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EVALUATION OF THE PERFORMANCE OF COMMERCIAL SOIL AND PLANT TESTING LABORATORIES FOR ANALYSIS OF SULPHUR AND NITROGEN

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EVALUATION OF THE PERFORMANCE OF COMMERCIAL SOIL AND PLANT TESTING LABORATORIES FOR ANALYSIS OF SULPHUR AND NITROGEN

by

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ABSTRACT

Ten UK laboratories participated in a study to evaluate variability of analysis for total concentrations of nitrogen (N) and sulphur (S) in plant materials and extractable S in soil samples. Four plant and two soil samples were prepared at IACR-Rothamsted and distributed to the participants. Laboratories were in reasonable agreement for analysis of total N in plant materials, but the variability for total S was considerably higher. Large differences between laboratories were also reported for extractable S in soil. Different analytical methods were used by laboratories for both soil and plant analyses, but this does explain the large variability in results. Diagnosis of S deficiency based on S analysis may be of questionable validity if S analyses results for plant and soil are not accurate. To improve analytical reliability, more method development and standardisation are needed.

1. INTRODUCTION

Decreasing sulphur (S) inputs from the atmosphere and fertilisers have led to increasingly widespread S deficiencies in grassland and arable crops in the UK (McGrath *et al.*, 1996) Due to adoption of pollution control measures, sulphur dioxide emissions in the UK have decreased by about 50% since the early 1970's. In other western European and North American countries similar trends have been observed. Furthermore, traditional fertilisers containing considerable amounts of S such as ammonium sulphate and single superphosphate have been largely replaced by high analysis fertilisers containing little or no S.

The problem of increased S deficiency has led to a greater need for plant tissue and soil testing to diagnose whether applications of S fertilisers are necessary. Compared with phosphorus, potassium and nitrogen (N) testing, testing for S is relatively new in the UK and at present only a relatively small number of laboratories are equipped to do this work routinely. It is therefore important to ensure that the laboratories involved in this type of work are using the most appropriate methods of analysis and thereby capable of providing reliable data. Decisions about fertiliser application based on unreliable data could prove costly for arable producers, both in terms of low yield or unnecessary fertiliser use.

To test laboratory performance, an inter-laboratory trial was set up and nine major commercial UK plant and soil analytical laboratories were invited to participate along with IACR-Rothamsted. Laboratories were asked to analyse four selected plant materials for total S and total N and to extract and analyse two soils for available S.

2. REVIEW OF THE METHODS USED FOR PLANT AND SOIL ANALYSIS

2.1 Plant tissue analysis

Several diagnostic indices have been suggested for diagnosis of S deficiency using plant tissue analysis for example, total S, N:S ratio, sulphate and sulphate:total S ratio (McGrath *et al.*, 1996). These indices involve analysis of total S, total N and sulphate-S. To date, there is no general consensus as to which index is best, the choice to some extent being determined by the analytical capabilities of the individual laboratories. Another factor in determining the most

appropriate index to use is crop species. For example, N:S ratio has been found useful for cereals and grass but not for oilseed rape. To improve reliability of the diagnosis, it is advisable to obtain results for more than one index.

The most widely used methods for determination of total S in plant tissues involve the initial destruction of organic matter by digestion with mixtures of nitric and perchloric acid, or ashing in the presence of magnesium nitrate in a muffle furnace followed by dissolution of the ash in dilute acid. The total S in solution may then be analysed by Inductively Coupled Plasma (ICP) Emission Spectrometry or turbidimetry (Zhao *et al.*, 1994).

For analysis by ICP, the ionic form of S is unimportant, because at the extremely high temperature (8,000-10,000 ^{o}K) which occurs in the plasma, all forms of S are determined as long as they are in solution. The ICP method has a number of advantages over other chemical methods in the analysis of S. In particular, the ICP method has a high sensitivity for S (detection limit<0.02 ppm in solution), suffers little interference, and offers the capability of simultaneous determinations of other major and trace elements. Two wavelengths can be used for the determination of S, i.e., 180 and 182 nm, but depending on the instrument, usually only one of them is available. The 180 nm wavelength provides a greater sensitivity than the 182 nm wavelength, but has the disadvantage of being affected by the spectral interference of calcium (Ca). Because Ca is ubiquitous and often present in high concentrations in plant and soil samples, the 182 nm wavelength is preferred for the S analysis. If the 180 nm wavelength is used, careful corrections for the Ca interference are required.

The turbidimetric method is based on precipitation of barium sulphate. Incomplete digestion may leave organic matter in solution, which tends to increase barium precipitation, resulting in overestimation of S. On the other hand, incomplete conversion of organic S to sulphate during the digestion or ashing stage will result in underestimation of S.

