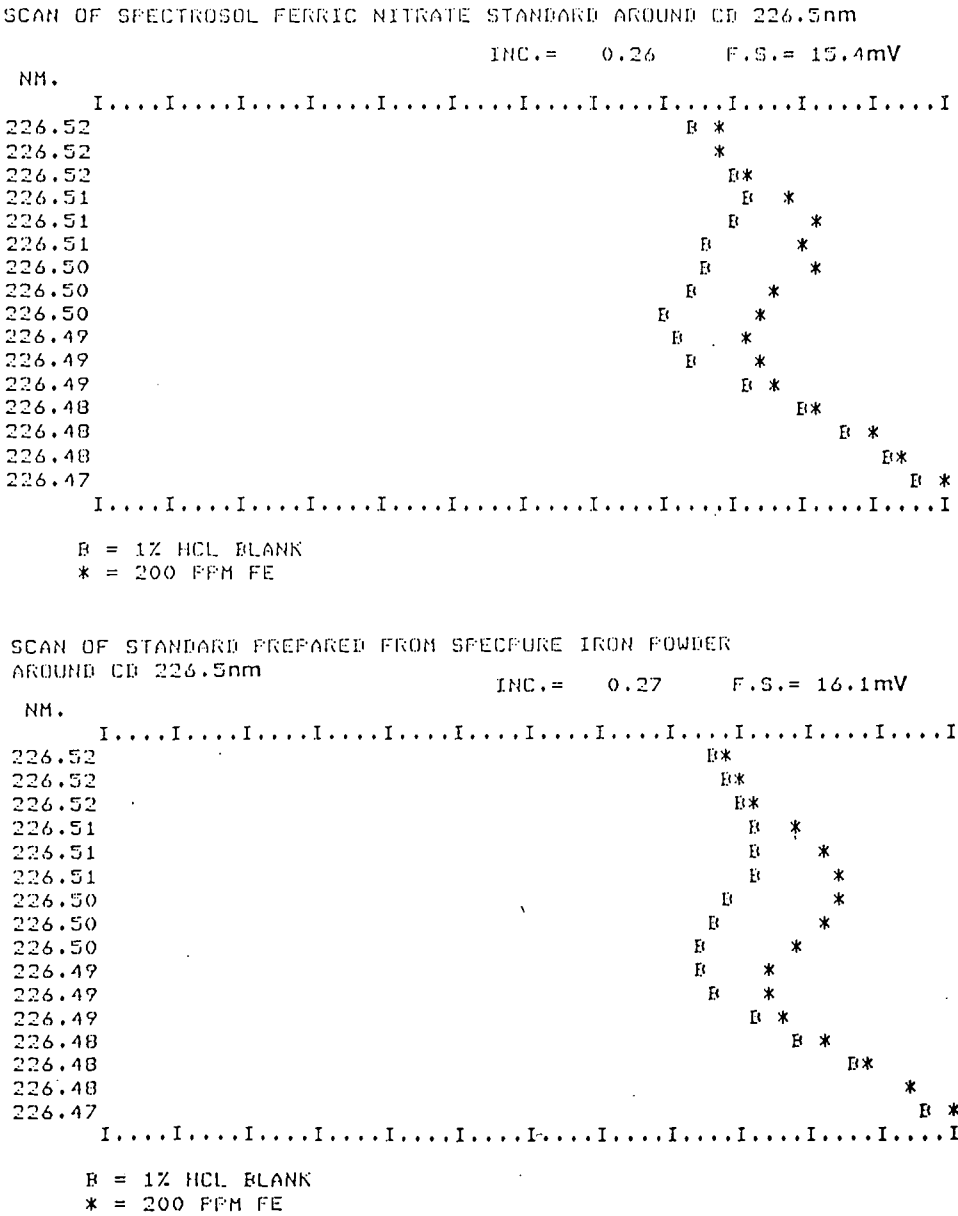
Figure 2. Scans around the 226.50 nm Cd line of a BDH iron solution and a standard prepared from Specpure iron powder give very similar responses. This is due to the presence of a weak iron line also at 226.50 nm

Chemical sensors for environmental research

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1 Introduction

Analytical chemistry is an essential part of environmental research. Chemical systems are of great significance in the lithosphere, hydrosphere, biosphere and atmosphere: qualitative and quantitative estimation of these systems is a primary requisite to the understanding of the ecology of each of these areas. Hitherto, the analytical chemistry has been laboratory-based, samples being brought from the field for analysis. Although this arrangement has served the needs of environmentalists adequately for many years, portable analytical techniques that can be taken out to the sample will open up new areas for probing and understanding the environment.

A general class of devices that could provide such a service are the chemical sensors. The term 'chemical sensor' is rather diffuse; just what constitutes a chemical sensor is rather difficult to define. At the very least, it should encompass devices that can carry out an analytical procedure with the minimum of intervention from an operator. In addition, it should be portable and capable of operating *in situ*. A chemical sensor may be the size of a pinhead or may weigh several kilograms. Its manufacture may be so simple that an ordinary laboratory could produce them (piezoelectric crystal gas monitors are good examples of this type); alternatively, complex manufacturing facilities may be required such as those needed for the production of ion-sensitive FETS. Finally, a chemical sensor may be located anywhere within the environment, from the human blood stream to the bottom of the North Sea.

In spite of the diversity of chemical sensors discussed in the previous paragraph, all chemical sensors have a number of similar characteristics. Somewhere in the sensor is a transducer, that transforms a chemical property of the analyte (usually concentration) into an electrical signal. Some transducers are inherently selective; for example, the LEDs that are used in non-dispersive IR gas sensors emit the wavelengths at which the target gas molecules absorb. Other transducers are not selective, and selectivity must be provided by incorporating a piece of selective chemistry into their structure. Often the simplest way to do this is by coating the transducer with a membrane of some form in which the specific chemistry is immobilized. ISEs are an example of this sort of approach. Similarly, all sensors should be rugged devices that respond sensitively and rapidly to the target analyte. A few selected examples of chemical sensors are presented in this paper to give an idea of the range and current status of chemical sensing.

2 Gas sensing with piezoelectric crystal monitors

Not only must a chemical sensor respond to a particular molecular species, or group of related compounds, but it must also discriminate between the target analyte and the full range of other chemical species in the immediate environment. In the case of gas or vapour sensors based on piezoelectric crystals, chemical coatings are applied to the crystal in an attempt to introduce some degree of selectivity. Water vapour, which is at a relatively high concentration in the atmosphere, tends to interact in a non-specific way with these coatings. The aim of this work was to build a system that could be operated at 'realistic' temperatures and humidities, ie a sensor that could be operated in the environment, without the need for thermostatting, removal of water vapour, or frequent calibration. This paper discusses a method of doing this, in which archival data are collected, and used interactively with real-time data, to produce a corrected response to the target analyte (ammonia in this case).

2.1 Apparatus

The use of piezoelectric transducers as gas monitors has been described elsewhere (Edmonds & West 1982) and a review of the field has recently appeared (Alder & McCallum 1983). Details of the crystals, oscillating circuitry and cell design are presented by Edmonds and West (1982). The cells used in this study were made of stainless steel; a small temperature sensor was located in the base of one of the cells. A 2-stage gas dilution system was used to provide a continuous stream of clean, dry laboratory air which could be suitably loaded with water vapour from a humidifying line, and ammonia from a certified air/ammonia mixture supplied in a pressurized cylinder by Rank Hilger. The humidifier and the detector cells were located in a thermostat water bath. The entire gas rig, the detector cells, humidifier, water bath and Shaw 'Bradford' Yellow Spot humidity probe were enclosed in a constant temperature box. The temperature in this box could be maintained at a constant value with a tolerance of ±0.5°C. The signal from each of the 2 sensors used in this study was read by a frequency meter (AMF Venner 7737), and was subsequently passed to a

microcomputer system based on an Intel 80/10B.

2.2 Results and discussion

Two sensor crystals were used, one coated with polyvinyl pyrollidone (PVP), and the other with silver chloride. PVP-coated crystals responded rapidly and sensitively to ammonia and water vapour in the gas stream: silver chloride-coated crystals responded to water vapour only. Extensive studies of these 2 sensors were carried out, with a view to using them in tandem for the determination of atmospheric ammonia concentrations. In this work a number of variables were identified that determined the response of the PVP-coated crystals to ammonia. These variables are summarized in Table 1.

Table 1. Variables affecting the response of the PVP-coated crystal to ammonia

Variable	Comments
Coating mass of PVP	The amount of ammonia is a positive function of the mass of PVP on the crystal. This variable must be accounted for in a real system.
Temperature	Both the sorption of water and ammonia were strongly influenced by temperature. An optimum was noted in each case, and no simple relationship was observed. This variable must be accounted for in a real system.
Humidity	Not only did the PVP sorb water, but also the amount of ammonia sorbed was decreased for a given gas stream concentration, indicating some competition for sites between the 2 species. This variable must be accounted for in a real system.
Flow rate	The response to ammonia was a complex function of the flow rate of the carrier gas through the detector cells. In a portable system this variable could be held at a constant value.

Clearly, to build a sensor system that could be operated in the real world, the response from the PVP-coated crystal needed to be corrected for the variables shown in Table 1. Such a system could only be constructed around the processing power of a microcomputer, in which real-time data from various sensors could be used with archival data generated at an earlier stage, to produce a corrected response from the PVP-coated crystal. This, indeed, was our approach. In some instances, we were able to write a simple algorithm that related to the response of the crystal to a variable, eg coating mass. In other cases, the relationship between the variable and the response was far from clear. Under these circumstances, look-up tables were constructed

in the microcomputer's firmware. These tables contained, for example, the response *versus* concentration profile for ammonia at various temperatures at a PVP-coated crystal. The data used for these algorithms and look-up tables were generated over a period of time and for a range of conditions. Wherever possible, the individual data points represented the mean of many readings, the intention being to generate a true archival data set. Once this data set had been generated, it could be used with real-time data from the other sensors in the system (temperature probe read-out, and response from the silver chloride-coated crystal), and the coating mass of the PVP crystal to generate from the response of the PVP-coated crystal a value for the concentration of ammonia.

There are several stages involved in calculating the ammonia concentration from the response of the PVP-coated crystal. First, the response is corrected for the additive effect of water vapour. Real-time data from the silver chloride-coated crystal and the temperature probe are used here, in conjunction with archival data relating the response of the 2 different piezoelectric sensors to water vapour. This procedure also generates data that can be used to assess how much water has been sorbed by the PVP coating, and hence to calculate a correction factor for the diminished sorption of ammonia that occurs in the presence of water sorption. The coating mass is then used to correct for the variation in response that arises for different coating masses. Finally, the temperature is used again, along with the water vapour and mass-corrected response from the PVP-coated crystal, to select an appropriate value for the ammonia concentration from the look-up table. Results from such a set of calculations are shown in Table 2.

Table 2. Results for the determination of ammonia at a PVP-coated crystal

Sample concentration ppb	Analysed concentration ppb	% difference
100	100	0
480	286	41
4000	4400	10
4000	3625	9.5
400	370	8.3
1000	800	20
9	10	10
1000	598	40
4320	6	—
300	6	—
100	6	—
480	6	—

2.3 Conclusions

The results in the first part of the Table are acceptable: a wide range of ammonia

concentrations are adequately assessed at various humidities and several temperatures. In the second part of the Table, one of the results was obtained for a freshly prepared crystal, the other for a crystal at the end of its working life (approximately one month for a PVP-coated crystal and 3 months for a silver, silver chloride-coated crystal). A variable that has not been accounted for in this system is ageing of the coating. This is a complex phenomenon, that alters the PVP-crystal's response to both ammonia and water vapour. All the archival data were obtained for crystals in between the 2 age extremes, as were the results in the first part of the Table. The final 2 results in the Table are not explicable.

There is a need to improve and refine the methods that have been used. The effect of coating ageing should be included, as well as applying a coating mass and temperature correction to the response of the silver chloride crystal before using it for water vapour correction. Not only should a wider range of data be used for this work, but more sophisticated methods of processing the data should be tried.

3 pH measurement with low-cost fibre optic chemical sensors

Fibre optic probes are chemical sensors, but, compared to, say, piezoelectric crystals or ion-sensitive electrodes, the overall chemical transducer is rather complicated. The fibre optic probe is a complex transducer consisting of 2 optical/electronic arrangements associated with the light source and the signal detector. However, a workable fibre optic probe can be constructed using much simpler light source and detector systems than have been employed hitherto.

3.1 Apparatus

In keeping with the objective of simplicity and low cost, step-index polymer cable consisting of a polymethyl methacrylate core with fluorinated polymer cladding was selected. The core/cladding diameter (1.00 mm), numerical aperture (0.47) and attenuation (150 dB kM at 600 mm) render this cable unsuitable for transmission of data over long distances (on account of both the attenuation and the modal dispersion), but for short lengths of cable (in this case less than 50 cm) the cable is adequate. An obvious source to use was a light-emitting diode (LED). LEDs exhibit a virtually linear relationship between radiated power and forward current, and this, coupled with their rapid rise time (typically 100 ns), simplifies the design of the power supply (an appropriate voltage source and limiting resistor is adequate) and obviates the need for mechanically chopping the light, should a pulsed system be desirable. In this work, a Honeywell 'Sweetspot' diode was used with a peak emission wavelength

of 665 nm and a bandwidth of 22 nm. The LED was run at a continuous forward current of 50 mA. The bandwidth is typical of surface-emitting LEDs; edge-emitting devices may have a narrower band pass. The 'Sweetspot' range of optoelectric devices is designed to couple with fibre optic system via 'DNP' connectors. The relatively narrow bandwidth and ease of coupling to fibre optic cables are 2 further advantages of LEDs. On the debit side, the emitted power of an LED is subject to variation due to both ageing effects and temperature, and the restricted range of wavelengths available is a severe limitation too. The detector was a 'Sweetspot' PIN photodiode. In common with most devices of this type, the diode is sensitive to light throughout the visible region and the near infra-red, but is relatively more sensitive at longer wavelengths. When the diode is reverse-biased, it acts as a high impedence variable current source, the current being a linear function of the intensity of light incident on its active surface. The circuitry for amplifying this signal is straightforward, consisting of a JFET operational amplifier configured as a current to voltage converter with a gain of 10^6.

3.2 Probe design

All the probes that were used consisted of independent source and detector optical fibres, brought into close physical contact at the sensor end. The sensor consisted of an immobilized zone of coloured indicator. Two methods were used for immobilization: sorption of the indicator on to XAD-2 beads (cross-linked styrene divinyl benzene copolymer) (Kirkbright et al. 1984a, b); and incorporation of the indicator into a porous membrane such as 'collodion' (cellulose nitrate), usually with 5 μm beads of XAD-2 to improve the
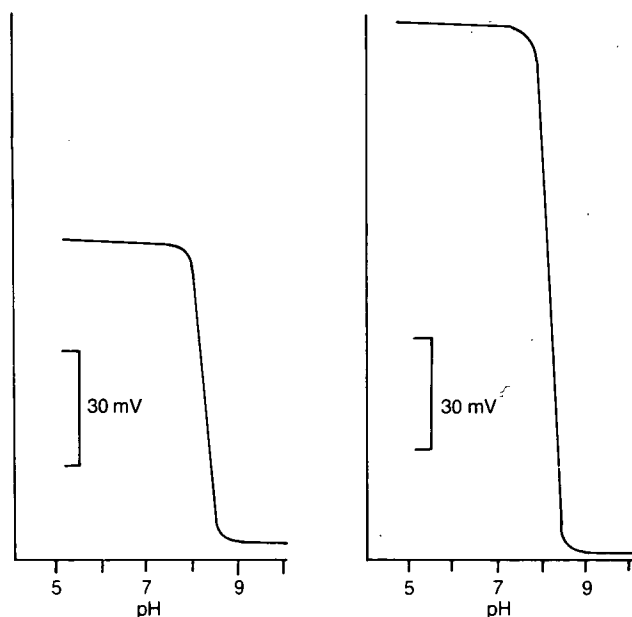

Figure 1. pH response of collodion membrane probes

light scattering in the membrane. The target in this work was fabrication of a device that would respond to pH in the 'environmental' range of 4.0–8.0. Bromothymol blue was chosen as the indicator, having a λmax at 620 nm and pH transition interval from 6.0 to 7.6. Typical responses to pH for collodion membrane sensors are shown in Figure 1. Two facts are immediately obvious from these curves. First, the pH transition range is not in the 6.0–7.5 region, but takes place some 1.5 pH units later. This is not unexpected; a shift in the value of pH transition region, and/or a shift in the absorption spectrum, due to absorption of indicator on to colloids, is well known (Klotz 1947), and has been deliberately used to improve spectrophotometric methods (Dagnall et al. 1967). The variation in response for the 2 probes also comes as no surprise. Conservative calculations indicate that less than 0.5% of the incident light is reflected back along the detector fibre, and this inefficiency, coupled with the rather rudimentary probe design, is bound to lead to poor probe-to-probe irreproducibility. The time taken to reach an equilibrium response is a function of membrane thickness (see Table 3).

One of the probes was tested for reproducibility by taking readings in buffers of pH 4.0, 7.0 and 10.0 respectively. The results are shown in Table 4.

We have investigated the effect of immobilization on λmax and the pK_{ind} of a range of pH indicators. The results are summarized in Table 5.

3.3 Conclusions

The simple low-cost system described here has demonstrated that low-cost fibre optic chemical sensors may be constructed. Clearly, a number of features need to be improved, not least being the probe design, both from the point of view of efficiency and reproducibility. The source and detector electronics should be pulsed and tuned respectively to enable the discrimination of the chemical signal from the ambient light. (All the work reported here was carried out with the sensor in a light-tight box.) Reproducibility and drift in the system should be investigated more rigorously, but a reference channel (ie a green LED) should improve the system performance here. Perhaps the most interesting

Table 3. Response time of fibre optic pH probe for different membrane thicknesses

Membrane thickness (mm)	Response time (min)
3	30
2	15
1	7.5

Table 4. Reproducibility of fibre optic pH probe

| Buffer solution pH | Response to pH (mV) | | | |
	1	2	3	Mean
4.0	138.0	140.0	139.0	139.8±1.2
7.0	137.8	137.7	137.5	137.7±0.2
10.0	66.0	67.6	67.8	67.1±1.1

Table 5. Effects of immobilizing pH-sensitive indicators

Indicator	Form	Colour change (increasing pH)	λmax (nm)	Δλmax (nm)	pK_{ind}	ΔpK_{ind}
Methyl red	Aqueous/methanolic immobilized	Red-yellow Red-yellow	525 540	+15	5.1 8.7	+3.6
Bromothymol blue	Aqueous/methanolic immobilized	Yellow-blue Yellow-blue	616 620	+4	7.2 10.9	+3.7
Bromocresol green	Aqueous/methanolic immobilized	Yellow-blue Yellow-blue	616 615	+1	4.6 4.6	0
Bromophenol blue	Aqueous/methanolic immobilized	Yellow-purple Yellow-royal blue	590 600	+10	4.0 4.6	+0.6
Phenol red	Aqueous/methanolic immobilized	Yellow-red Yellow-red	560 580	+20	7.7 9.0	+1.2
Cresol red (1)	Aqueous/methanolic immobilized	Pink-yellow Pink-yellow	520 540	+20	1.0 4.3	+3.3
Cresol red (2)	Aqueous/methanolic immobilized	Yellow-red Yellow-red	570 590	+20	8.1 9.1	+1.0

conclusion from this work is that there is every possibility of modifying the chemistry of the sensor to suit the rather restricted wavelength range available from commercial LEDs. Thus, acid-base indicators whose pH transition seemed too low for the production of an 'environmental' pH probe may yet be of use, because of the shift of pK_{ind} arising from immobilization in a membrane. A concomitant shift in the λmax also makes the choice more appropriate. We noted a more extended pH response when the indicator was incorporated in a membrane in which amine groups were known to be present in significant numbers. Our interpretation of this finding is that some form of buffering occurs within the membrane. If this is so, then other exciting possibilities are opened up for tuning the chemistry of the system.

4 Ion sensing with coated wire electrodes
Ion-sensitive electrodes represent a relatively mature sensor technology, and their applications in soil, plant and food analysis are legion. In spite of their frequency of use, these devices (ISEs) suffer from some serious problems, not least being their lack of selectivity. Other problems include drift and deterioration of electrode performance with time, both these effects leading to the frequent need to recalibrate the electrode. A related type of sensor exhibits similar disadvantages, but has the singular merits of being small and disposable. Consequent upon this, the whole design philosophy of chemical monitoring is altered. Instead of frequent and often labour-intensive recalibrations, the monitoring system can switch from one sensor to another, as the end of the reliable calibration life-span of the sensor is approached. An alternative strategy concerns the use of the sensor as a one-shot disposable probe for situations where sample integrity must be maintained and/or cross-contamination avoided.

The device concerned is the coated wire electrode (CWE). Essentially, it could be viewed as a simplified ISE in which the internal reference electrode and filling solution have been eliminated. CWEs may be fabricated by simply coating a metal conducting wire with an appropriate membrane-forming material (such as PVC) in which a suitable receptor system is located. Typical receptor systems include: valinomycin for potassium ions (Cattrall *et al.* 1974); sodium, ammonium or potassium salts of tetraphenylborate for ammonium ions (Hopirtean & Stefaniga 1976); Aliquat 336S (suitably prepared as the salt of the target anionic species) for detergent and simple anionic species such as nitrate (Fujinaga *et al.* 1974; Kneebone & Freiser 1973). More recently, the PVC membrane has been omitted, and sensor species such as antigens or antibodies, or polymer films, have been coated directly on to the wire (Solsky 1982; Heineman *et al.* 1980). A review of CWEs has recently appeared in Cattrall and Hamilton (1984).

CWEs suffer from 2 distinct problems, not shared by their more conventional counterparts. There is an absence of a formal thermodynamically describable internal reference system. In conventional ISEs, the potential that develops across the sensing membrane is sensed by, and measured with respect to, 2 reference electrodes, one internal and one external to the electrode. The measured potential for the entire system can then be thought of as containing 2 components. An analyte-activity-insensitive potential, which is assumed constant for all measurements, arises from the potentials of the reference systems and other sources. The analytically significant signal arises from the boundary potentials at the 2 membranes solution interfaces, and any diffusion potential caused by variation of the counter ion concentration through the membrane. In the CWE, the former potential is determined by the well-defined potential of the external reference electrode, and by a rather loosely defined system that arises at the internal metal/membrane interface. This is of less consequence when compared to the second problem associated with CWEs: their response is sensitive to the mole fraction of receptor species in the membrane. To a first approximation, this is not so for ISEs, and has the implications that the response of a CWE will drift as reagent is leached from the membrane. This response will be accentuated for some electrodes at low concentrations of the primary analyte ion. In addition, the response of a CWE will be a function of the degree of water absorption from the analyte solution.

4.1 Apparatus
The coated wire electrodes were prepared by an adaptation of a method proposed by Freiser (1980). A piece of copper wire approximately 6 cm long was cut, and one end was 'sanded' smooth with fine-grade emery paper. The wire was then washed with laboratory detergent and water, thoroughly washed with distilled water, and dried with acetone. It was then rinsed with chloroform and allowed to dry.

The wire was hung in a vertical position; the smooth end of the wire was dipped rapidly in the coating solution several times, and the film of the coating solution left on the wire was allowed to dry in air for about 30 seconds. This dipping and drying procedure was repeated until a plastic bead approximately 2 mm in diameter was obtained. This bead was allowed to dry in air for about 30 minutes; the exposed part of the wire was wrapped in 'Vitafilm' (Goodyear) and the sensor bead was placed to soak overnight in a 10^{-1} M solution of a nitrate salt (usually sodium).

After soaking, the electrode tip was washed with distilled water, dabbed dry with a tissue,.and was then ready for use.

Electrodes incorporating 3 basic types of electroactive material were tested:

i. quaternary ammonium salts based on large organic groups
ii. transition metal-organic ligand complexes
iii. nitron-nitrate precipitate.

i. Two different commercially available quaternary ammonium salts were used:

Tetradodecyl ammonium bromide (Fluka)

Aliquat 336S (Tricaprylmethyl ammonium chloride) (Aldrich)

ii. The transition metal-ligand complexes tris (1,10-phenanthroline), iron (II) nitrate (Cattrall *et al.* 1974), bis (neocupreine) copper (I) nitrate (Hopirtean & Stefaniga 1976), Nickel (II) biquinolyl nitrate (Fujinaga *et al.* 1974) and tris (2,2-Bipyridyl) nickel (II) nitrate were prepared from the appropriate metal salts and chelating agents, as described in the literature. These complexes were characterized, where possible, by their UV-visible absorption spectra.

iii. The precipitate of nitron-nitrate was prepared from commercially available nitron (1,4-diphenyl-4,5-dihydro-3,5-phenylimino-1,2,4-triazole hydroxide, inner salt) (BDH chemicals) and a solution of a nitrate salt as described in the literature (Cope & Barab 1917; Collins 1907).

The electrode coating solutions were prepared by dissolving the electroactive material in tetrahydrofuran (or cyclohexanone) along with polyvinyl chloride powder (ICI) and a suitable plasticizer (a dialkyl phthalate) if required.

All potential measurements were made on a Radiometer PHM 64 digital pH meter against an EIL saturated calomel reference electrode. The measurements were made in unstirred standard solution at ambient temperature (which was recorded).

Nitrate standards were prepared by the serial dilution of a 10^{-1} M stock solution made up with analytical reagent grade sodium nitrate in distilled water. All nitrate standards were made up in 10^{-1} M potassium dihydrogen orthophosphate solution, which has the effect of keeping the ionic strength of the standard solutions constant, and thus allows the calibration of the electrode directly in concentration units.

4.2 Results and discussion
4.2.1 Calibration
The responses of the electrodes constructed were measured in standard solutions containing 10^{-1} M nitrate. As the phosphate ions present in the standards do not contribute to the potential produced by the electrode, the response of the electrodes in the standard nitrate solutions can be described by a form of the Nernst equation, ie

$$E = E^* - 2.303\frac{RT}{F}\log(NO_3^-) \quad \text{.........................} \text{(a)}$$

where E^* is a constant which depends on the type of external reference electrode chosen, internal metallic conductor and electroactive material used. R, T and F all have their usual meaning.

Calibration curves were thus obtained by plotting the potential E (millivolts) against the negative logarithm of the nitrate ion concentration (p NO_3^-). The best response characteristics were shown by electrodes incorporating the 2 quaternary ammonium salts as ion exchangers.

Thus, tetradodecyl ammonium bromide (TDDAB)-based electrodes having the membrane composition: 4% TDDAB, 29% PVC powder and 67% di-n-butyl phthalate platicizer in tetrahydrofuran, showed a linear range of 10^{-1} M to 10^{-5} M nitrate, with an electrode slope (found by the least squares method) of between 90% and 95% of the theoretical response (see Figure 2).

4.2.2 Selectivity
The effect of the following anions, Cl^-, Br^-, I^-, SO_4^{2-} and NO_2^-, was investigated using the mixed solution method (Method III of Srinivason & Rechnitz 1969). The effects were measured at 10^{-1} M and 10^{-3} M levels of NO_3^- and 10^{-1} M levels of interferent. The results are shown in Table 6. The selectivity coefficients compare well with those obtained for commercial electrodes.

Table 6. TDDAB-based electrode: selectivity coefficients

Interferent, X	Concentration of interferent (Mole l^{-1})	Concentration of nitrate (Mole l^{-1})	$K_{NO_3,X}$
Cl^{-1}	10^{-1}	10^{-1}	3.96×10^{-3}
Cl^{-1}	10^{-3}	10^{-3}	3.39×10^{-3}
NO_2^-	10^{-1}	10^{-1}	6.10×10^{-2}
NO_2^-	10^{-1}	10^{-3}	6.14×10^{-2}
NO_2^-	10^{-3}	10^{-3}	6.10×10^{-2}
SO_4^{2-}	10^{-1}	10^{-3}	1.39×10^{-3}
Br^-	10^{-1}	10^{-1}	0.218
Br^-	10^{-1}	10^{-3}	0.225
Br^-	10^{-3}	10^{-3}	0.213
I^-	10^{-1}	10^{-1}	10.3
I^-	10^{-1}	10^{-3}	10.6
I^-	10^{-3}	10^{-1}	9.94
I^-	10^{-3}	10^{-3}	10.2

4.2.3 Long-term stability and time-dependent response

The TDDAB electrodes were immersed in a 10^{-2} M nitrate solution, and the mV response measured over a period of 6 days. After an initial rapid drop of around 10 mV in the first 30 hours, the electrode response stabilized, and showed a −1 mV change in the next 5 days. Electrodes that were made and then stored in a dry condition for 3 months were reactivated by soaking in 10^{-1} M nitrate solution for 30 min. The performance of these electrodes was similar to those that were freshly made.

Figure 2. Response of 13 coated wire electrodes to NO_3^-. Solid line represents the average response, the error bars are ± one standard deviation. Individual slopes ranged from 53.4 to 55.6 mV per decade, and correlation coefficients from 0.9998 to 1.000

4.3 Conclusions

Certainly within the limits of the very simple experiments that have been carried out, CWEs look like promising devices. The results of Figure 2 are temperature-corrected, but otherwise undoctored for 13 different electrodes that were made on 3 different dates. No attempt has been

made to provide internal reference system for these electrodes. (A range of these are suggested, including coating a silver wire with a bead of AgBr followed by an epoxy/bromide mixture. The PVC coating is cast on top of this pseudo-reference electrode.) This lack of internal reference element may help to explain the shift in response to 10^{-2} M nitrate that was observed over a period of time.

5 Current problems in chemical sensing in the environment

The brief and rather selective compilation on sensors presented above made no mention of many other chemical sensor technologies. ChemFETs (Janata & Huber 1980) are devices based on chemically modified field effect transistors (FETs). They can be fabricated using the manufacturing techniques of the electronic industry, and several different sensors may be integrated on a single chip. These and other sensors will, perhaps, occupy significant niches in the array of sensors available to the environmental analyst. A vast catalogue of sensor types is more likely to be confusing than helpful, when considering the current state of chemical sensing. In many respects, the piezoelectric crystal monitor is a model sensor, in that the sensing and transduction functions are so clearly identifiable. It is also a model sensor in that, although there are many laboratory applications for the piezoelectric detector, there are very few real applications. Even well-tried sensors such as ISEs must be judged as successful laboratory devices, but real sensing applications are few and far between.

Undoubtedly, the reasons for the poor showing of chemical sensors in situations other than laboratory applications arise from 3 distinct failures.

i. Most chemical sensors are insufficiently selective.
ii. Most chemical sensors are not stable.
iii. Most chemical sensors have limited working lives.

The essence of chemical sensing in the environment is that the probe can be located in the desired spot, and then ignored. Constant recalibration, maintenance, or replacement impose as significant a burden on manpower as does sampling. In a recent report on sensors in agriculture (ARC Working Party 1982), the following point was made: 'What is required, ideally in every case, is a small robust transducer (sensor) that can be coupled to a modern solid-state data logger so as to facilitate repeated/ multiple measurements'. Few devices have reached the stage of development where they could be left connected to a data logger for
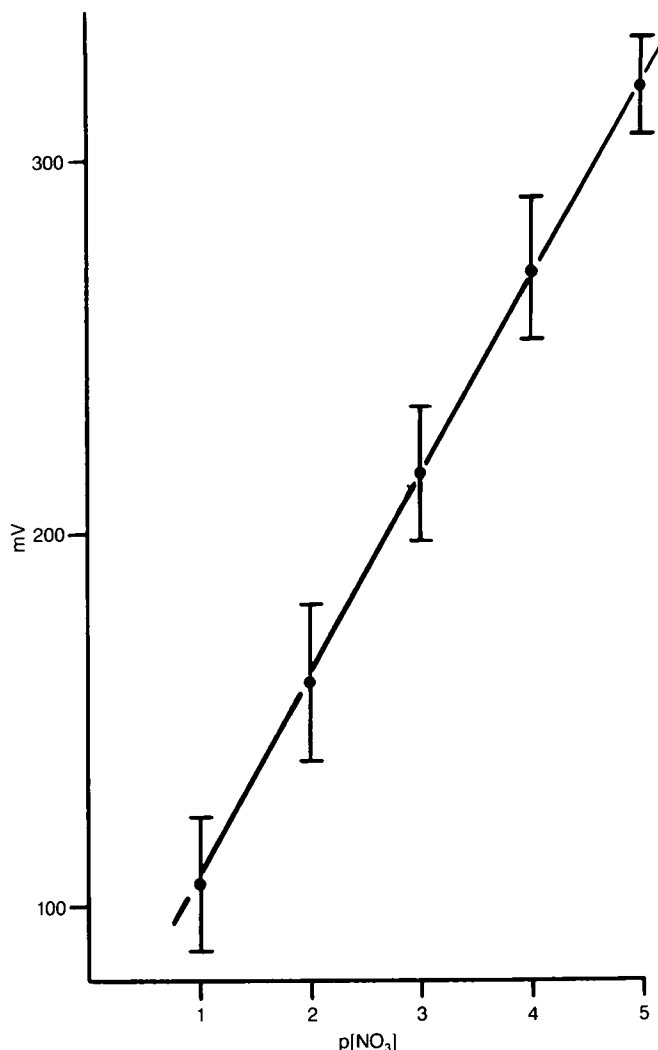
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anything other than a fairly restricted period.