Another method used for the determination of total S in plant tissues is combustion analysis based on the Dumas method. Plant tissue is rapidly oxidised at high temperature in a combustion analyser, S is determined by infrared detection, and nitrogen can also analysed by this method using thermal conductivity detection. For total nitrogen analysis, the Kjeldahl method, which involves digestion of the sample with boiling sulphuric acid in the presence of a catalyst, is the most widely used method. Many laboratories are moving to combustion analysis for total N and S because the procedure is rapid and less hazardous. This technique is not

without problems; if a satisfactory simultaneous analysis of N and S is to be obtained, care must be taken to ensure that sample oxidation is complete and that an appropriate calibrant is selected which is similar to the sample material analysed. If the instrument has been calibrated with a pure compound there can be problems with the analysis especially when dealing with materials that are difficult to oxidise. This problem is usually resolved by drift correcting the instrument with a reference material of similar composition to the sample analysed, but for the following reasons this approach is far from ideal: reference materials are expensive, reference materials for all sample types do not exist, and the uncertainties of the reference value are much greater than those associated with a pure compound.

Sulphate usually accounts for between 10-50% of the total S in plant tissues. There is evidence that the sulphate pool in plant tissues responds more sensitively to the S availability and therefore may be a better indicator of S deficiency (Zhao *et al.*, 1996). Sulphate in plant materials can be extracted readily with water and determined using ion chromatography (IC). If the aim is to determine sulphate only, then the IC method is preferred because it is specific for sulphate and other inorganic anions (such as nitrate), whereas ICP measures both sulphate and soluble organic S. The turbidimetric method can be unreliable for this type of sulphate analysis because water extracts of plant tissues are usually brown or green in colour, which can interfere with measurement. Determination of plant sulphate was not included in the present project.

2.2 Soil analysis

Most methods which measure the amounts of soil S available for plant uptake involve extraction of soil with weak salt solutions. For determination of water soluble S the most widely used extractants are water, $CaCl_2$ and LiCl, and for water soluble plus adsorbed S KH_2PO_4 , $Ca(H_2PO_4)_2$ and NH_4OAc+HOAc are used. The extracted S is then measured by a reduction -colorimetric procedure or turbidimetry. Both procedures are tedious and suffer from the effects of chemical interference. Increasingly, instrumental methods of S determination such as ion chromatography and ICP are being used. Ion chromatography is sensitive and specific to inorganic SO_4-S; ICP is rapid and precise measuring both SO_4-S and dissolved organic S. On average, ICP results are 30-60% greater than those of IC (Zhao and McGrath, 1994). It is clear that different methods of extraction and determination will produce different values of

extractable S for the same soil. If a method is to be used for estimating soil S availability, the method has to be calibrated with field experiments. A previous HGCA funded project has shown that the critical values for winter oilseed are about 3 mg kg^{-1} soil for the IC method and 5-6 mg kg^{-1} soil for the ICP method (Withers *et al.*, 1995a and 1995b). Because the critical values of soil available S are small, it can be difficult to determine accurately unless the most sensitive analytical techniques are used.

3. MATERIALS AND METHODS

Ten laboratories including IACR-Rothamsted participated in the study. Four crop materials, grass (PL1), wheat grain (PL2), winter rape (PL3) and wheat flour (PL4) were selected for analysis. The materials PL1, PL2 and PL3 were prepared at IACR. They were selected for their range of total N and S concentrations. Sample PL4 was a reference material purchased from the Bureau of Analysed Samples Ltd (NIST 1567A) with a certified total S concentration. Samples PL1, 2 and 3 were dried at 80°C for 16h before being ground to pass a 0.5 mm sieve, and thoroughly homogenised by a process of coning and quartering. Subsamples of PL1, 2 and 3 were taken for repeated analysis of total N and S to test homogeneity. The four samples, each about 20 g, were distributed to the participating laboratories. Laboratories were instructed to dry the plant materials at 80 °C for 4h prior to subsampling for analysis, and they were asked to use their routine methods for the determination of total S and N. IACR-Rothamsted also carried out analysis of total S using the $HNO_3/HClO_4$ digestion followed by ICP determination. The wavelength 182 nm was used, and the instrument was calibrated with standard solutions purchased from Fisher Scientific Ltd. Total N was determined using a combustion method (LECO CNS 2000), which was calibrated with standard EDTA. Comparisons between the Kjeldahl and combustion methods were done.

Two soils selected for their contrasting available S concentrations were prepared at IACR-Rothamsted. Soil SO1 was from Woburn and SO2 from Rothamsted; both were sampled from the top 15 cm of the soil profile. The soils were air dried, passed through a 2mm sieve and thoroughly homogenised. Plant debris was removed by hand. About 120 g of each soil was sent to the laboratories. For determination of available S, laboratories were instructed to use KH_2PO_4 extraction (Zhao and McGrath, 1994), followed by their chosen method of analysis.

4. RESULTS AND DISCUSSION

4.1 Plant materials

Data for total N in the four different crop samples are presented in Figures 1 and 2, and plant total S in Figures 3 and 4. Concentrations are presented on the vertical axis, and the laboratory code (L1-10) and method of analysis on the horizontal axis. All laboratories, except L1, reported individual values of repeated analysis of the same sample (usually 4-5 replicates). The data are presented in the form of box plots, which show the mean value of replicates as a thick dark line inside each box, the median as a thin line (the 80th percentile of replicate data are within the box) and the entire range of replicates within the two outermost horizontal bars. Therefore, the narrower the range, the better the analytical precision for a laboratory. L1 reported only the mean value for each sample; therefore the precision of their analysis cannot be assessed.