6 Conclusions
Chemical sensing has developed in a rather piecemeal fashion, and throughout this development there has been an over-emphasis on the transducers. The heart of chemical sensing is the quantitative recognition of molecular and ionic species. Thus, the real need is to investigate and develop selective, sensitive, stable and robust chemical systems. Typical candidates might be immunoassays, immobilized enzymes, bi-lipid layer membranes, conducting polymers, polymer-bound sensor systems, host-guest systems and activated metal surfaces. Some chemistries, such as those concerned with modified electrodes, would have to be developed in conjunction with a particular transducer, but for many the question of probing the chemistry with a suitable transducer could assume a secondary significance. To a certain extent, this process is already taking place: immunoassay systems have been probed by both potentiometric and spectroscopic transducers; there is a growing interest in the chemistry of modified electrodes (Murray 1984).

A secondary approach to chemical sensing is to consider the response of a series of different non-specific, or partially selective, sensors to various chemical species. The output of such a system could then be examined by a range of powerful data processing techniques that are collectively referred to as chemometrics. Chemometrics may be defined (Kowalski 1977) as 'the development and application of mathematical and statistical methods to extract useful chemical information from chemical measurements'. Such an approach has been applied with some success to simple binary and ternary systems of sensors.

Finally, one should not lose sight of the fact that for many sensors the actual information required may be a simple 'yes' or 'no' answer to the question 'Is the analyte present at a particular concentration?' Alternatively, the sensor may be required to monitor a trend rather than make a 95% certain discrimination between 50.00 and 50.01 ppm of the analyte. These considerations, at least, bring the future of reliable chemical sensing in the environment a little closer.

7 Summary
Three types of chemical sensor are described in this paper: piezoelectric crystal gas monitors; fibre optic probes; coated wire electrodes. In each case, the sensor consists of a non-specific transducer on which a chemically sensitive membrane or surface is formed. The principles of the transducers are discussed, along with chemical and other methods of improving their performance. The limitations of current sensors

are reviewed, and suggestions made for future work.

8 Acknowledgements
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Measurement of microbial biomass in soil

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1 Introduction

The soil microbial biomass (ie the sum total of fungi, bacteria, protozoa, algae and other micro-organisms in soil) has been estimated to be many times the living weight of all other animals (vertebrate and invertebrate) in grassland ecosystems (Clark 1970). The biomass is also highly labile compared with other soil components, and thus plays a key role in the cycling of plant nutrients, especially N, P and S. For example, Brookes et al. (1984) calculated that the annual flux of P through the biomass in grassland soils averaged 23 kg P ha^{-1} yr^{-1}, equal to the annual P uptake in the herbage.

The study of microbial biomass has been aided by recent developments of methods which allow the soil microbial biomass to be studied *in situ*. Many of these new techniques will be reviewed here, and particular attention given to the advantages and disadvantages of the main approaches used.

2 Methods for measuring the soil biomass

2.1 Direct microscopy

Microscopic counting and sizing of micro-organisms in soil preparations remains the standard technique for quantifying microbial biomass. Although time-consuming and prone to several sources of error, agreement between measurements by direct microscopic observation and indirect methods confirms that the latter measure biomass, and not some other soil constituent. In this section, we will briefly discuss some of the major considerations for direct microscopic measurements of microbial biomass. More detailed and technical aspects are reviewed elsewhere (Jenkinson & Ladd 1981; Schmidt & Paul 1982).

A microscopic technique for directly counting micro-organisms in a soil suspension was introduced by Conn (1918). The major problem was the varied thickness of the suspension, which prevented a truly representative count. The problem can be overcome, however, by using a plastic adhesive material with a hole of known diameter on the slide (Schmidt & Paul 1982), to ensure an even thickness throughout the smear.

Another more widely used slide preparation method is the agar film technique (Jones & Mollison 1948), which involves the suspension of soil in dilute agar. A small volume of suspension is pipetted on to a hemocytometer slide, covered with a cover slip, and allowed to solidify. The agar film of known thickness is then stained with phenolic analine blue before microscopic examination.

More recently, a membrane filter technique (Hanssen et al. 1974) has been introduced. Micro-organisms from a soil suspension are trapped on a membrane filter of small pore size and then observed directly on the filter. This method is more rapid than the agar film technique and can be more easily adapted to fluorescent staining.

These direct microscopic methods have 3 major problems: the method used to disperse the soil organisms, the type of stain used, and the factors used to convert microscopic counts to biomass carbon.

The 3 most commonly used procedures for preparing soil suspensions (water, detergent, and/or agar) are: shaking (with or without previous soil grinding), blender mixing (Babiuk & Paul 1970), or ultrasonifying with stirring (Jenkinson et al. 1976). All three methods were compared by Jenkinson et al. (1976). They found that, although the methods were ranked in the order: blending > ultrasonics > shaking and grinding, for total numbers of organisms extracted, the ultrasonic dispersion method gave the highest biovolumes because of the greater dispersion efficiency of hyphal and large spherical organisms. The advantage of the ultrasonic method may be less for coarser-textured soils, and ultrasonifying for too long may cause injury to organisms (Ramsey 1984).

Various staining procedures have been used. Phenolic analine blue (PAB) has the advantage of staining both bacteria and fungi and is considered to stain only live organisms, ie those filled with protoplasm (Jones & Mollison 1948). Frankland (1974, 1975) concluded that the fungal hyphae stained with PAB under bright field was an *under-estimate* of total hyphae present, because not all hyphae stained, although PAB *over-estimated* live hyphae because some dead hyphae stained. She consequently developed a method for measuring live fungal biomass by determining the proportion of hyphae with cell contents under phase contrast without staining (Frankland 1975). One disadvantage of phase contrast, however, is that it is not suitable for counting bacteria in the presence of inorganic soil particles (Jenkinson & Ladd 1981).

Acridine orange (AO) is a commonly used

the extra CO_2 evolved from a previously chloroform-fumigated soil, following fumigant removal and aerobic incubation, compared to that evolved from a similarly incubated but non-fumigated soil, results from the decomposition of microbial cells killed by the fumigant (Figure 2). The method involves fumigation of soil samples with alcohol-free chloroform for 24 hours under vacuum, fumigant removal, and aerobic incubation of fumigated and unfumigated samples for 10 days at 25°C. Biomass carbon is calculated from F_c/k_c, where $F_c =$ [(CO_2-C evolved from the fumigated soil) minus (CO_2-C evolved from the unfumigated soil)]. The constant, k_c, is taken to be 0.45 at 25°C, on the basis that 45% of the carbon in micro-organisms added to fumigated soils is evolved as CO_2-C during 10 days of aerobic incubation (Jenkinson & Ladd 1981). A similar value for k_c (0.41 at 22°C) was found by Anderson & Domsch (1978a) by measuring decomposition rates of 15 species of ^{14}C-labelled fungi and 12 species of ^{14}C-labelled bacteria and calculating a weighted average decomposition rate, assuming a 3:1 fungal/bacterial ratio in soil.
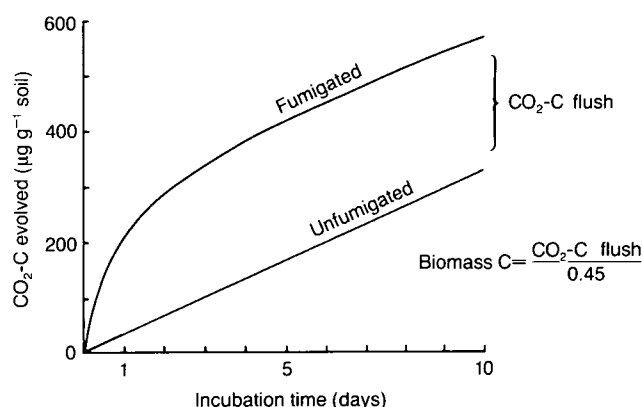

Figure 2. Respiration curves (CO_2-C evolution) for $CHCl_3$-fumigated and unfumigated forest soil samples (pH 7.2)

One of the basic assumptions of the fumigation method is that chloroform does not alter the decomposability of non-biomass soil organic matter. Enhanced decomposability of non-biomass organic matter could contribute to the 'flush' of respiration observed in fumigated samples. The validity of this assumption is supported by the observation that different biocidal treatments: $CHCl_3$, methyl bromide, and gamma irradiation (Powlson & Jenkinson 1976), and carbon disulphide (Kudeyarov & Jenkinson 1976), produce flushes of similar size. Other supportive evidence comes from experiments on soil that had been non-uniformly labelled with [14]C (by incubation with ^{14}C-labelled ryegrass for one year): similar amounts of CO_2-C with similar specific activities were evolved, whether the soil was irradiated or fumigated with $CHCl_3$ (Jenkinson 1966, 1976). This result implies that

both agents are acting on the same soil fraction. This fraction is probably the soil microbial biomass because treatments as different as irradiation and $CHCl_3$ fumigation are unlikely to render the same non-biomass fraction decomposable to about the same extent. Estimates of microbial biomass measured by the fumigation method were found to be very similar to the amounts measured by direct microscopy (Jenkinson et al. 1979; Oades & Jenkinson 1979) for a wide range of soil types (Figures 3a & 3b).
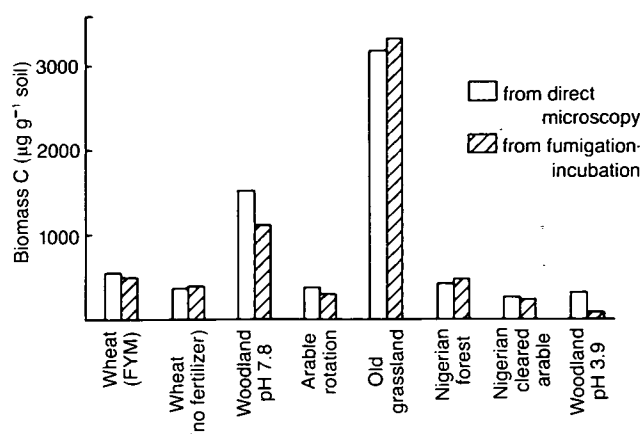

Figure 3a. The relationship between biomass C measured by direct microscopy and by $CHCl_3$ fumigation-incubation (from Jenkinson et al. 1976)
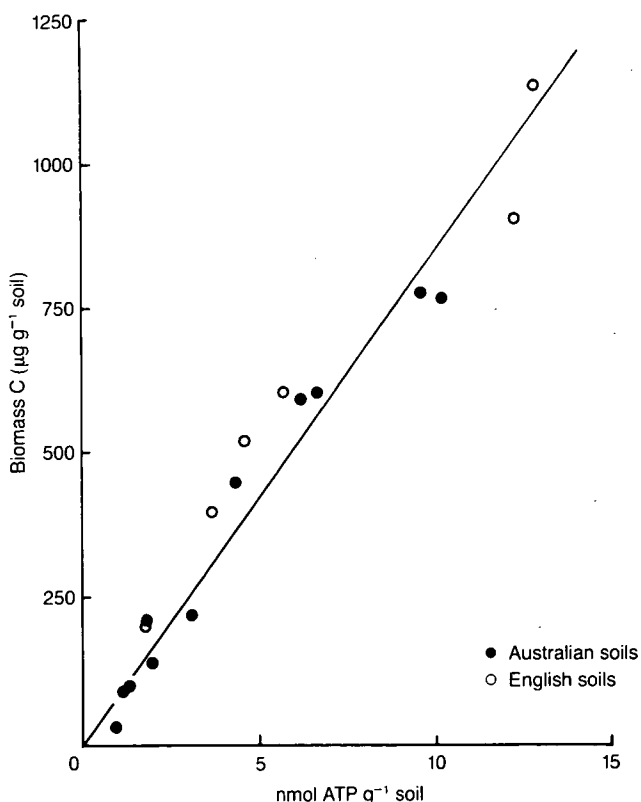

Figure 3b. The relationship between biomass C measured by $CHCl_3$ fumigation-incubation and soil ATP concentration for 11 Australian and 6 English soils (from Jenkinson et al. 1979; Tate & Jenkinson 1983)

Another assumption in the fumigation method is that the small microbial population which recolonizes the fumigated soil (about 10% of the original population) has the same ability to decompose non-biomass soil organic matter during the 10-day aerobic incubation period as the native soil population. This is implied in the calculation of biomass, in which the CO_2-C evolved from the unfumigated soil is subtracted from CO_2-C evolved from the fumigated soil. This assumption has been criticized (Voroney & Paul 1984) because the recolonizing population in fumigated soils is known to be smaller in amount (Jenkinson & Powlson 1976a), diversity, and metabolic capability (Voroney 1983). Because of this, Voroney and Paul (1984) proposed that biomass carbon be calculated from the CO_2-C evolved from the fumigated soil alone, assuming that there is negligible attack on non-biomass soil organic carbon by the recolonizing population in fumigated soil over the 10-day incubation period. The major argument against this theory is that fumigated soils, despite their reduced microbial population, respire at similar rates to unfumigated soils *once* the flush is over (Jenkinson & Powlson 1976a). Furthermore, Voroney (1983), using a soil in which the biomass was much more heavily labelled with ^{14}C than the soil organic matter as a whole, found that the specific activity of the CO_2 evolved by the fumigated soil fell after only a few days' incubation to a value very similar to that of the unfumigated control soil. This result again suggests that non-biomass CO_2-C was being evolved from both fumigated and non-fumigated soils.

Another variation of the fumigation method involves the use of mathematical models to predict the size of the mineralizable biomass C and its mineralization rate (Chaussod & Nicolardot 1982). They also proposed a modification to the original fumigation method, with the flush taken as the difference in CO_2-C evolved between the 0—7 day and 7—14 day incubation periods of the fumigated samples only.

There are some limitations to the use of the fumigation method. As with most soil biological assays, soils must not be air dried because this process solubilizes native organic matter, rendering it decomposable, and kills a portion of the biomass (Powlson & Jenkinson 1976). In addition, the fumigation method may not give valid measurements of biomass immediately after the addition of fresh substrates (Jenkinson & Powlson 1976b).

Using ^{14}C-labelled substrate additions, Martens (1985) found that the rates of decomposition of added substrates (eg glucose and wheat roots) differed in fumigated and unfumigated soil. Biomass calculations in the amended soils were only valid after substantial degradation of the substrates had occurred, 3 days for glucose, 14 days for cellulose, and 28 days for wheat roots.

There may also be interference from abiotic CO_2 production when the fumigation method is used on calcareous soils (Jenkinson & Powlson 1976b). This effect can be largely overcome by pre-incubation of the moist soil (40% WHC) in the presence of soda lime for 10 days before fumigation (Jenkinson & Powlson 1976b). The biomass thus measured is that in the soil at the end of the pre-incubation period, and may well be a little less than that present at the beginning.

The present fumigation method has not been applicable to strongly acid soils like those commonly found under forests and other natural vegetation. The problem was first detected by Jenkinson et al. (1976), who found that biomass measured by fumigation was only about a seventh of that measured by direct microscopy in a strongly acid (pH 3.9) woodland soil, although there was relatively good agreement between the 2 methods for 7 soils of higher pH. In the same soil, a similar discrepancy between biomass measured by fumigation and from soil ATP concentration was found subsequently (Jenkinson et al. 1979). Low biomass estimates using the fumigation method have been found in other strongly acid soils (Adams & Laughlin 1981; Adams & Adams 1983) and acid organic soils (Williams & Sparling 1984).