4.2 Total nitrogen

The N concentrations for the plant materials ranged from 1.4 to 2.4%. Mean results for total N in samples PL1-3 agree well among laboratories, with data from most laboratories falling within a narrow range of around 0.2% N, and coefficients of variation (CVs) in the range of 2.7-4.1% (Figures 1-2, Table 1). The data for PL4 were more variable, covering a much wider range of 0.4% N, and a CV of 5.4%. It is not clear why the inter-laboratory variability for the analysis of PL4 was greater than for the other three materials, particularly as this was a certified reference material which was very homogeneous. Participating laboratories used two different methods, Dumas and Kjeldahl, to analyse total N. However, a closer examination reveals that the discrepancy between laboratories was not related to the analytical method used.

Within laboratory variability for most laboratories was small with the exception of L3, L6 and L9. Data from these laboratories were consistently more variable and it is worth noting that they used the Kjeldahl method of analysis. Our experience with this procedure shows that the method is less precise than the Dumas combustion method. Agreement between Kjeldahl and Dumas is generally very good, although there is a tendency for Dumas to produce slightly

larger values, probably because the combustion procedure will determine all forms of N. Table 2 shows comparisons between the Kjeldahl and Dumas methods for total N concentrations of a range of soil and plant materials analysed at IACR-Rothamsted. Because of the good agreement with the Kjeldahl method, a superior precision and rapid analysis, the Dumas method is becoming more widely used.

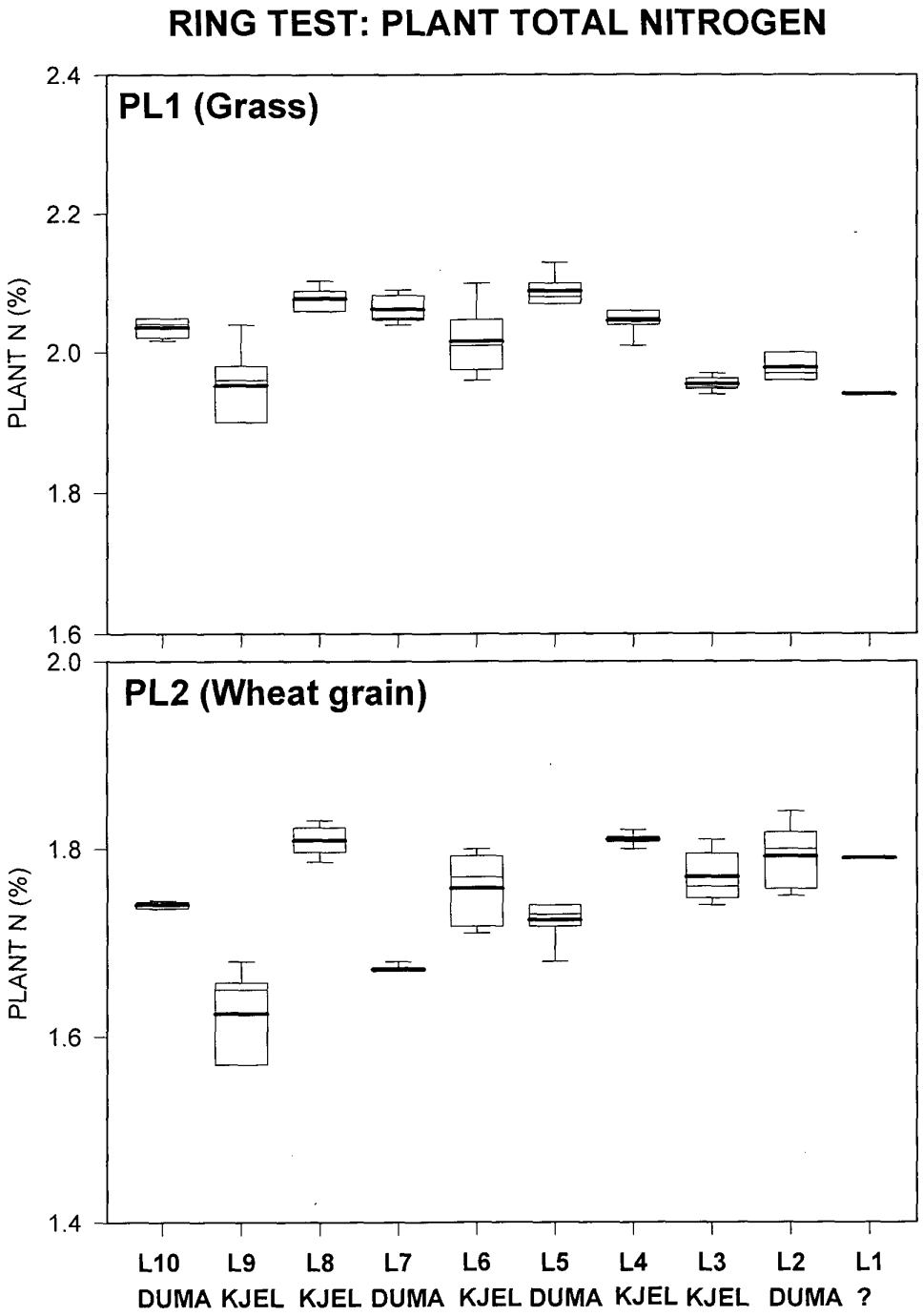

Figure 1. Analytical results for total N concentrations of plant samples PL01 and PL02. KJEL=Kjeldahl, DUMA=Dumas; ?=Method unknown.