There are 2 components to the CO_2-C evolution from a fumigated soil during fumigation-incubation, viz CO_2-C evolved due to decomposition of biomass killed by the fumigant and CO_2-C evolved due to basal respiration of non-biomass soil organic matter. In the case of a neutral soil, it is assumed that the microbial recolonizing population mineralizes both the killed biomass (assumption 1), and also non-biomass soil organic matter (assumption 2) *to the same extent* as that by the native microbial population in non-fumigated soil. Some of the evidence for both assumptions has already been reviewed in this paper. There is evidence to suggest that assumption 1 is also correct in acid soils. Added yeast cytoplasm extracts decomposed to only a slightly lower extent in a fumigated, strongly acid (pH 3.9), soil than in a fumigated neutral soil (Powlson & Jenkinson 1976). Assuming that killed soil biomass decomposes in a similar way to yeast extract, it follows that k_c values for acid soils are only slightly lower than those for neutral soils.

However, the shape of the respiration curve in a fumigated acid soil is usually very different to that of a neutral soil. Such curves for a typical neutral and an acid forest soil respectively are shown in

Figures 2 and 4. In the neutral soil, once the flush is over (ie about day 4), rates of CO_2-C evolution in both the fumigated and non-fumigated soil are very similar, ie assumption 2 is again satisfied. However, the shape of the respiration curve of the acid soil (Figure 4) suggests strongly that assumption 2 is not satisfied in this case, ie there is reduced mineralization of non-biomass soil organic carbon in the fumigated soil compared to the non-fumigated soil. Thus, after an initial lag phase, there is a rapid rise in CO_2-C evolution, similar to that in a neutral fumigated soil, and attributable to the mineralization of the biomass killed by the fumigant. However, after this flush is over (ie about day 5), the rates of CO_2-C evolution in the control and fumigated soil continue to *diverge*, rather than become similar. The reason for the divergence is that respiration in the non-fumigated soil continues at a steady rate, but respiration in the fumigated soil is negligible, even up to 15 days after the initial flush is over. We suggest that this is because the microbial recolonizers in an acid fumigated soil are capable of decomposing the biomass killed by the fumigant, but are incapable of much mineralization of non-biomass soil organic matter, even 15–20 days after fumigation.
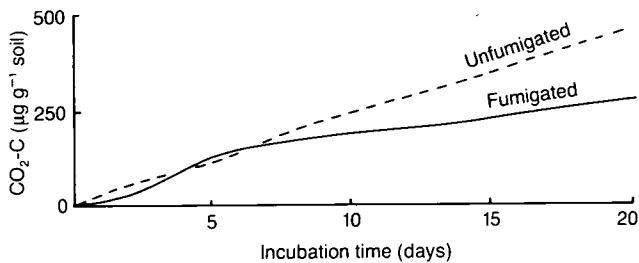

Figure 4. Respiration curves for $CHCl_3$-fumigated and unfumigated samples of a strongly acid soil (pH 4.2)

We are testing this hypothesis at Rothamsted by measuring decomposition of micro-organisms grown *in vitro* and by comparing biomass measurements by direct microscopy, ATP, and fumigation in a range of forest soils varying from pH 3.2 to 7.2. Some preliminary results, presented in Figure 5, show the relationship between biomass measured by direct microscopy and by a modified version of the fumigation method. Soils are treated as follows: when soil pH is below 4.2, CO_2 evolved from unfumigated soils is not subtracted from that evolved from fumigated soils. Above this pH, CO_2 production from the unfumigated sample is subtracted from fumigated soil CO_2 production in the usual way. The relationship between biomass measured by the 2 methods remained fairly constant, with an average ratio close to 1:1 over a wide range of both pH and biomass content.

An equally good relationship was found between biomass calculated from soil ATP concentrations and by the modified fumigation method. The concentration of ATP in the biomass averaged 9.1 × 10^{-6} M ATP g^{-1} biomass C, very similar to that previously measured in a range of neutral and near-neutral soils (Jenkinson *et al.* 1979; Oades & Jenkinson 1979). These results also strongly indicate that biomass ATP concentrations are similar in neutral and acid soil, so that ATP can serve as a reliable measure of soil biomass content in acid soils.

Our present results suggest that the fumigation method, as originally proposed, breaks down at a critical pH of about 4.2, because at or below this pH the recolonizers cannot mineralize non-biomass soil organic matter even 15–20 days after fumigation. The simple modification to the method that we propose needs to be tested on other acid soils before its general applicability can be confirmed.

2.4 The physiological method
The physiological method for measuring microbial biomass, introduced by Anderson and Domsch (1978b), uses the initial respiratory response of the soil microbial population to glucose additions as a measure of the amount of biomass present. After the concentration of glucose needed to give maximum response is determined, the respiration rate of the glucose-amended soil is measured over a period (usually one hour) too short to allow microbial proliferation. The rate of evolution of CO_2 was significantly correlated ($r=0.96$) with biomass measured by the fumigation method for 50 different soils. One advantage of the physiological method is that it can be used with selective inhibitors of bacterial or fungal activity, allowing the biomass to be separated into the 2 components (Anderson & Domsch 1978b). This
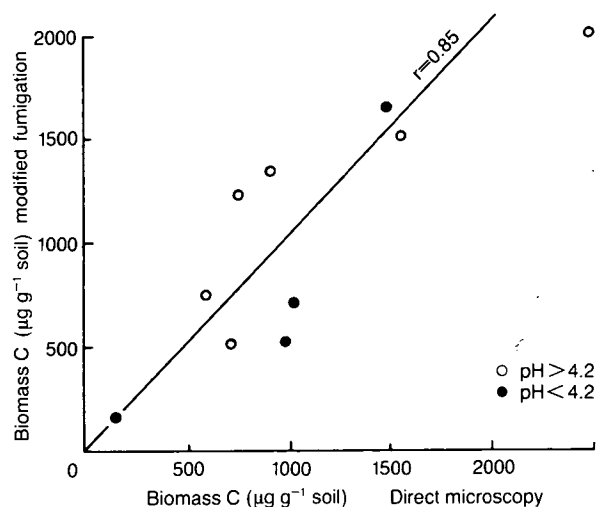

Figure 5. The relationship between biomass measured by modified fumigation-incubation and by direct microscopy for acid and neutral forest soils

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approach, however, has been challenged (Ross et al. 1981). Biomass measured by the physiological method has been found to correlate well with other measures of biomass and activity (eg Domsch et al. 1979).

The physiological method is based on the assumption that the various components of the microbial population, which may respond differently to the glucose addition, are present in the same proportions in different soils (Jenkinson & Ladd 1981). Because the method is calibrated against the fumigation-incubation method, results from the physiological method must be interpreted with caution when applied to soils where the fumigation method breaks down (eg strongly acid soils or soils with fresh substrate additions). Williams and Sparling (1984) found that biomass calculated using the physiological method did not agree well with results from the fumigation method in acid organic soils.

2.5 Measurement of nutrients immobilized in the soil microbial biomass

Quantities of nutrients contained in the soil biomass have traditionally been estimated by measuring microbial biomass carbon and calculating other nutrient concentrations from values determined from laboratory-grown micro-organisms (Ausmus et al. 1975; Baath & Söderström 1979; Anderson & Domsch 1980). The use of the chloroform fumigation technique to measure biomass carbon has led to other, related, procedures for measuring nutrients in the biomass, notably N, P and S. Fumigation has been known for a long time to release inorganic N from organic forms in soil, both directly, and during subsequent aerobic incubation (Russell & Hutchinson 1909; Waksman & Starkey 1923). The idea that the inorganic N and C thus released by fumigation came from the biomass led to the development of methods for measuring carbon and other nutrients in microbial biomass. The term 'stress labile N', used to denote nitrogen released in a fumigated soil during 10-day aerobic incubation, was introduced by Ayanaba et al. (1976). This term was used as an indication of the 'quality' of organic N and was found to be related to cropping history. A close linear relationship was shown between 'stress labile N' and biomass C for 20 Nigerian soils studied. Ross et al. (1980) found that ratios of biomass C to mineral N flush varied from 8 to 23 in 9 New Zealand soils under grassland, however, and questioned the use of the N flush to calculate microbial biomass.

The estimation of biomass N by the fumigation-incubation procedure has proved to be more uncertain than biomass C, first, because the N content of microbial cells is much more variable than the C content, and, second, because some of the N mineralized during the incubation of

fumigated soil is reimmobilized (Jenkinson 1976; Marumoto et al. 1982). These 2 factors are probably related.

Paul and Juma (1981) measured nitrogen mineralized in fumigated soil and assumed a k_N value of 0.25 to calculate microbial biomass N and the flux of N through the biomass in soils receiving labelled substrates. Two approaches have recently been introduced to overcome the problem of N immobilization in the fumigation-incubation procedure. Voroney and Paul (1984) labelled the microbial biomass in situ by adding ^{14}C-glucose and ^{15}N-KNO_3 to soil, incubating and measuring C and N mineralization following fumigation. From these studies, they proposed a variable k_N, using an adjustment factor calculated from the ratio of CO_2-C evolved to N mineralized during incubation to correct for N immobilization.

A second approach to overcoming the problem of N immobilization during biomass N determinations was introduced by Shen et al. (1984), who assumed that a constant fraction of the N released from the killed population was reimmobilized. Making this assumption, k_N was calculated to be 0.68 from the ratio of C flush to N flush, taking the average C/N ratio of micro-organisms to be 6.7 and a k_c value of 0.45.

A new method for measuring biomass N has been developed, based on the extra N (organic + inorganic) extracted by 0.5 M K_2SO_4 from a fumigated soil compared to an unfumigated control (Brookes et al. 1985a, b). Total N rendered extractable by fumigation was strongly correlated with F_N determined by the fumigation-incubation method in 37 soils. Because this new method does not require an aerobic incubation, it overcomes the problem of N immobilization and can be done in one day instead of 10. However, the problem remains of establishing an agreed factor to convert N rendered extractable by fumigation to biomass N.

2.6 Measurement of phosphorus and sulphur in the biomass

Direct extraction methods have also been developed for measuring biomass phosphorus (Brookes et al. 1982; Hedley & Stewart 1982) and sulphur (Saggar et al. 1981). For biomass P, soil is either fumigated with $CHCl_3$ vapour (Brookes et al. 1982) or liquid $CHCl_3$ (Hedley & Stewart 1982) to lyse microbial cells, followed by extraction with 0.5 M $NaHCO_3$. The 2 methods differ further in that Brookes et al. (1982) used a known addition of KH_2PO_4 during extraction to correct for adsorption of inorganic P released by fumigation. Hedley and Stewart (1982) found that removal of inorganic P from the soil, using exchange resins prior to extraction, increased the accuracy of the method. However, both groups obtained values for k_p of about 0.4.

Brookes *et al.* (1982) showed that about 90% of the total P (P_t) released by $CHCl_3$ after a 24 h fumigation was inorganic (P_i), presumably because of enzymic hydrolysis of organic P in the lysed cells. On the basis of this finding, it was suggested that $NaHCO_3$-extractable P_i alone might be used for measuring biomass P. However, recent work using forest soils suggests that in acid soils P_i makes up a much lower percentage of P_t (Table 1). The extractable organic P released by $CHCl_3$ in acid soils must be investigated further to determine whether it is actually derived from the biomass, or from non-biomass soil organic matter. There is evidence that $CHCl_3$ may extract P from fresh plant material added to soil (McLaughlin & Alston 1985; Sparling *et al.* 1985).

Table 1. Ratios of inorganic P (P_i) to total P (P_t) released by $CHCl_3$ and extracted by 0.5 M $NaHCO_3$, for 10 forest soils

Soil number	pH	$CHCl_3$-released P_i as % of P_t
1	7.2	99
2	6.1	70
3	5.2	78
4	5.2	85
5	5.1	71
6	4.2	76
7	4.1	45
8	3.8	26
9	3.5	42
10	3.4	33

The original method for estimating biomass sulphur in soil (Saggar *et al.* 1981) used liquid $CHCl_3$ as a lysing agent, followed by measurement of total S extracted by $CaCl_2$ or $NaHCO_3$. Strick and Nakas (1984) obtained more reproducible results for biomass S contents of forest soils with high uptake adsorption capacity by (i) using NaH_2PO_4 as the extractant, (ii) using $CHCl_3$ vapour instead of liquid $CHCl_3$, (iii) using moist soil that had not been air dried and rewetted, and (iv) pre-leaching soil to remove non-biomass soil S. They obtained k_s values of 0.37 and 0.25 for 0 horizons under coniferous and hardwood forests, respectively.

3 Summary
The soil microbial biomass is the agent of breakdown of organic material in soils, mineralizing organically bound nutrients into inorganic forms which can be used by plants. The biomass thus provides a labile sink for N, P and S, and plays a key role in the cycling of these plant nutrients.

Methods have been developed to measure microbial biomass in soil and the nutrients held in the biomass. Direct microscopic counting is the standard method and allows some separation of the microbial population into live, dead, active, and inactive fractions based on selective staining. A more rapid method is the measurement of soil ATP which only occurs in living cells. Extractants for ATP should be acidic, or contain other agents capable of inactivating ATPases, which would otherwise hydrolyse ATP during extraction, and hence give apparently low soil ATP concentrations.

The fumigation-incubation method is widely used. CO_2 evolved during decomposition of micro-organisms killed by chloroform fumigation is related to the amount of soil biomass originally present. Special modifications are needed, however, to apply the method to strongly acid (ie <pH 5) soils. Biomass measured by fumigation correlates well with that measured by direct microscopy and with soil ATP concentration.

The respiratory response of the soil biomass to glucose amendment (the physiological method) is closely related to the amount of microbial biomass measured by fumigation-incubation. The physiological method has been used with selective inhibitors to separate the microbial biomass into fungal and bacterial components.

Modifications of the fumigation-incubation procedure permit the measurement of nutrients (N, P, S) contained in the biomass. For example, biomass N can be estimated from the flush of mineral N following soil fumigation and aerobic incubation. Under some conditions, N can be immobilized or denitrified, but this can be overcome using a recently developed procedure, which involves determination of the organic + inorganic soil-N released by fumigation and extracted by K_2SO_4, without aerobic incubation. Similar extraction methods have also been developed to measure biomass S and P in soil. These direct extraction methods have the advantage that soils do not need pre-incubation before analysis. This step is required in the fumigation-incubation and ATP methods to allow microbial activity to stabilize following disturbance due to sampling.

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Assessment of stress to microbial biomass

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1 Introduction

The fundamental role of the microbial biomass in the cycling of organic matter and in controlling active nutrient pools strongly suggests that the effect of any environmental stress on the microbial biomass will be intimately related to stress effects on crops, on natural vegetation, and on ecosystem productivity.

To date, most assessments of the effects of an environmental stress on the microbial biomass have been based on assay of respiration or enzyme activity. Recent studies of effects of acid rain on biomass have used respiration with or without substrate amendment (eg Bryant et al. 1979; Hovland et al. 1980; Strayer & Alexander 1981) or activity of dehydrogenase and other soil enzymes (Francis et al. 1980), as likely indicators of possible effects. Recent work by myself and others suggests, however, that these parameters are not the best ones to determine the true impact of environmental stress on the functioning of the microbial biomass. Physiological studies of water stress, for example, have shown that soil micro-organisms divert an increasing proportion of energy from growth into maintenance of cell integrity as stress increases (Killham & Firestone 1984). In this case, the energy diversion is needed for solute transport to counteract osmotic stress. This diversion of carbon from biosynthesis to maintenance energy requirements will occur for all environmental stresses which are adverse to the microbial cell. It might well be expected, therefore, that an increasing proportion of carbon uptake will be respired with increasing stress. Indeed, in marine micro-organisms, increasing C diversion into respiration has been shown to be a function of increasing stress (Griffiths et al. 1984). It is suggested, therefore, that CO_2 evolution alone cannot be used as a suitable indicator of environmental stress to microbial biomass.

A factor which incorporates partitioning of assimilated carbon into biomass growth as well as respiration is clearly necessary. This paper reports, by means of experiments using labelled C substrates and different microbial populations, a comparison between the ratio of respired C/biomass-incorporated C with respired C and dehydrogenase activity as indicators of environmental stress to microbial biomass in a range of habitats.

2 Materials and methods

2.1 Soil and leaf litter samples

2.1.1 Soil

A sandy loam (Tarves series) under arable cultivation was sampled from Turriff, 20 miles north of Aberdeen, Scotland (National Grid reference NJ734506). The soil was sieved (<2 mm), packed into 15 cm diameter pots, and then the pots were sprayed twice-weekly with a range of treatment solutions. Three control pots received distilled water, 3 received 0.15 M NaCl, and 3 received 0.3 M NaCl. The treatments were designed to represent irrigation water of varying salinity. Treatment period was 20 weeks and the treatments were equivalent to 35 cm of rainfall. After 20 weeks, the surface soil (0—2 cm depth) of each pot was removed and mixed thoroughly in plastic bags.

2.1.2 Leaf litter

Needles of Ponderosa pine *(Pinus ponderosa)* were collected by litter trap over a 24-h period from a site in the western Sierra Nevada, northern California, USA. The needles were cut into approximately 2 cm lengths, and 10 g portions of litter were put into 2 mm mesh litter bags which were then placed just below the surface of the litter layer of intact forest floor profiles. Plastic cylinders (15 cm diameter, 40 cm length) were used to contain the profiles. Profiles were sprayed with simulated rain (pH 3.0, 4.0 and 5.6) prepared using an acid mix of HNO_3/H_2SO_4 in a proportion of 3:2 and with base ions to simulate northern Californian precipitation (McColl 1981).

Table 1. Quantities of heavy metals added to simulated rain ($\mu g \ ml^{-1}$)

Heavy metal load	Cu	Ni	Pb	Zn	Fe	Co
None	—	—	—	—	—	—
Low	0.3	0.2	0.1	0.1	1.0	—
High	4.2	6.0	0.2	0.1	1.1	0.1

All rain treatments had the following initial ionic composition (10^{-3} M): Mg^{2+}, 3; Ca^{2+}, 3.5; NH_4^+, 14; Na^+, 15; K^+, 1.5 (plus the required concentration of the 3:2 acid mix to produce the desired pH). For each rain acidity, 3 heavy metal loads ('none', 'low', and 'high') were added, making 9 rain treatments in all (Table 1). The compositions of the 'high' and 'low' heavy metal loads were designed to approximate depositions observed downwind of the Coniston Ni/Cu smelter, Sudbury, Ontario, at about 1.6 and 16 km, respectively (Hutchinson & Whitby 1976). Treatments were applied twice-weekly to 3 replicate profiles over a 22-month period; treatments totalled 150 cm of rainfall.

2.2 Incubation of samples with ^{14}C glucose

Triplicate samples of soil (25 g) and of leaf litter (5 g) were placed in 500 ml sealed Erlenmeyer flasks with an oxifluor CO_2 trap and the samples amended with ^{14}C glucose (1:11 mCi mg^{-1}). Glucose (1000 µg g^{-1}) was added as an aqueous solution, evenly pipetted over the surface of the samples, to achieve a sample moisture content of 30% w/w: the glucose concentration used was found to give maximum C uptake during incubation. After 3 h incubation at 25°C, the oxifluor CO_2 trap was removed and concentration of CO_2 determined. The samples were then placed on a membrane (0.45 µm), washed (quarter strength Ringers solution used to prevent osmotic stress to microbial cells) to remove residual glucose not taken up by the microbial populations, and the concentration of ^{14}C glucose in the washings determined. Radioactive carbon was quantified by liquid scintillation counting using a Beckman LS-100C. Subtraction of ^{14}C glucose not taken up by the microbial biomass and remaining in the soil from initial substrate concentration enabled calculation of ^{14}C glucose uptake (equation 1). Then, by subtracting respired ^{14}C from ^{14}C uptake, biomass-incorporated ^{14}C was calculated (equation 2).

$$^{14}C \text{ uptake} = \text{initial } ^{14}C \text{ substrate concentration} - \text{residual } ^{14}C \text{ in soil} \quad (1)$$

$$^{14}C \text{ uptake} - \text{respired } ^{14}C = \text{biomass } ^{14}C \quad (2)$$

These calculations are based on the assumption that washing is effective in removing all residual glucose from the samples (see below) and that, in the short period, there was not significant efflux of ^{14}C-labelled metabolites (other than CO_2) from the microbial cells.

2.3 Effectiveness of extraction of glucose

To test the efficiency of quarter strength Ringers solution in removing all residual glucose not taken up by the microbial community, several soil and leaf litter samples were sterilized (autoclaved for 1 h on 3 successive occasions) and then amended with 1000 ppm ^{14}C glucose. Samples were washed, as previously described, and the extract used for determination of ^{14}C activity. In addition, the samples were then extracted with 0.2 M HCl (shaken for 30 min, then filtered) and the acid extracts also tested for ^{14}C activity. This procedure was carried out for 6 soils of varying pH, clay and organic matter content to determine the suitability of water extraction of glucose for a wide range of soils. The saline-sodic soil (no. 5) was also amended with sodium borate (50 ppm B) and sodium molybdate (50 ppm Mo) prior to glucose amendment to investigate whether these salts, sometimes occurring in sodic soils, complex added glucose.

2.4 Determination of soil dehydrogenase activity

Soil dehydrogenase activity was determined by supplying 2,3,5-triphenyltetrazolium chloride (TTC) as an artificial terminal H$^+$-acceptor, and measuring reduction of the dye (Casida et al. 1964).

2.5 Sample storage

Soil samples and contents of litter bags were stored in polythene bags at 4°C prior to incubation with ^{14}C glucose or determination of dehydrogenase activity.

2.6 Statistical analysis

An SNK multiple range test was used for statistical analysis of the data (Zar 1974).

3 Results and discussion

There are 2 main conclusions concerning assessment of the effect of environmental stress on the microbial biomass of soil and leaf litter which can be drawn from the data shown in Figures 1—4.

i. Increasing environmental stress generally caused a reduction in soil and leaf litter respiration, a reduction in soil dehydrogenase activity, and an increase in the ratio of respired C/biomass-incorporated C for both soil and leaf litter.

ii. The most sensitive indicator of environmental stress was the ratio of respired C/biomass-incorporated C. For a given increase in stress, the increase in the above ratio was, on average, twice as great as the magnitude of the concomitant reduction in either respiration or dehydrogenase activity.

It would appear, therefore, that the effect of environmental stress on microbial populations can be much better assessed by determining the allocation of C uptake into both biomass and respiration, rather than by the widely used methods of determining either rates of respiration or dehydrogenase activity. To partition assimilated C in the above manner, samples of soil and leaf litter, which had previously been subjected to various environmental stresses, were incubated with ^{14}C glucose as substrate for their microbial populations. ^{14}C activities in respired and biomass-incorporated fractions were then determined. A substrate concentration of 1000 ppm C was identified as producing maximum reaction velocity (CO_2 evolution), and an incubation period of 3 h was selected to preclude the possibility of significant quantities of ^{14}C-labelled metabolic products (amino-acids, organic acids, enzymes, etc) other than CO_2 effluxing from microbial cells. Possible strategies of longer-term stress responses of microbial communities, involving leakage of metabolites as well as non-
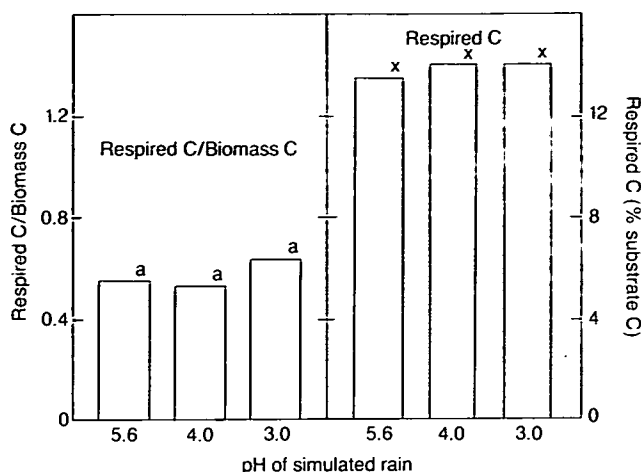
Figure 1. Effect of pH of simulated rain on partitioning of assimilated substrate C by the microbial biomass of Ponderosa pine needle litter. Letters indicate significant (P<0.05) differences between means
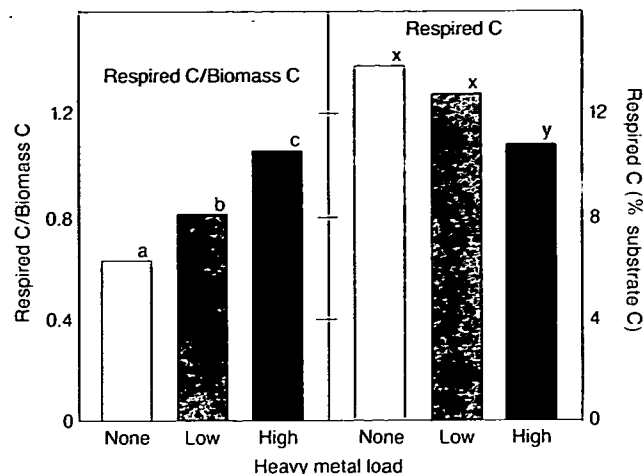

Figure 3. Effect of heavy metal load, applied in simulated rain (pH 3.0) on partitioning of assimilated substrate C by the microbial biomass of Ponderosa pine needle litter. Letters indicate significant (P<0.05) differences between means
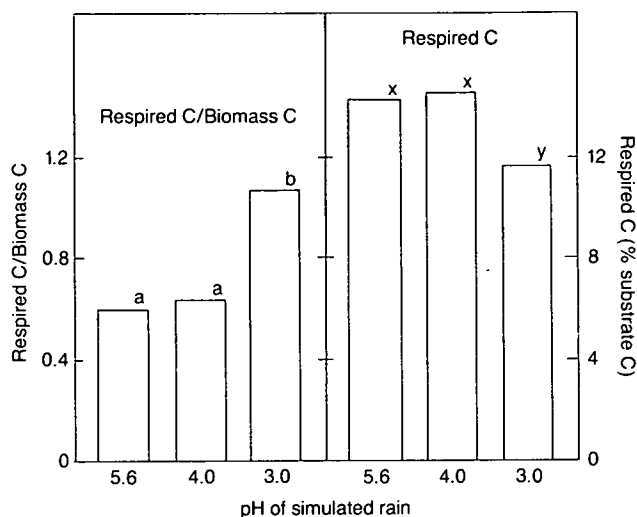

Figure 2. Effect of pH of simulated rain, containing the 'high' heavy metal load, on partitioning of assimilated substrate C by the microbial biomass of Ponderosa pine needle litter. Letters indicate significant (P<0.05) differences between means
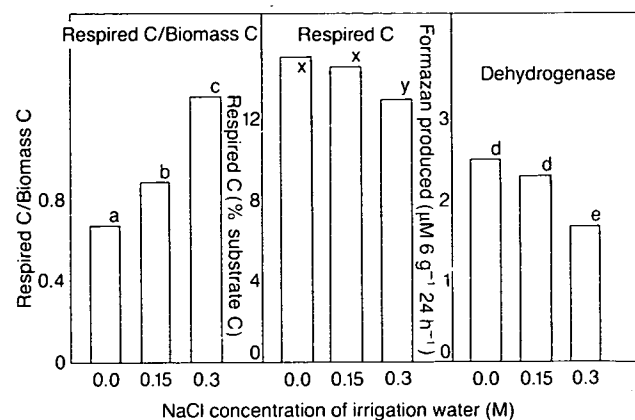

Figure 4. Effect of NaCl concentration of irrigation water on partitioning of assimilated substrate C and on dehydrogenase activity of soil microbial biomass. Letters indicate significant (P<0.05) differences between means

physiological mechanisms of response, have been reviewed (Daigger & Grady 1982).

In the case of the pine litter samples exposed to treatments designed to simulate possible pollution loads downwind of a metal smelter, neither acidity nor heavy metal load (in isolation) of the treatments caused significant (P<0.05) stress (as determined by changes in respiration or in respired C/biomass-incorporated C) to the microbial biomass (Figures 1, 2 & 3). Even the extreme pH 3.0 treatment, when applied without an additional heavy metal load, had no significant effect. It was only when heavy metal loads were applied in the most acid (pH 3.0) simulated rain that significant stress was detected. When the 'high' heavy metal load was applied in the pH 3.0

treatment, the ratio of respired C/biomass-incorporated C increased by 75%, while respired C alone decreased by only 28%, compared to controls (pH 5.6, no metals). This greater sensitivity of the ratio of respired C/biomass-incorporated C as a stress indicator was also evident for soils exposed to the pH 3.0, 'low' metals treatment, where the ratio increased by 30% despite respiration not being significantly changed (Figure 2). There was no glucose uptake in sterile, control soil samples under any of the treatments used. In the case of the soil samples irrigated under regimes of varying salinity, stress to the microbial biomass was detected even when the least saline of the saline treatments (0.15 M NaCl) was used (Figure 4). Again, however, stress-induced changes in the ratio of respired C/biomass-incorporated C were considerably greater than corresponding changes in either respiration or dehydrogenase activity. In fact,

when soils were irrigated with 0.15 M NaCl, stress to the microbial biomass could only be detected by determination of respired C/biomass-incorporated C, there being no significant change in respiration or dehydrogenase compared to control soils receiving distilled water.

A marked discrepancy between the effects of environmental stress on the ratio of respired C/biomass-incorporated C and on respiration or dehydrogenase activity raises fundamental questions concerning our future approach to assessing the impact of environmental stresses, and concerning interpretation of previous studies which relied solely on respiration or dehydrogenase as the determinants of stress-induced changes in microbial activity. One can easily hypothesize a situation where the respiration rate of a microbial community is effectively unchanged by some environmental stress, despite the fact that both C uptake and C flow into growth of biomass have been drastically reduced. In the context of the findings of this study, such a situation is far from inconceivable. Growth of the microbial biomass could even be stopped entirely, despite the fact that respiration data alone would suggest no adverse impact of the environmental stress. Such a situation is represented by Figure 5 which shows a hypothetical response of the energy budget of microbial biomass to an increasing environmental stress. Growth of the biomass ceases when the entire energy budget is used for maintenance requirements. At higher stresses, cell damage/death will result because more energy is required for maintenance than may be available.

The validity of the technique reported here is based on the assumption that a simple quarter strength Ringers solution is effective in removing all residual glucose, not taken up by the microbial population, from the soil and leaf litter samples. This assumption was tested by glucose amendment of sterile samples and, after the normal washing procedure, extracting with 0.2 M HCl and assaying the acid extracts for ^{14}C activity. Washing was highly effective in removing residual glucose, not only from the soil and leaf litter samples used in this study, but also from several other soils of varying pH, clay and organic matter

content (Table 2). With the exception of the high organic matter peat soil (no. 4), at least 95% of the glucose added to the sterilized soils was recovered. Also, when borate or molybdate was added to the saline-sodic soil of high pH 9 (no. 5), complexing of glucose occurred and recoveries were substantially reduced. That borates and molybdates can complex glucose is well established (Foster & Stacey 1953; Murayama 1977; Cheshire et al. 1979). It is proposed, therefore, that this new technique for determining stress to the microbial biomass will be suitable for most soil types, as well as leaf litter samples. Soils of exceptionally high organic matter content as well as saline or saline-sodic soils containing borates or molybdates will be less suited, without modification of the method.

The technique used in this study offers a rapid, simple, and much more sensitive alternative to assaying respiration or dehydrogenase activity as an indicator of physiological stress to microbial biomass. Greatly increased sensitivity in identification and determination of stress suggests that extrapolations of respiration or dehydrogenase data alone to the ecological ramifications of environmental disturbances should be treated with great caution.