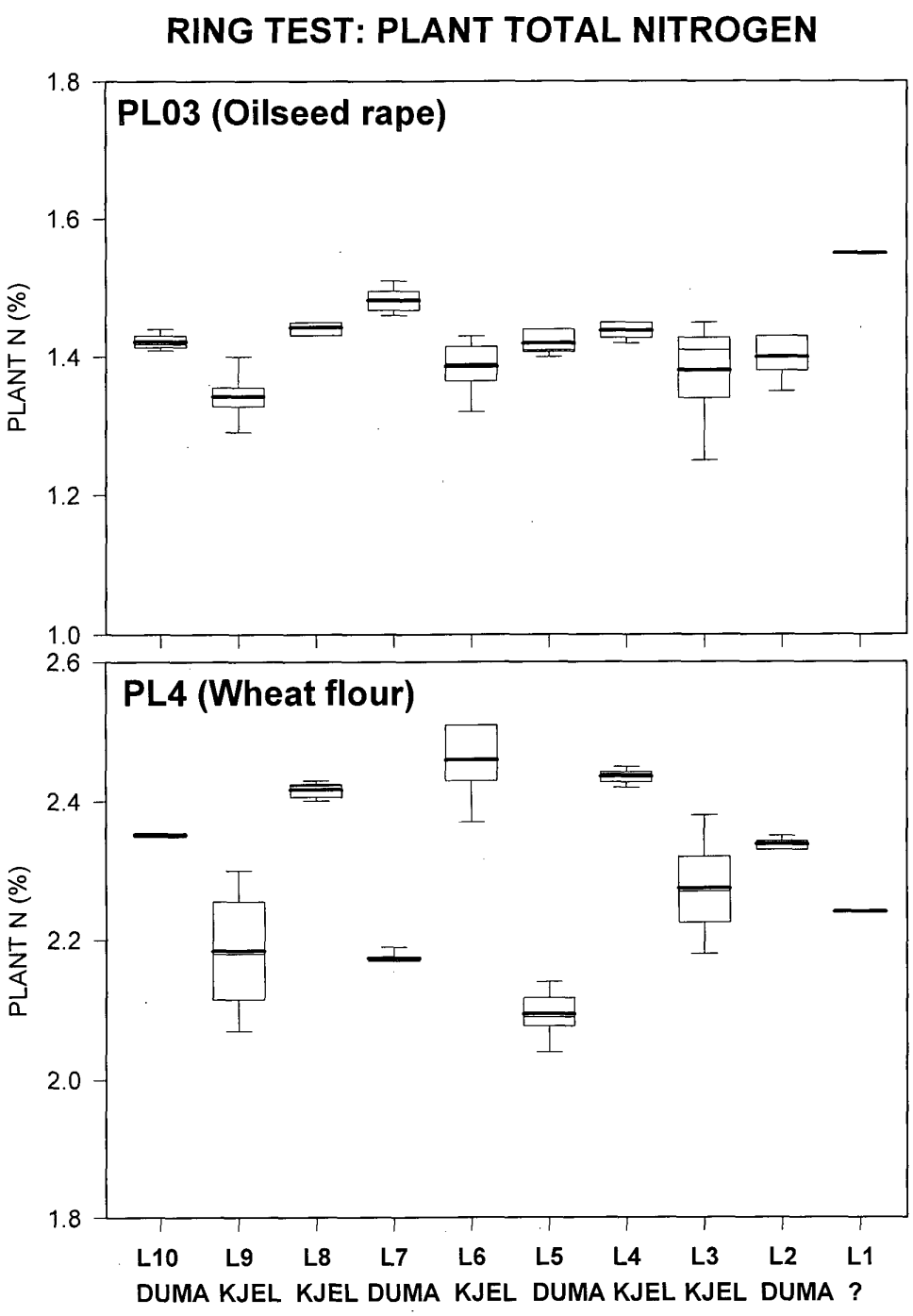

Figure 2. Analytical results for total N concentrations of plant samples PL03 and PL04. KJEL=Kjeldahl, DUMA=Dumas; ?=Method unknown.

Table 1. Inter-laboratory CV% for total plant N and S and soil extractable S

Materials	Inter-laboratory CV% for total N	Inter-laboratory CV% for total S	Inter-laboratory CV% for N:S	Inter-laboratory CV% for extractable S
PL1	2.74	13.12	13.68	
PL2	3.50	19.53	20.28	
PL3	4.08	8.22	8.1	
PL4	5.39	20.27	21.87	
SO1				46.38
SO2				47.53

Table 2. Comparisons of the Dumas and Kjeldahl methods for total N analysis

Materials	Dumas* N%	Kjeldahl N%	Dumas* CV%	Kjeldahl CV%
Grass	1.48	1.47	0.59	3.22
Wheat straw	0.50	0.44	1.66	4.88
Wheat grain	1.98	1.95	0.43	2.10
Lupin grain	5.61	5.49	0.60	2.35
Soil	0.26	0.25	1.56	2.68

*LECO CNS model 2000.