4 Summary
By supplying ^{14}C-labelled glucose as substrate, the ratio of respired C/biomass-incorporated C was determined for the microbial biomass of samples of soil and leaf litter after their exposure to a range of environmental stresses. The ratio of respired C/biomass-incorporated C was found to be a much more sensitive indicator of the impact of stress than either respiration or dehydrogenase
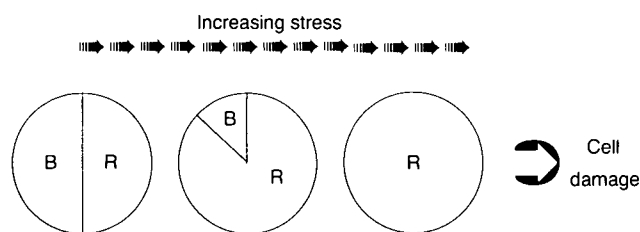
Figure 5. Possible response pattern in terms of carbon-allocation strategy, of the microbial biomass to increasing environmental stress

Table 2. Efficiency of extraction of glucose

Soil	pH	% organic C	% clay	% glucose recovered
1. Turriff	4.4	3.3	8.4	98
2. Tillycorthie	5.8	22.9	10.1	97
3. Cruden bay clay	5.1	3.1	17.7	96
4. Red moss peat	3.4	46.4	0.0	88
5. Ethiopian saline soil	8.0	2.3	10.3	95
6. Soil 5 + 50 μg B(g^{-1} soil)	8.0	2.3	10.3	79
7. Soil 5 + 50 μg Mo (g^{-1} soil)	8.0	2.3	10.3	86

activity, which are currently most widely used as stress indicators for microbial biomass. The ratio decreased as the magnitude of environmental stress increased, suggesting that there is an increasing diversion of carbon in the biomass from biosynthesis to maintenance energy requirements. Patterns of response of the microbial biomass to environmental stress are discussed in relation to carbon allocation by the biomass, and to biomass growth.

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Mineralization of organic phosphorus in relation to soil factors, determined using isotopic ^{32}P labelling

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1 Introduction

The mineralization of organically bound phosphorus, which forms a major fraction of the soil P capital (Harrison 1979), is essential to the maintenance of the phosphorus cycle and the replenishment of the available P in the soil in forest ecosystems (Harrison 1985). The trees and ground flora, in a local phosphorus-deficient deciduous woodland — Meathop Wood — take up about 11.4 kg P ha^{-1} yr^{-1} from the soil, and this amount is about the average annual uptake for temperate woodlands and forests. If it is assumed that all the P taken up by the vegetation is derived from mineralization of organic phosphorus in the top 5 cm of the soil profile, then the average rate of mineralization required through the year is only about 95 mg P m^{-2} month^{-1} or 4 μg P g^{-1} soil month^{-1}. This amounts to about 0.2% to 1% of the organic phosphorus pool in Lake District woodland soils each month. Furthermore, small differences in mineralization rates at the level of <5 μg P g^{-1} soil month^{-1}, caused by variation in soil conditions or site management factors, could be quite important in influencing the productivity of both the local woodlands and other UK forest plantations, as they are frequently P-deficient. These rates of mineralization are extremely low.

These conclusions are probably relevant to most ecosystems, for only a small proportion of the soil organic phosphorus needs to be mineralized to provide significant quantities of inorganic phosphorus to plants (Greb & Olsen 1967). With such low rates of mineralization being potentially important, how do we attempt to determine the factors, such as soil and environmental conditions and site management, which have a controlling influence on the rate of soil organic phosphorus mineralization?

At ITE's Merlewood Research Station, we have tried to determine organic P mineralization rates using the conventional incubation methods, but the results from such techniques are far from satisfactory for local woodland soils. Often reductions in the labile organic phosphorus, that extractable in Olsen's bicarbonate (Bowman & Cole 1978a), during incubation are not paralleled by an apparent and equivalent increase in inorganic phosphorus, so we cannot be confident that mineralization is actually taking place. Only the net changes in the labile organic and inorganic phosphorus are being measured by these techniques, and both may change in their extractability during incubation. Also, error terms on the measurements are large, particularly in relation to the low rates of the mineralization required in the ecosystem (see Halstead *et al.* 1963), so that few statistically significant changes in organic or inorganic phosphorus contents of soils are detected on incubation, even after incubation for a period of 6 months (Harrison 1982a). The conventional techniques of incubation appear to be too insensitive and imprecise to answer the problem of how soil and environmental conditions and site management factors influence organic phosphorus mineralization rates in woodland and forest soils.

Consequently, there is a need for another approach to the assay of comparative rates of organic phosphorus mineralization in these woodland soils. I have developed a method in which ^{32}P-labelled ribose-nucleic acid (RNA is a component of the labile fraction of soil organic phosphorus — Bowman & Cole 1978b) was added to soils, which were subsequently incubated. The amount of mineralization was determined following the recovery of the ^{32}P from soils and its partitioning into organic and inorganic forms. The main attribute of the method is the considerably improved sensitivity due to the use of ^{32}P as a tracer and its capacity to provide gross, rather than net, conversion rates. It was sensitive enough to detect highly significant differences in rates of mineralization in the woodland soils, after only 24 h incubation at 13°C, the period being shorter and the temperature being lower than in commonly used incubation methods.

2 Soils and methods

Soil samples were collected from the 0—5 cm depth (after the removal of the litter layer) from brown earth, brown podzolic and rendzina profiles from 13 different Lake District woodlands. Twenty-five samples (1—3 per woodland) were collected in October 1973, and a similar set was obtained the following May. Soils were sieved (2 mm) to remove stones and root material, but were otherwise used in a fresh condition. They varied considerably in their physical and chemical properties (Harrison 1982b).

Subsamples of each fresh soil were compacted into polystyrene 'sterilin' tubes, so that each tube contained 10 cm^3 soil at its natural bulk density. After equilibrating the soils at 13°C (the average soil temperature at the times of the year sampled)

for one day, 1 ml of ^{32}P-RNA (containing 97 µg RNA $\equiv$ 7.8 µg P, with average molecular weight 30 000 and a specific activity of c 130 GBq per 10^{-3} M) was distributed in the soil of each tube, using a hypodermic syringe. The ^{32}P-RNA was added in an amount less than generally found to be present in soils. The tubes were incubated for 24 h at 13°C, after which the soil in each tube was mixed and divided into 4 equal weighed sub-samples, each of which was added to 100 ml of an extractant solution in a polypropylene bottle. The suspension was then subjected to ultrasonic vibration (50 W, 20 kHz at 6—8 µ amplitude) for 5 min. Bottles were shaken subsequently overnight on a reciprocal shaker in a waterbath at 18°C. Subsamples of the suspension were centrifuged and the ^{32}P-labelled phosphorus in the super-natant partitioned into inorganic and organic forms, using a method similar to that of Martin and Doty (1949); inorganic ^{32}P transferred into the isobutanol-benzene layer with an efficiency of >99.5%. Total ^{32}P recovered in the original supernatant and inorganic ^{32}P recovered in the organic layer were determined, after addition to fluor, by liquid scintillation spectrometry. To determine the degree of ^{32}P-RNA hydrolysis caused by the extraction and separation procedures, control samples were also prepared for all soils; ^{32}P-RNA was added to soil, immediately extracted, and partitioned into organic and inorganic forms. Recoveries of ^{32}P from the 50 soils ranged from 56±2 to 104±6%, with only 10 soils having recoveries of <80%; recovery was negatively related to the amounts of extractable calcium (r^2=0.71**) and clay (r^2=0.42**) in the soils. In the calculations of the mineralization rate, it has been assumed that ^{32}P not recovered from the soil was apportioned between organic and inorganic forms in the same ratio as that extracted. Further details of the methodology are presented in Harrison (1982a).

3 Results

The rates of net mineralization of the ^{32}P-RNA in the 50 soils ranged from −29 to 190 ng P cm^{-3} soil day^{-1}, with a least significant difference of about 10 ng. These rates of mineralization are equivalent to 0.75 to 5.7 µg P cm^{-3} soil month^{-1}. The negative values are attributable to net immobilization of the small amount of inorganic ^{32}P present in the ^{32}P-RNA preparation. Net immobilization of inorganic P occurred in 12 of the October samples, with no May samples having negative mineralization rates. Partially as a result, soils collected in October had lower rates of mineralization (9.2±7.8 ng P cm^{-3} soil day^{-1}) than those collected in May (45.9±9.8 ng P cm^{-3} soil day^{-1}). Soils over limestone, irrespective of the time of the year collected, had higher rates of mineralization (56±14.2 ng P cm^{-3} soil day^{-1}) than those soils over slates, shales and andesite-

tuff rocks (10±4.2 ng P cm^{-3} soil day^{-1}). There were also strong interactive effects of the time of year and the underlying geology, as shown by covariance analysis, on the relationships between mineralization rate and soil chemical and physical properties (Table 1). In general, however, strong and significant positive relationships were found with soil pH and extractable calcium contents, and available phosphorus contents (expressed as a combined function of water-soluble, isotopically exchangeable and Olsen's bicarbonate extractable inorganic phosphorus) across all soils (Table 2). Fifty per cent of the variation in ^{32}P-RNA mineralization rate was accounted for by the 3 'available' P variables, sometimes more when the soils were partitioned according to the geology and time factors. A significant negative relationship was also found between mineralization rate and the Olsen's bicarbonate extractable organic phosphorus content (particularly when expressed as a percentage of the total organic phosphorus) of soils; the latter is considered to be the labile fraction of soil organic phosphorus (Bowman & Cole 1978a). Relation-ships with phosphatase activity and respiration rate in the soils were found only for soils overlying slates, shales and andesite-tuff rocks. Around 90% of the variation in rates of mineralization in the soils was accounted for by significant soil properties (not all the measured soil properties are mentioned in this paper). Further details of the relationships between the rate of ^{32}P-RNA mineralization and soil properties are presented in Harrison (1982b).

4 Discussion

The question of whether or not analytical procedures to be applied to soils are sensitive

Table 1. Effects[1] of geology and time of sampling on relationships between ^{32}P-RNA mineral-ization rate and properties in 50 soils

Property	Geology[2] Slope	Geology[2] Elevation	Time[3] Slope	Time[3] Elevation
pH	NS	NS	*	***
Extractable Ca	NS	NS	**	*
^{32}P-exchangeable P_i	NS	NS	NS	NS
Water-soluble P_i	NS	***	**	**
Extractable P_i	*	***	NS	*
Extractable P_o	*	NS	NS	**
Extractable P_o % P_t	NS	NS	*	**
Phosphatase activity	*	***	NS	**
Soil respiration	NS	**	NS	*

[1] As shown by covariance analysis
*, **, *** Significant at P<0.05, P<0.01 and P<0.001 respectively
[2] Group 1 Soils on slates, shales and Andesite—tuffs
Group 2 Soils on limestone
[3] Group 1 October sampling
Group 2 May sampling

Table 2. Relationships between ^{32}P-RNA mineralization rates and some soil properties[1]

Property	All soils		Geology Slates & Andesites		Limestone		Time October		May	
	±	r^2	±	r^2	±	r^2	±	r^2	±	r^2
pH	+	0.42**	(+)	0.48**	+	0.24*	+	0.30**	+	0.67**
Extractable Ca	(+)	0.49**	+	0.62**	+	0.27*	(+)	0.44**	+	0.73**
^{32}P-exchangeable P$_i$	+	0.42**	+	0.35**	+	0.27*	(+)	0.56**	+	0.47**
Water-soluble P$_i$	+	0.14**	+	0.24**	(+)	0.36*	+	0.77*	NS	
Extractable P$_i$	+	0.27**	+	0.24**	+	0.28*	+	0.79**	NS	
Extractable P$_o$	(−)	0.24**	NS		NS		NS		(−)	0.42**
Extractable P$_o$ % P$_t$	(−)	0.43**	(−)	0.32**	−	0.24*	−	0.27**	(−)	0.52**
Phosphatase activity	NS		(+)	0.60**	NS		NS		NS	
Soil respiration	+	0.10*	(+)	0.51**	NS		NS		NS	

[1] Properties expressed cm^{-3}; ( ) significant quadratic term

enough to measure the likely biologically significant differences in the parameters of particular interest in a study should always be asked, preferably before embarking on the study. In the case of the present method, the use of ^{32}P as a tracer has markedly increased the sensitivity obtainable and also made it possible to measure both mineralization and microbial immobilization processes at the same time. The mineralization rates measured, using this method, are of the same order as those likely to be significant in the replenishment of available phosphorus in the local woodland soils. The method also has the following advantages: (i) it generates reasonably precise estimates of mineralization in soils; (ii) the pattern of the relationships between mineralization rate and soil properties appears to be theoretically acceptable; and (iii) it is sensitive enough to be able to detect interactive effects of geology and season in those relationships as well. Moreover, a very high proportion of the variation in the ^{32}P-RNA mineralization rates between soils could be accounted for by the measured soil properties.

As a consequence, it appears to be quite suitable for studies determining the various influences of soil conditions, crop type, environmental and land management factors on rates of organic phosphorus mineralization in soils. It could also provide answers to questions about the relative uptake by plants and micro-organisms of phosphorus from the labile organic phosphorus pool during the growing season (Tate 1984). Analysis of the effects of all these factors, through the development of predictive models, is important, as the mineralization of organic phosphorus is a key process in the phosphorus cycle of managed or 'natural' terrestrial ecosystems and the maintenance of soil fertility (Harrison 1985, 1987).

5 Summary

An isotopic method, to examine the mineralization of labile organic phosphorus in soils at the low rates likely to be significant in the nutrition of forest trees, is briefly outlined. The method uses ^{32}P-labelled RNA, added at 780 ng P cm^{-3}, and allows adjustments to be made for its hydrolysis induced during the soil extraction and organic-inorganic separation phases of the procedure. Rates of mineralization in 50 soils, with pH ranging from 3.1 to 7.5, varied from −29 to 190 ng P cm^{-3} when incubated for 24 h at 13°C. The rates of mineralization were primarily related to soil pH and extractable calcium, and nearly 50% of their variation was related to the availability of P in the soils. Using covariance analysis, interactions of site geology and time of the year were found in the relationships between mineralization rate and a range of soil properties. Over 90% of the total variation in rates of mineralization could be accounted for by soil chemical, physical and biological properties. The method is considered to be useful in understanding the influences of soil conditions and environmental factors on the rate of organic phosphorus mineralization in soils.