4.3 Total sulphur

The inter-laboratory variability for total S was considerable for all plant materials (Figures 3-4; Table 1). For materials PL1, PL2 and PL4 the inter-laboratory variability was around 0.09% S in samples which have S concentrations of less than 0.3%. In sample PL3 the variability was around 0.17% S in a material which has a total S concentration of around 0.6%. Inter-laboratory CVs for total S ranged from 8.2 to 20.3%, again indicating poor agreement between laboratories. For all four plant samples, mean results from five laboratories (L2, L3, L4, L5 and L10) appeared to agree reasonably well, whereas the other five laboratories reported more extreme results.

Inter-laboratory variability was much greater for S analysis than N analysis (Table 1). This may indicate that S determination is a more complex and difficult procedure. For plant materials PL1, PL2 and PL3, laboratories L1, L6 and L7 tended to report smaller values, and this does not seem to be related to method of measurement, as the laboratories used either turbidimetry or ICP. This problem could relate to the method of digestion or extraction. Laboratory L9 also reported smaller S concentrations in PL3 and PL4 using ICP. In the certified reference material sample PL4, the certified S concentration is 0.165±0.002%. Only laboratory L2, which reported a mean S content of 0.167% was in line with the certified value. L8 was close with a result of 0.174%.

From a purely practical standpoint, farmers want to use these data for deciding whether S fertilisers should be bought and applied or not. The effects of lack of reliability of the analytical processes involved (leaving aside sampling problems) can be illustrated using these data.

Again, leaving aside any argument about which plant tissue to sample and what the critical values should be at certain stages for different crops, if a critical value of 0.20% S is taken for grass, then the results for PL1 in Figure 3 may have been used to conclude that the sample was clearly S deficient in four cases (L1, L6, L7 and L9). It would be seen as borderline using results from four other laboratories (L2, L3, L4 and L5); and not deficient with results from L8 and L10. Using the same rationale, a critical value of 0.12% for wheat grain (PL2) gives a similar picture, with four laboratories again clearly indicating deficiency, and the same four laboratories giving these low results. Moving next to the certified sample in Figure 4 (PL4), we have the benefit of knowing in this case that the result should be about 0.16%, i.e. not deficient, yet the same four 'low' laboratories would indicate that the sample was in fact deficient. Forty percent of the laboratories would therefore be 'wrong' from the farmer's point of view and cost him/her money. Taking the rape sample PL3 last, and a critical value of 0.40% in the leaves, no laboratory would lead to the interpretation that S was needed, but nevertheless two laboratories reported results which were very low.