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Reference materials for ecological analysis

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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 Auto-Analyzer 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 $NaH_2PO_4.2H_2O$, 0.6714 g Na_2SiF_6 and 2.1980 g $CaCl_2.6H_2O$. Mix together all solutions except $CaCl_2.6H_2O$, 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.)
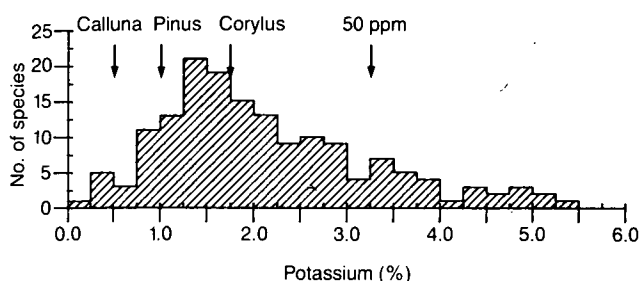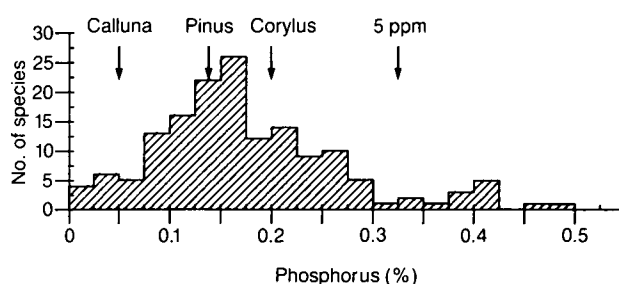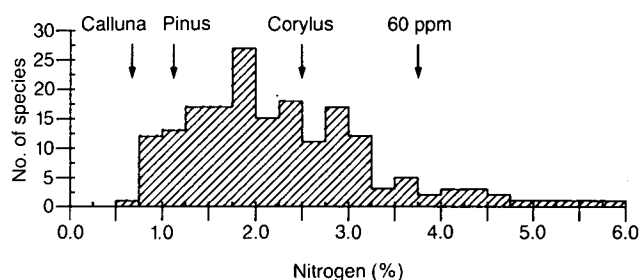

Figure 1. Frequency distributions for N, P and K in vegetation (the ppm value is the concentration in solution after dilution for analysis)
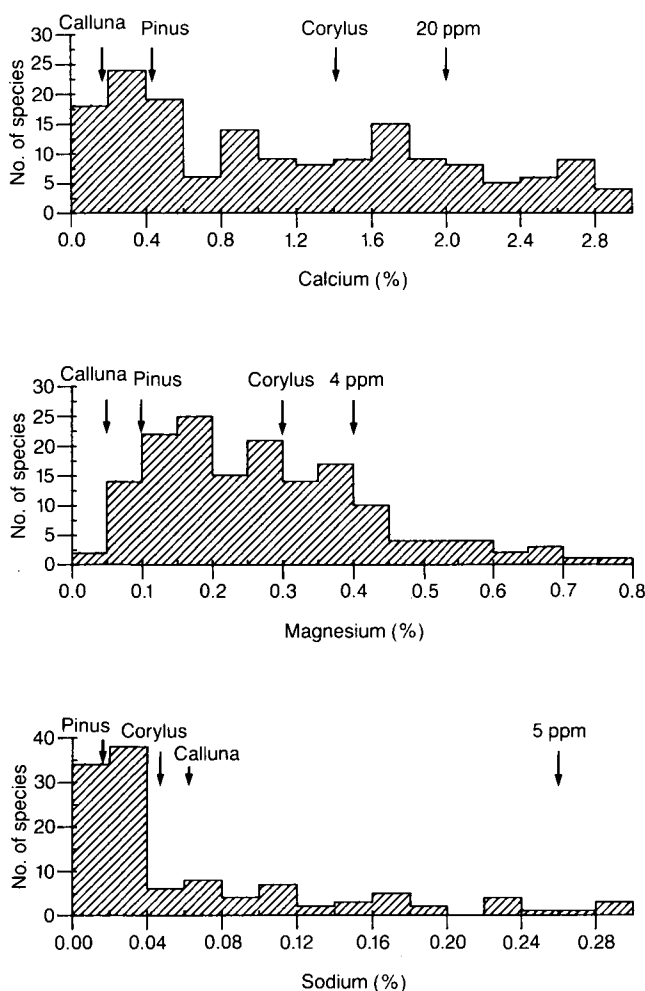
90

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

Figure 3. Frequency distributions for Fe, Mn, Cu and Zn 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.

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.

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The determination of total phosphorus in soils by acid digestion

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1 Introduction

Phosphorus is of major significance in plant nutrition and the various forms in soil have long been studied intensively. Such investigations often require knowledge of the total content in soil, particularly as primary mineral levels are often quite low for this element. Hence, it is important that reliable estimates of the total level can be made.

Absolute chemical methods for bringing minerals, particularly silicates, into solution have always depended on fusion or hydrofluoric acid procedures, although it is well known that the commonest variants (Na_2CO_3 fusion and HF+ $HClO_4$) cannot bring all minerals into solution. Fortunately, the mineral phosphates are, as a group, relatively soluble in strong acids, and this property, coupled with a growing need for convenient procedures in soil analysis, led, many decades ago, to substitute methods being proposed for phosphorus. These methods were based on acid dissolution, and perchloric acid was a favourite because it is also a strong oxidizing agent for soil organic matter.

A common perchloric acid procedure involved digesting 2 g dried soil with 30 ml acid, with the option of a nitric acid pre-treatment if the organic content was high (Jackson 1958). However, although this digestion has been widely used, some workers have suggested that the phosphorus recovery might sometimes be low (Muir 1952; Syers *et al.* 1968). Various mechanisms could, in theory, be responsible for this loss, but there is evidence to suggest that incomplete release from the sample is involved (Sommers & Nelson 1972).

In addition to these problems, there appeared to be a safety risk in the large-scale use of neat perchloric acid, particularly if perchloric salts were allowed to accidently dry out. In more recent years, the need for adequate fume cupboard facilities when using this acid has also been stressed. The neat perchloric acid digestion was therefore not used on a routine basis at ITE's Merlewood Research Station. Instead, a nitric-perchloric-sulphuric system was adopted for soil phosphorus and was taken from the procedure already in use in the laboratory for vegetation. This system followed the method of Piper (1950), but on a smaller scale. Although it was a strong oxidizing system, it was safer to use because the presence of sulphuric acid greatly reduced the risk of drying out.

However, some comparison with a hydrofluoric acid method was required for soils analysed at that time. These tests involved 24 chalk soil samples from 6 sites in southern England, whose mean percentage total phosphorus content (with standard error) was 0.094±0.005, following a hydrofluoric-perchloric digestion of 0.1 g sample, and 0.096±0.004 with a nitric-perchloric-sulphuric digestion of 0.05 g. These particular digestions both included 1 ml sulphuric acid instead of 0.5 ml and dilution to 100 ml not 50 ml (see below). It was concluded that the 2 systems were equally effective for these soils.

At a later stage, the nitric-perchloric-sulphuric procedure was replaced by a sulphuric-peroxide system for vegetation. Although this system had somewhat less oxidizing power and required a catalyst, it had the advantage of recovering nitrogen in the same digest as used for other elements (Allen *et al.* 1974; Parkinson & Allen 1975). Recently this method has been adopted at Merlewood for estimating nitrogen and phosphorus in soils (Rowland & Grimshaw 1985). This paper records comparisons between the 2 systems which were made for the recovery of total phosphorus from a range of soil types, and includes supplementary data obtained with a hydrofluoric-perchloric system. Factors relevant to the overall performance of the sulphuric-peroxide method are also examined briefly.

2 Materials and methods

2.1 Soils and minerals

Samples (103) covering 8 soil types in the United Kingdom were originally taken as part of a soil fertility study led by Dr A F Harrison of Merlewood. The soil types were described pedologically by Dr M Hornung of ITE's Bangor Research Station. Samples were dried (40°C), sieved (<2 mm) and a subsample ground (<0.15 mm) prior to analysis. The approximate organic contents were estimated by loss-on-ignition at 550°C (2 hours). Mean values of the types ranged from 7% to 85% of the dry matter. Mineral samples of fluorapatite $Ca_5 (PO_4)_3F$ (from Norway) and monazite (W Australia) were supplied by University College of North Wales (Bangor) and ground to a fine powder.

2.2 Nitric-perchloric-sulphuric digestion

An air-dried ground sample (0.05 g) was weighed into a 50 ml Kjeldahl flask and digested with 3 ml 5+1 nitric acid (SG 1.42) + perchloric acid (60%) and 0.5 ml sulphuric acid (SG 1.84). The digestion was controlled to avoid rapid loss of acid or excess heating. The flasks were gently boiled for about one hour with only a gradual loss of mainly nitric acid. As the white fume stage approached, heating was increased a little to remove white perchloric fumes and continued for about 15 minutes until mainly sulphuric acid remained. The digest was now more viscous but drying out was not permitted. When cool, the digests were diluted to 50 ml (using a Zippette), mixed and the silica residue allowed to settle. Blank solutions (acids only) were also digested. The final solution was 1% acid (v/v) and used without further dilution to estimate phosphorus.

2.3 Sulphuric-peroxide digestion

The digestion mixture was prepared by mixing 350 ml hydrogen peroxide (100 vol), 14 g lithium sulphate and 0.42 g selenium powder (catalyst), followed by the careful addition of 420 ml sulphuric acid. The mixture was stored at 2°C and was stable for 3—4 weeks. For digestion, 0.2 g air-dry ground samples were weighed into a 50 ml Kjeldahl flask. The digestion mixture was added (4.4 ml) and digested at low heat until the initial vigorous reaction subsided. Heat was then gradually increased and continued for 2 hours. When cool, the digest was diluted to 50 ml, mixed and the silica residue allowed to settle. Blank solutions (digestion mixture only) were also digested. The final solution was 5% acid (v/v) and was further diluted 5 times before estimating phosphorus.

2.4 Hydrofluoric-perchloric digestion

An air-dried ground sample (0.05 g) was weighed into a platinum crucible and 7 ml hydrofluoric acid (40%) and 1 ml perchloric (60%) added in succession. Crucibles were heated gently at 200—220°C for 2 hours with the lid on, followed by evaporation to white fumes. When cool, 3 ml nitric acid and 0.5 ml sulphuric acid were added and digested until white fumes reappeared and continued for about 15 minutes. Digests were finally made up to 50 ml in a volumetric flask.

2.5 Estimation of phosphorus

Phosphorus was determined using an automated molybdenum-blue colorimetric procedure with an AutoAnalyzer, as described by Allen et al. (1974). Standards were compensated with 1% sulphuric acid or digested sulphuric-peroxide reagents as appropriate.

2.6

Moisture contents were determined on separate subsamples of air-dry soil to correct analytical results to a dry (105°C) basis.

3 Results and discussion

3.1 Comparison of digestion procedures

The data in Table 1 show the mean phosphorus contents of each soil type, as recorded by both digestion systems. Differences between the 2 systems were minor for all soil types and none significant at $P<0.05$. The standard errors were also very similar for a given soil type. The digestions appeared to be equally effective, regardless of the organic content of the soil types. Further examination showed that the phosphorus levels themselves were not significantly correlated at $P<0.05$ with the organic contents over 103 soil samples ($r=-0.029$). This result, in turn, may reflect the fact that, on average, only 65% of the total phosphorus was organically bound as shown by data not presented here. It was concluded that the 2 digestion systems recovered comparable amounts of phosphorus from the soils tested. Further evidence was required to show if this was a full recovery.

3.2 Precision

Table 1 includes standard errors which represent the overall variations between 13 soil samples for

Table 1. Comparison of digestion procedures for 8 soil types showing mean percentage total phosphorus with standard errors (dry matter basis)

Soil type	Sites	LOI (%)	% phosphorus 'Peroxide'[1]	'Perchloric'[2]
Pleaty gley	13	35	0.073 ± 0.009	0.069 ± 0.009
Gley	13	10	0.105 ± 0.015	0.100 ± 0.015
Acidic brown earth	13	15	0.074 ± 0.008	0.074 ± 0.008
Basic brown earth	13	7	0.085 ± 0.014	0.080 ± 0.012
Podzol	13	9	0.018 ± 0.004	0.019 ± 0.004
Peaty podzol	13	50	0.064 ± 0.006	0.060 ± 0.006
Brown podzol	13	13	0.058 ± 0.007	0.063 ± 0.008
Peat	12	85	0.069 ± 0.008	0.062 ± 0.008
Overall mean ($\bar{x}$)	103		0.068 ± 0.004	0.066 ± 0.004

[1] 'Peroxide' = sulphuric-peroxide digestion
[2] 'Perchloric' = nitric-perchloric-sulphuric digestion

each soil type. These errors include both site and sampling variations, as well as the analytical component. A measure of the latter which will include both digestion and colorimetric variation can be obtained by digesting replicates of one sample. Four samples were selected as being representative of 4 soil types and 10 replicates from each sample were digested by the sulphuric-peroxide system and analysed as before. The mean values of the 10 replicates and their standard errors are given in Table 2. Although these errors inevitably include variation within a sample, Table 2 still indicates a very satisfactory level of precision for this digestion method.

3.3 Sample preparation
It is a general recommendation in the texts that only finely ground samples should be handled at the weights required for these types of digestion. The greater homogeneity of the ground sample allows a weight of 0.2 g to be reasonably representative of the sample, as shown by the narrow standard error limits in Table 2. Comparable data were obtained for seived but unground material which are not presented here, but they confirm that the precision is less satisfactory. These data also suggest that a slightly higher (6—9%) recovery of phosphorus was obtained from the ground samples of the soil types (peat and peaty gley). This was considered to be due to an increase in surface area presented to the sulphuric-peroxide mixture.

3.4 Digestive conditions
Digestion of 0.1 g and 0.2 g portions of soil showed that comparable recoveries were obtained. However, weights in excess of 0.2 g are not recommended for soil because excess mineral matter is undesirable for smooth control of the sulphur-peroxide system, although up to 0.4 g is acceptable for vegetation (Parkinson & Allen 1975). Although temperature control is not critical, only minimal heating should be used during the initial vigorous reactions and increased gradually after this stage, but avoiding excessive loss of sulphuric acid fumes.

The time required for the digestion of soil was also examined for periods up to 4 hours. Four soil types were selected and the data are presented in Table 3. The results suggested that the digestion is complete after 2 hours. This period was adopted in digesting the 103 soil samples, and only 2 digests had a yellowish tint at the conclusion. In these 2 cases, the colour was considered to be mineral rather than organic in origin.

3.5 Mineral phosphate
Data for mineral phosphate are presented in Table 4. They show that the sulphuric-peroxide digestion gave a phosphorus recovery of 103% from a fluorapatite sample of known composition, whilst hydrofluoric-perchloric and nitric-perchloric-sulphuric systems recovered approximately 95%. Recovery from the monoazite sample was more variable, and no theoretical phosphorus content was available because unknown amounts of the rare earth elements cerium, lanthanum and neodynium were present in this particular material. It left a white precipitate following the sulphuric-peroxide digestion which may have been a peroxide of one or more of the rare earth elements.

The results were satisfactory regarding fluorapatite because of the recovery and because the apatite group (including the chlor- and

Table 2. Precision of the sulphuric-peroxide digestion. Mean percentage phosphorus and standard errors for 10 replicates of one sample of a soil type (dry matter basis)

Soil type	% phosphorus
Pleaty gley	0.063 ± 0.000
Gley	0.044 ± 0.001
Podzol	0.0096 ± 0.0005
Peat	0.046 ± 0.000

Table 4. Recovery of phosphorus from replicates of a single fluorapatite mineral sample by various methods

Method	% phosphorus
Theoretical	18.4
Peroxide[1]	18.9
Perchloric[2]	17.6
HF-HCIO$_4$	17.5

[1,2] see Table 1

Table 3. Recovery of total phosphorus from soil by the sulphuric-peroxide digestion over various time intervals. Mean percentage phosphorus for 3 replicates of one sample of each soil type

Soil type	Time (hours)					
	1.0	1.5	2.0	2.5	3.0	4.0
Gley	0.045	0.044	0.046	0.045	0.046	0.047
Acid brown earth	0.037	0.037	0.035	0.034	0.033	0.035
Podzol	0.012	0.012	0.012	0.012	0.012	0.012
Peat	0.076	0.080	0.082	0.080	0.080	0.080

hydroxy-forms) is the commonest source of primary mineral phosphorus in nature (Mason & Berry 1968). However, problems encountered in digesting monazite suggested that rarer forms of mineral phosphorus might not be fully recovered by these digestion procedures.

4 Conclusions

It was concluded that both digestion systems could recover total phosphorus satisfactorily from the 8 types tested. This would be a full recovery if apatite was the dominant form of primary mineral phosphorus in these soils, although direct evidence for this latter assumption was not available at the time. Forms of phosphorus which appear to be difficult to digest may be sufficiently scarce to suggest that they will rarely lead to a significantly low recovery. However, further work is required on soils containing non-apatite phosphate minerals to evaluate this point.

The paper ends with a caution. It was noted earlier that the sulphuric-peroxide digestion was adopted for vegetation because nitrogen can be determined in the same digest as is used for several other elements. This statement is valid for soil only as regards the estimation of nitrogen and phosphorus. Other mineral elements are not recovered from soil by wet digestion methods, unless hydrofluoric is present, because they are often bound up in silicates or other resistant minerals which, unlike phosphates, are largely insoluble in acids.

5 Summary

Two wet oxidation procedures are compared with regard to their effectiveness in the estimation of total phosphorus in soils. One method is a conventional procedure based on nitric, perchloric and sulphuric acids. The second is based on sulphuric acid and hydrogen peroxide, and has been widely used for plant materials but so far little used for soils. Eight soil types plus 2 mineral phosphate samples are examined and sample preparation is taken into account. Reference is made to a classical hydrofluoric plus perchloric acid procedure. The data suggest that a sulphuric-peroxide system is effective in assessing total phosphorus in soils.

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A comparative study of ashing procedures for the analysis of N, P and K in small amounts of plant materials

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1 Introduction

Many growth studies require chemical analysis of plants for N, P and K. Ashing of plant samples is required to remove organic matter and the 2 methods most commonly used involve dry and wet digestion. Either of these 2 methods can cause problems during the subsequent analysis (Gorsuch 1970; Parkinson & Allen 1975; Watling & Wardale 1979). Dry ashing is relatively simple but N is lost completely, whilst ashing samples above 500°C can result in losses of P. In contrast, wet ashing involves the use of various concentrated acids and catalytic agents. This method may be tedious and can result in matrix interference and contamination. The limited amounts of plant sample produced in many growth studies are an added complication. It was, therefore, essential to compare various ashing procedures for the analysis of all the 3 elements in small amounts of plant materials. As a further check, the recoveries of added N, P and K in a plant sample were determined by both manual and auto-analytical methods. Also, because of its involvement in wet ashing, the effects of Cu on standard solutions of N and P were investigated.

2 Materials and methods

Shoot material of annual meadow-grass *(Poa annua)* was chosen from one of our growth experiments carried out in solution culture. It contained relatively higher concentrations of nutrients than most field-grown plants. Therefore, most of the elements were at a high enough concentration to be detected in small amounts of ashed samples.

2.1 Comparison of ashing procedures

2.1.1 Dry ashing

A 100 mg plant sample was weighed into a porcelain crucible (36 mm diameter) and then placed in a muffle furnace at room temperature. The sample was ashed at 490°C for 17 hours or overnight. The ash was dissolved in 4% v/v H_2SO_4.

2.1.2 Wet ashing

i. Kjeldahl digestion
   Plant samples of 100 mg were weighed into 150 mm long × 15 mm diameter pyrex glass tubes. To each tube was added 1 ml H_2SO_4 and one of the following digestion mixtures:
   a. 100 mg mixture of K_2SO_4 + $CuSO_4$ + Se powder (100:10:1)
   b. 100 mg mixture of Na_2SO_4 + Se powder (100:1)
   c. 100 mg mixture of Na_2SO_4 + $CuSO_4$ + Se powder (100:10:1)
   d. 250 mg mixture of Na_2SO_4 + Se powder (100:1)

They were then digested on an aluminium Thermal Block at 315°C for 4—5 h. The digest was diluted with distilled water to 4% v/v acid.

ii. Acid oxidation
   A 100 mg plant sample was weighed into a glass tube as described in 2.1.2 (i). It was then digested with 0.5 ml 60% perchloric acid and 0.5 ml H_2SO_4 at 260°C for 2—3 h.

2.2 Recovery of added N, P and K

A solution of 0.5 mg N as $(NH_4)_2SO_4$, 0.08 mg P as $NaH_2PO_4.2H_2O$ and 0.2 mg K as K_2SO_4 was added to tubes containing 0 mg and 20 mg plant samples. These and another set of tubes containing only 20 mg plant samples were also ashed by the method described in 2.1.2 (i) (c).

2.3 Effect of copper on N and P

A range of Cu concentrations, ie 0—100 mg l^{-1}, added to 5 mg l^{-1} P and 10 mg l^{-1} N standard solutions respectively.

2.4 Comparison of different sample sizes

A range of 5—100 mg samples was ashed by the micro-Kjeldahl digestion procedure as described in 2.1.2 (i), using 100 mg mixture of Na_2SO_4 + $CuSO_4$ + Se powder (100:10:1). The ashed solutions were analysed for N by the indophenol blue method, and for P by the modified method of sulphomolybdic acid and $SnCl_2$ on AC1 (Pye Unicam, Automatic Chemistry Unit). N was also determined by the manual Kjeldahl distillation procedure and P by the sulphomolybdic acid method (Allen *et al.* 1974). K was determined by flame photometry. All standards were made with the addition of respective digestion mixtures of acids and salts.

3 Results and discussion

3.1 Effect of ashing procedures

The contents of N, P and K analysed in plant digests (Table 1) showed that in samples ashed dry N was completely lost and the K levels were lower than in wet digests. In contrast, the results for N, P and K in samples ashed wet, either by the

Table 1. Concentrations of N, P and K in 100 mg plant samples ashed by various methods. Mean of 5 replicates as mg g^{-1} (±SD)

Ashing method	N	P	K
1. Dry	ND	6.00	49.9
2. Wet			
i. Micro-Kjeldahl			
a.	42.4	5.47	C
	(1.4)	(0.13)	
b.	42.4	6.23	55.0
	(3.5)	(0.28)	(0.3)
c.	43.5	6.15	60.7
	(2.3)	(0.52)	(0.9)
d.	40.5	5.31	62.4
	(1.6)	(0.08)	(0.9)
ii. Acid oxidation	42.5	6.16	55.0
	(2.2)	(0.16)	(1.0)
Merlewood	43.5	6.05	58.0
	(0.8)	(0.10)	(0.0)

ND = not detectable
C = contamination from digestion mixture

micro-Kjeldahl digestion procedure using 1 ml H_2SO_4 and 100 mg of Na_2SO_4 + $CuSO_4$ + Se powder (100:10:1), or by the acid oxidation procedure, were similar to the values independently obtained by ITE's Merlewood laboratory. The results of other wet digests showed that K_2SO_4 caused contamination of K but was otherwise acceptable. When Cu was absent from the digestion mixture, amber-coloured solutions were produced. A larger weight of digestion mixture containing $CuSO_4$ reduced P content (see Section 3.3). This result indicates that, though ashing of plants by wet acid oxidation is generally adequate, the micro-Kjeldahl procedure is most suitable. The use of perchloric acid is not advised because it can cause explosions.

3.2 Recovery of N, P and K
The results in Table 2 show that 93—101% recovery of all the added elements was achieved

Table 2. Percentage recovery of added N, P and K into 20 mg plant samples wet digested and analysed by manual and automatic methods. Means of 3 replicates (±SD)

	Manual		Automatic		Flame photometer
Sample	N	P	N	P	K
Plant + NPK	101	93	101	101	98
	(2)	(2)	(1)	(2)	(0)
NPK	99	100	95	95	100
	(2)	(1)	(2)	(2)	(0)

in the presence and absence of plant samples. There was little or no difference in the results of N and P analysed by manual or auto-analytical methods, indicating that there is no matrix interference from the digestion mixture on the chemistry of N and P.

3.3 Effect of Cu interference
The results in Table 3 show that Cu did not interfere with the determination of N, whereas P was depressed in the presence of Cu. The depression increased with increasing levels of Cu to a maximum of *c* 10—15%. However, although the amount of Cu added in the 100 mg digestion mixture was more than 100 mg l^{-1} Cu, it had no deleterious effect on the estimation of P (see Tables 1 & 2). In contrast, the addition of larger weights of digestion mixture containing $CuSO_4$ again caused low recovery in the P estimation. This suggests that the amount of Cu added in the digestion mixture should be reduced to the minimum level required to produce an adequate end-point.

Table 3. Effect of copper on the N and P standard solutions

Level of Cu added (mg l^{-1})	N	P
0	10.0	5.0
10	10.0	4.65
20	10.0	4.50
30	10.0	4.45
45	9.9	4.35
50	9.9	4.35
100	10.0	4.35

Table 4. Concentrations of N, P and K in different plant weights ashed by wet Kjeldahl digestion method. Mean of 5 replicates as mg g^{-1} (±SD)

Sample weight (mg)	Digestion mixture 1 ml H_2SO_4 + 100 mg (NA_2SO_4 + $CuSO_4$ + Se)		
	N	P	K
5	52.4	6.49	58.5
	(3.6)	(0.30)	(3.8)
10	49.2	6.42	57.5
	(2.0)	(0.42)	(1.8)
20	48.0	6.03	57.4
	(0.9)	(0.05)	(1.8)
50	44.3	5.92	55.0
	(0.6)	(0.04)	(0.0)
100	43.5	6.15	60.7
	(2.3)	(0.52)	(0.9)

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3.4 Effect of different sample sizes

The results in Table 4 show that the N content increased with decreased sample weights, ie N was c 20% higher in 5 mg and 8—10% higher in 20 mg, than in 50 mg samples. This increase occurred because the error of estimation was exaggerated by increased sample dilution. The differences in the levels of P and K found were small and showed no consistent trends due to sample weight, suggesting that a sample weight of as little as 5 mg can be digested adequately and analysed, providing that small samples are diluted to the minimum volume required for analysis.

4 Conclusion

The destruction of organic matter in plants is not a problem — the important consideration is whether the ashing procedure chosen produces accurate and reproducible results. Second, the ashing procedure has to be safe and without contamination on the subsequent analysis of N, P and K. Analytical discrepancies can arise in the chemical analysis of small amounts of plant material because of a variety of processing and instrumental errors. The most important, in this instance, is the effect of large dilutions on small-size samples, which affects N results more than the other elements. It is clear from the results that the plant material ashed by the micro-Kjeldahl digestion procedure using 1 ml H_2SO_4 and 100 mg digestion mixture of Na_2SO_4 + $CuSO_4$ + Se powder (100:10:1) is most suitable. This procedure gave c 93—101% recoveries of added N, P and K in plant samples by both manual and auto-analytical methods. Also, both the small- and large-size samples were analysed with reasonable accuracy. It appears that low results may occur if samples are digested with diges - tion mixture containing large amounts of Cu salts. It is, therefore, advisable to reduce to a minimum the use of Cu in the digestion mixture.

It is suggested that the digestion of plants in pyrex tubes on the thermal block increases turnover, and that the subsequent analysis of N and P on the AutoAnalyzer reduces the cost.

5 Summary

A comparative study of various dry and wet ashing procedures using 100 mg plant samples was made on the nutrient levels of N, P and K. Samples digested with 1 ml H_2SO_4 and 100 mg Kjeldahl digestion mixture of Na_2SO_4 + $CuSO_4$ + Se powder (100:10:1) gave similar levels of N, P and K to those obtained by ITE's Merlewood laboratory. When less than 50 mg plant weight was digested, results for N increased by 5—10%, whereas there was no such effect on P and K levels. Recoveries of added N, P and K in wet digested plant samples gave 93—100% recovery of all the elements, suggesting that the wet micro-Kjeldahl digestion method is suitable for the analysis of N, P and K in very small amounts of plant samples.

Large numbers of samples can be digested in pyrex tubes by the micro-Kjeldahl digestion procedure on thermal blocks. Analysis of N and P on semi-automatic or AutoAnalyzer systems also increases turnover, which is an important consideration under present economic conditions.

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A controlled flow rate leaching method for the determination of exchangeable acidity and aluminium in soils

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1 Introduction

A 1 M KCl solution is considered suitable for displacing exchangeable acidity and aluminium from acid soils (McLean 1965; Pratt & Bair 1961; Sivasubramaniam & Talibudeen 1972), but several methods (see references) including leaching and/or extraction have been used. The methods vary in soil to solution ratio from 1:10 to 1:500, in length of contact time of soil with solution from 1 min to 24 h, and in whether the soil and solution are shaken or allowed to stand. While some studies comparing leaching with extraction methods show little difference (Dewan & Rich 1970), others show that there is more variation in extraction than leaching and that, for some soils, leaching removes more Al (Bache & Sharp 1976a; Lee *et al.* 1985). The longer the leaching period, the more Al is released in total (Bhumbla & McLean 1965), but with progressive leaching there is a gradual decrease in the release of Al (Bache & Sharp 1976a). Pratt and Bair (1961) suggest that soils should be filtered immediately to prevent including non-exchangeable Al in the measurement, while Lin and Coleman (1960) and Sivasubramaniam and Talibudeen (1972) prefer slow leaching. The amount of Al released depends on the flow rate of the extracting solution through the soil and, if the flow rate is not controlled, it will vary with soil type, and spurious results will be obtained.

A leaching method with a controlled flow rate is described which is more efficient and reliable than a manual method previously used at the Hill Farming Research Organisation.

2 Experimental method

Eleven soils were leached in duplicate by a manual method and a controlled flow rate method. Exchangeable acidity was measured by titration against 0.02 M NaOH (Logan *et al.* 1985a) and exchangeable Al by coloration with Alizarin red (Logan *et al.* 1985b).

2.1 Manual leaching

A 10 g sample of air-dry soil (<2 mm) was weighed into a 100 ml beaker, mixed with 50 ml 1 M KCl solution and left to stand overnight. After filtering through a Whatman no. 1 filter, the soil was washed with small portions of 1 M KCl until 100 ml leachate was collected. Filtering should take at least 2 h (McLean 1965). The washing was continued until a further 2 x 100 ml leachates were collected. Each 100 ml leachate was analysed separately and summed to give total soil exchangeable acidity and Al.

2.2 Controlled flow rate leaching

A 2.5 g sample of air-dry soil (<2 mm) was weighed into a Whatman filter thimble (27 × 80 mm external), then placed inside a plastic syringe barrel (B-D Plastipak 50 ml) with a small bore tube leading from the base into a 250 ml volumetric flask. The soil was wetted with a little 1 M KCl solution, and the tube sealed with a bung through which 1 M KCl was continuously pumped at 0.6 ml min^{-1} until 250 ml leachate was collected.

3 Results and discussion

Both exchangeable acidity and exchangeable Al were significantly greater (P<0.001) when soil was leached by the controlled flow rate method (Table 1). This result can be explained by the greater soil/solution ratio of 1:100 compared with 1:30 for the manual method. Analysis of the third 100 ml manual leachates (Table 2) showed that they contained between 10% and 20% of the total manual exchangeable acidity and Al, suggesting that exchange was incomplete. This suggestion is supported by Bache and Sharp (1976a), where leaching was complete at a soil/solution ratio of about 1:100.

The standard errors of the 2 methods were the same, but the coefficient of variation was less for the controlled flow rate method.

4 Conclusions

With manual methods, leaching conditions differ with soil type; extractant flows quickly through a sandy soil (often less than 2 h), but very slowly through peat. The controlled flow rate method leaches soils for the same time period and soil/solution ratio, allowing better comparisons to be made of exchangeable acidity and Al in different soil types.

Exchangeable acidity analysis must be carried out as soon as possible (Bache & Sharp 1976b), so that there is a limit to the number of samples which can be analysed in a day. As the manual method requires 3 × 100 ml leachates, and hence 3 analyses for each sample, far fewer determinations can be made, compared with the controlled flow rate method.

The main advantages of the controlled flow rate

Table 1. Exchangeable acidity and exchangeable aluminium for 11 soils leached by the controlled flow rate and manual methods

Soil no.	Exchangeable acidity (meq 100 g^{-1}) Manual[1]	Controlled flow[1]	Exchangeable Al (meq 100 g^{-1}) Manual[1]	Controlled flow
1	5.35	6.60	4.87	6.45
2	4.97	6.13	4.44	5.15
3	1.26	2.00	0.94	1.89
4	5.19	6.43	4.77	6.39
5	5.21	6.64	4.62	6.31
6	2.30	3.28	1.75	1.60
7	3.03	4.19	2.49	3.86
8	4.84	6.25	4.17	6.69
9	1.23	1.79	0.76	1.32
10	5.23	6.66	4.34	6.69
11	5.15	6.68	4.26	6.13
x̄	3.97	5.15	3.40	4.77
SE[2]	0.13	0.13	0.12	—
CV%	3.22	2.49	3.38	—

[1] Values are means of duplicate leachates
[2] Pooled standard error of 11 pairs of duplicates

Table 2. Exchangeable acidity and exchangeable Al in first, second and third manual leachates for 11 soils (means of duplicate leaching)

Soil no.	Exchangeable acidity (meq 100 g^{-1}) I	II	III	Exchangeable Al (meq 100 g^{-1}) I	II	III
1	3.98	0.83	0.55	3.63	0.79	0.46
2	3.60	0.84	0.53	3.24	0.77	0.43
3	0.70	0.31	0.25	0.43	0.32	0.20
4	3.79	0.88	0.52	3.60	0.76	0.42
5	3.50	1.10	0.61	3.18	0.95	0.49
6	1.35	0.54	0.40	0.97	0.47	0.32
7	1.82	0.75	0.46	1.49	0.68	0.33
8	3.58	0.79	0.48	3.12	0.66	0.39
9	0.72	0.29	0.22	0.34	0.27	0.15
10	3.67	0.93	0.64	3.12	0.80	0.42
11	3.62	0.97	0.56	3.01	0.81	0.45

method are that exchange is more complete, flow rate is independent of soil type, and most of the leaching occurs overnight so that it is time-saving.

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