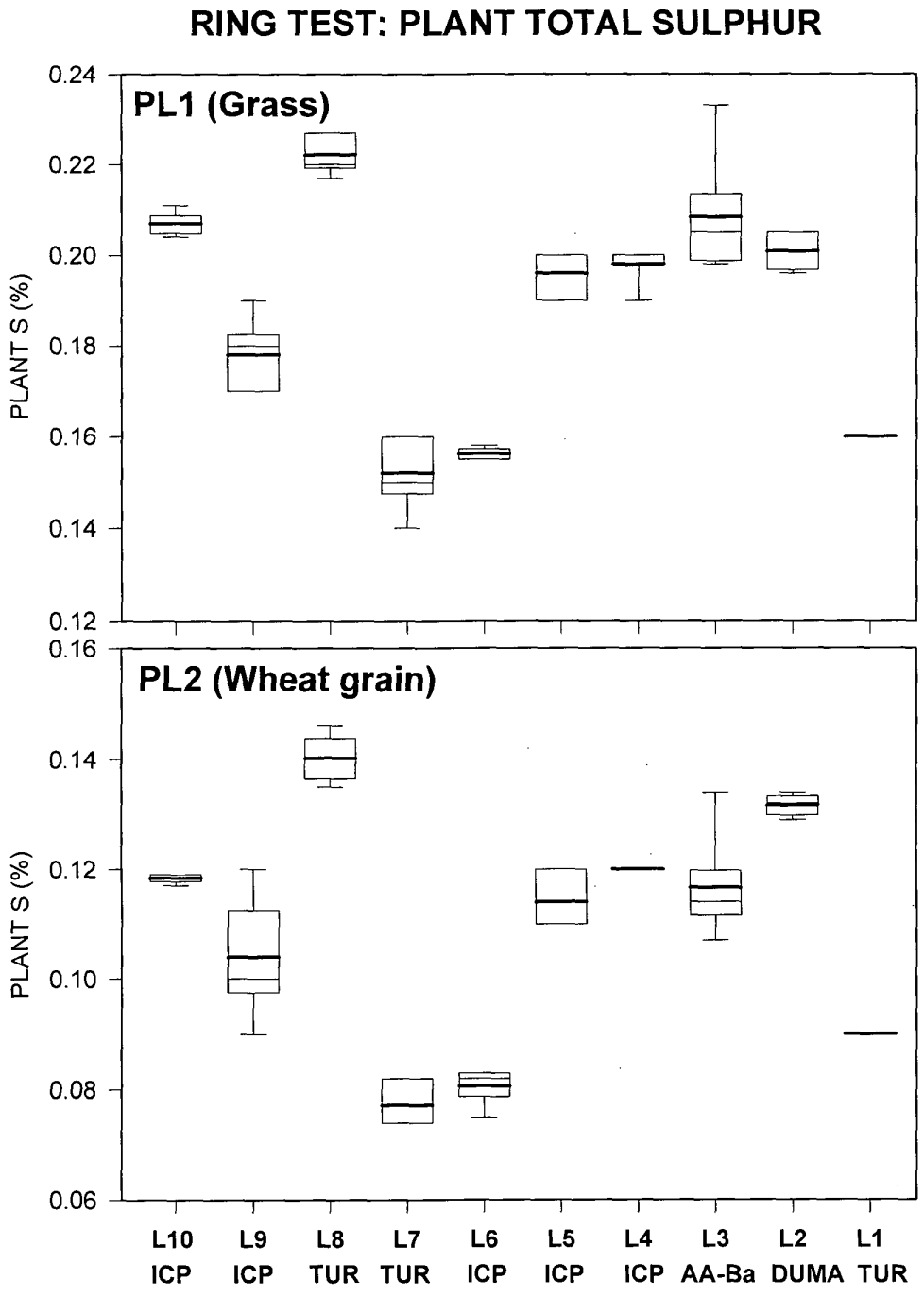

Figure 3. Analytical results for total S concentrations of plant samples PL1 and PL2. ICP=Inductively Coupled Plasma Spectrometry; TUR=Turbidimetry; DUMA=Dumas; AA-Ba=Barium precipitation +Atomic Absorption Spectrometry.

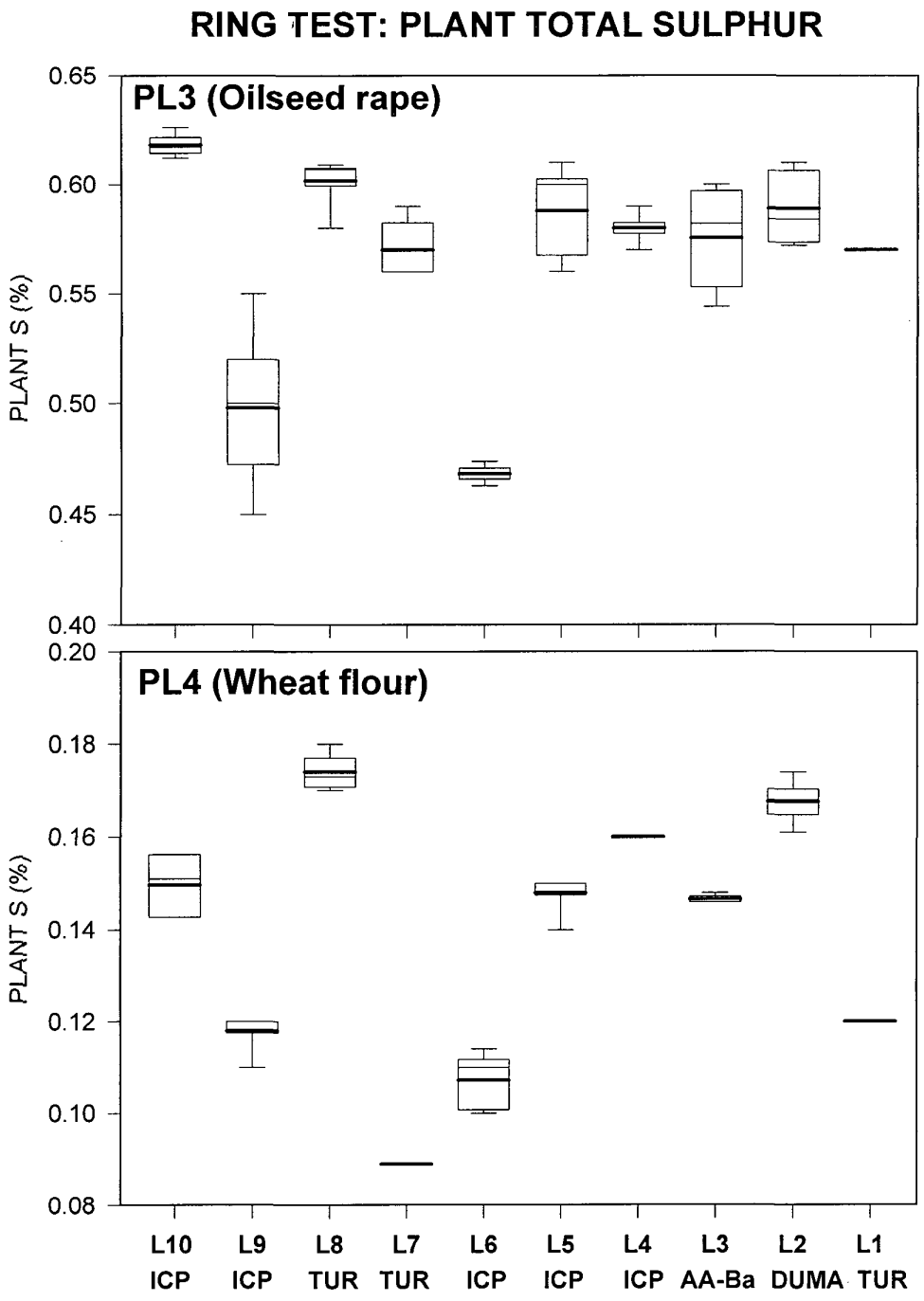

Figure 4. Analytical results for total S concentrations of plant samples PL3 and PL4. ICP=Inductively Coupled Plasma Spectrometry; TUR=Turbidimetry; DUMA=Dumas; AA-Ba=Barium precipitation +Atomic Absorption Spectrometry.

4.4 N:S ratios

These ratios are often used by the industry as a diagnostic tool, but they are probably useful only in the case of cereals and grass (Section 2.1). This ratio is not directly measured, but requires the results from two different analyses for its calculation. This means that two lots of analytical error are involved in its derivation. However, this project showed that the errors for N analysis were not too large, but they were substantial for S analysis. Table 3 shows the N:S ratios calculated from the N and S data which were reported by each laboratory. Inter-laboratory variability for the N:S ratios was large, and similar in scale to that for total S, with CVs ranging from 8.1 to 21.9%. For the four crop samples, between 40-70% of the laboratories produced results within 90-110% of the median values. Clearly, reliability of the N:S ratio is limited by the accuracy of the S analysis.

A ratio of 17:1 is usually considered as the critical value for S deficiency in wheat grain. Using this value, three laboratories would diagnose PL2 as very deficient in S, whereas the results from the other seven laboratories would indicate the opposite. For PL4, four laboratories reported S deficiency, whereas the results from the other six laboratories would indicate S sufficiency. Not surprisingly, the laboratories (L1, L6, L7 and L9) giving low S results reported high N:S ratios.

Table 3. N:S ratios of the crop samples PL1-4

Laboratory	PL1 (Grass)	PL2 (Wheat grain)	PL3 (Oilseed rape)	PL4 (Wheat flour)
L1	12.13	19.89	2.72	18.67
L2	9.85	13.62	2.38	13.95
L3	9.38	15.18	2.40	15.51
L4	10.33	15.08	2.48	15.23
L5	10.65	15.12	2.41	14.15
L6	12.91	21.81	2.96	22.95
L7	13.57	22.12	2.60	24.43
L8	9.35	12.90	2.40	13.89
L9	10.97	15.62	2.69	18.51
L10	9.83	14.70	2.30	15.71
Median	10.33	15.12	2.41	15.51
CV%	13.68	20.28	8.10	21.87

4.5 Soils extractable S

The results for extractable S show the greatest inter-laboratory variability of all of the analyses, both in samples SO1 and SO2, with CVs exceeding 45% (Figure 5 and Table 1). The results reported ranged from 2.5 to 12.5 mg kg^{-1} soil in SO1 and from 3.0 to 18.0 mg kg^{-1} soil in SO2. Laboratories L2, L6 and L9 all using ICP reported larger values than the other laboratories. However, laboratories L5 and L10, also using ICP, gave similar results to those obtained by the laboratories L3 and L7 which used the turbidimetric method. Laboratory L10 also reported results from IC determination, which were considerably smaller than the corresponding ICP values for the reasons given in Section 2, and also smaller than those given by the turbidimetric method. This indicates that the turbidimetric method gave results which were either too high due to analytical error or that it did not determine sulphate specifically as did the IC method. From the nine laboratories which measured the soils, five laboratories were in reasonable

agreement although the methods of measurement used were different. This suggests that some of the variability may arise from the way the extractions were performed.

If we take 6 mg S kg^{-1} soil as the critical value, in the case of soil SO1, which has been shown in previous HGCA projects (Withers *et al.*, 1995b) to require S fertiliser, seven laboratories gave results that would lead to the recommendation that fertiliser be applied, but three did not. Therefore, 30% of the results would lead to losses of crop yield and quality. Soil SO2 has an adequate current S status, but the results from two laboratories would have erroneously indicated that fertiliser S should have been applied. One of these used the IC method, and was small for reasons given in Section 2. However, farmers and their advisors would have to take account of this difference due to method of detection of the different chemical forms of S. Standardisation of methods is an obvious way of minimising such misunderstandings.

The analytical problem seems to be worse when soil sulphate concentrations are low. This is increasingly the case in this country (McGrath *et al.*, 1996), and analytical techniques need to be used which can reliably quantify as little as 0.04 - 0.2 mg S l^{-1} in the solutions which are produced from standard extraction techniques. A solution concentration of 0.2 mg S l^{-1} is produced from a soil at the critical value of 6 mg S kg^{-1}, but obviously to be accurate this must not be the detection limit, and the method must be capable of quantifying concentrations below this.

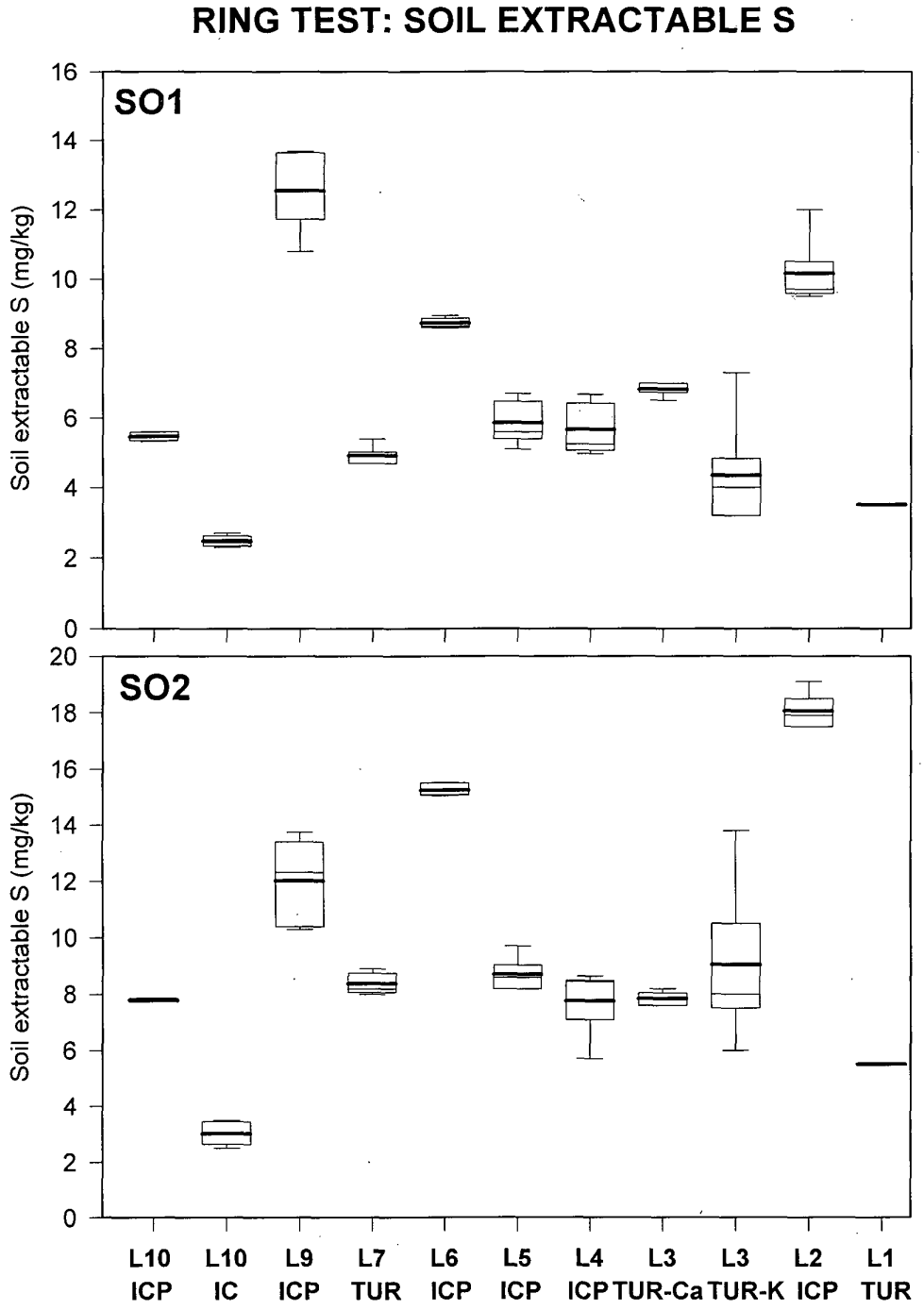

Figure 5. Analytical results for extractable S of soil samples SO1 and SO2. ICP=Inductively Coupled Plasma Spectroscopy; IC=Ion Chromatography; TUR=Turbidimetry; TUR-Ca=Calcium phosphate extraction + Turbidimetry; TUR-K=Potassium phosphate extraction + Turbidimetry.

5. CONCLUSIONS AND RECOMMENDATIONS

Plant total N analysis appears to be well-controlled and was determined reliably by all laboratories, probably because the methods are well developed and widely used. In contrast, total S analysis can be quite variable between laboratories. The procedures for plant S analysis are less well developed than for N, and are technically more demanding, requiring higher levels of skill and understanding. Determination of extractable S in soil was extremely variable, and the range of values obtained in this study demonstrated a serious problem in relation to interpretation of analytical data for diagnostic purposes, i.e. deciding whether to apply S fertilisers or not.

In the light of this study the following recommendations would seem appropriate:

1). Laboratories should standardise extraction and digestion procedures.

2). Where possible reference materials should be utilised (none currently exist for soils).

3). Analytical methods must be capable of determining low concentrations of S in soil extracts.

4). Methods must be calibrated for diagnostic purposes so that results obtained are comparable with those from other methods.

5). Laboratories should assess their analytical performance regularly by participating schemes such as International Plant/Soil Exchange (IPE/ISE) run by the Wageningen Agricultural University in the Netherlands. This costs only £520 per year, but coupled with the above recommendations, would improve the credibility of analytical laboratories for S determinations. This is critical for the farming industry, because information upon which decisions for fertiliser use are made need to be much more reliable than they are now, as this project has demonstrated.

6. ACKNOWLEDGEMENT

We would like to thank Fiona Woodhouse for technical assistance.

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