

THE MEASUREMENT OF EXTRACTABLE ORGANIC SULFUR BY NEAR-INFRARED SPECTROSCOPY

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Abstract

Extractable Organic Sulfur (EOS) is an estimate of the available sulfur pool in soil. It is an alternative to the widely used sulfate test, but does not suffer the same in-field variability that sulfate does. Sulfate is often elevated by dung and urine events, and has high temporal and spatial variation. While EOS does not suffer the same in-field variation, it traditionally suffers high analytical measurement variation.

Extractable Organic Sulfur (EOS), is typically determined from a potassium phosphate extraction, and taking the difference between the total extracted sulfur (TES) and the sulfate extracted. The total extracted fraction is typically measured by an Inductively Coupled-Plasma Optical Emission Spectroscopy (ICP-OES) instrument, and the sulfate by Ion Chromatography (IC). As the EOS measurement is the difference, the uncertainty in the measurement is coupled to the measurements of the other two. As typical with analytical measurements, there is a component of error associated to the concentration of the individual analytes, thus when sulfate (and therefore TES) is high, the uncertainty associated to this, is also high in absolute terms (though still low in relative terms). This means that for high TES or sulfate values, the analytical error in the EOS method can be very high, to the point of making the result meaningless.

Near-Infrared (NIR) has been used for many years to measure some other soil constituents at Hill Laboratories particularly those associated with the organic fraction in soil. It has been recently found that a successful calibration for EOS can be also constructed. Although, the NIR measurement is most probably an indirect measure of the EOS through the vibrations associated to the organic matter fraction, the error associated to the measurement does not scale with TES or sulfate concentration. Thus the NIR method for EOS is typically more accurate than the reference method for soils with high sulfate.

Introduction and Background

Sulfur is a soil nutrient that is required for plant growth. To maximise pasture growth sulfur needs to be within the optimal range and in a form that is bioavailable to plants. A range of analytical techniques exist to estimate this available sulfur pool including extractable sulfate and Extractable Organic Sulfur (EOS), with sulfate being immediately available to plants and EOS being available in the short term through mineralisation processes. Extractable Organic Sulfur (EOS) was first proposed in 1990 (Watkinson & Perrot, 1990), and typically uses a 0.02 M KH_2PO_4 extraction solution, followed by analysis using Inductively Coupled-Plasma Optical Emission Spectroscopy (ICP-OES) and Ion Chromatography (IC). These two instrumental techniques provide Total Extractable Sulfur (TES) and sulfate results respectively which are then used to calculate EOS (Eq. 1).

$$EOS = TES - \text{sulfateS} \quad (\text{Eq. 1})$$

Measured soil sulfate concentrations are prone to temporal and spatial variation due to influences of rain, animal grazing and fertiliser application whereas the soil EOS concentrations are relatively stable to these influences (Watkinson & Kear, 1996b). This, coupled with the equilibrium between the sulfate and the readily mineralisable organic pool (that is measured by EOS) mean that EOS can be used as a good indicator of soil sulfur concentrations and indeed has been shown to provide a superior relationship than sulfate (Watkinson & Kear, 1996a) (Watkinson & Kear, 1996b). Furthermore, EOS determines the immediate and medium term sulfur supply of a soil (Edmeades, Thorrold, & Roberts, 2005).

Typically analytical error has a component that is proportional to the measured concentration, when the concentration of an analyte becomes high the absolute error associated with measuring it becomes large. However, this is not usually practically significant to the measured analyte as the relative error may still be small. If two measured results are used to calculate a third the error associated with the calculated result is dependent upon the error of both of the measured results. This is particularly significant in subtraction calculation where the calculated result is generally much smaller than the two measured results used to generate it. This generates a result with a very large error in both absolute and relative terms. The consequence is that when a high concentration of sulfate is present in a soil the typically measured EOS value becomes less reliable, using the standard methodology.

Near-Infrared (NIR) has been used for many years to measure some other soil constituents at Hill Laboratories particularly those associated with the organic fraction in soil. If NIR could be used to determine the concentration of EOS, this would provide a direct (as opposed to calculated) method which would remove the impact of sulfate and TES on the error associated with the EOS measurement. This would potentially allow a more reliable result to be provided to clients especially at higher levels of sulfate (and TES).

Calibration and Validation

Calibration

Diffuse reflectance NIR spectra were generated for each sample in the calibration data set, which included approximately 17 000 samples. The spectra were first pre-processed using a first derivative Savitzky-Golay smoothing algorithm, vector normalisation and wavelet transformation.

The transformed spectra were then passed through a bootstrap aggregation (bagging) re-sampling model, which uses a Partial Least Squares (PLS) data reduction filter with 20 components, a local weighted Euclidean distance (500 nearest neighbours) and support vector machine (SVM) model. The bootstrap re-sampling creates 20 different random calibrations from the dataset for each on-the-fly prediction, and the mean of the 20 different predictions is reported as the NIR measurement.

As part of each measurement a series of qualification values are generated and used to determine if the measurement is acceptable. These values were firstly a residual value that determines the unmodelled variation as part of the PLS algorithm and secondly a local Mahalanobis distance measurement. The maximum of the two qualifiers, each standardised by the 95th quantile is used as the final qualifier (Q). A Standard Error (SE) is also generated as the standard deviation of the predictions from each of the 20 calibrations.

Validation

Approximately 20 000 samples were used to validate the NIR EOS calibration. The data set was comprised of routine laboratory samples, random samples excluded from the calibration data but collected at the same time, Inter Laboratory Comparison Programme (ILCP) samples

and a selection of samples analysed in duplicate through the reference and NIR methods. These data sets were analysed separately to determine the number and percentage of samples qualified, the bias and Standard Error of Prediction (SEP) for each set (Table 1). Samples were deemed qualified if the Q value and the SE value were below given thresholds. The bias was calculated as the average of the differences between the NIR and the reference results, and the SEP was calculated as the standard deviation of the differences between the NIR and the reference results.

Table 1. Summary of statistics for validation data sets.

Data Set	Total Count	Qualified (%)	Bias (mg/kg)	SEP (mg/kg)
Duplicate Set	352	80	-0.46	4.04
ILCP	206	93	-3.76	6.83
Random Set	558	96	0.12	1.82
Routine Set	18824	93	-1.24	2.85

Figure 1 shows a comparison of the NIR method results and the reference method results for the Duplicate set. This data set follows the same trends as the other data sets but due to the number of samples in the set it provides a good visualisation of the data. The two methods appeared to produce similar results in most cases with no significant bias. As expected, for samples that had sulfate concentrations greater than 20 mg/kg the NIR and reference method result were less likely to match due to increased error in the reference method.

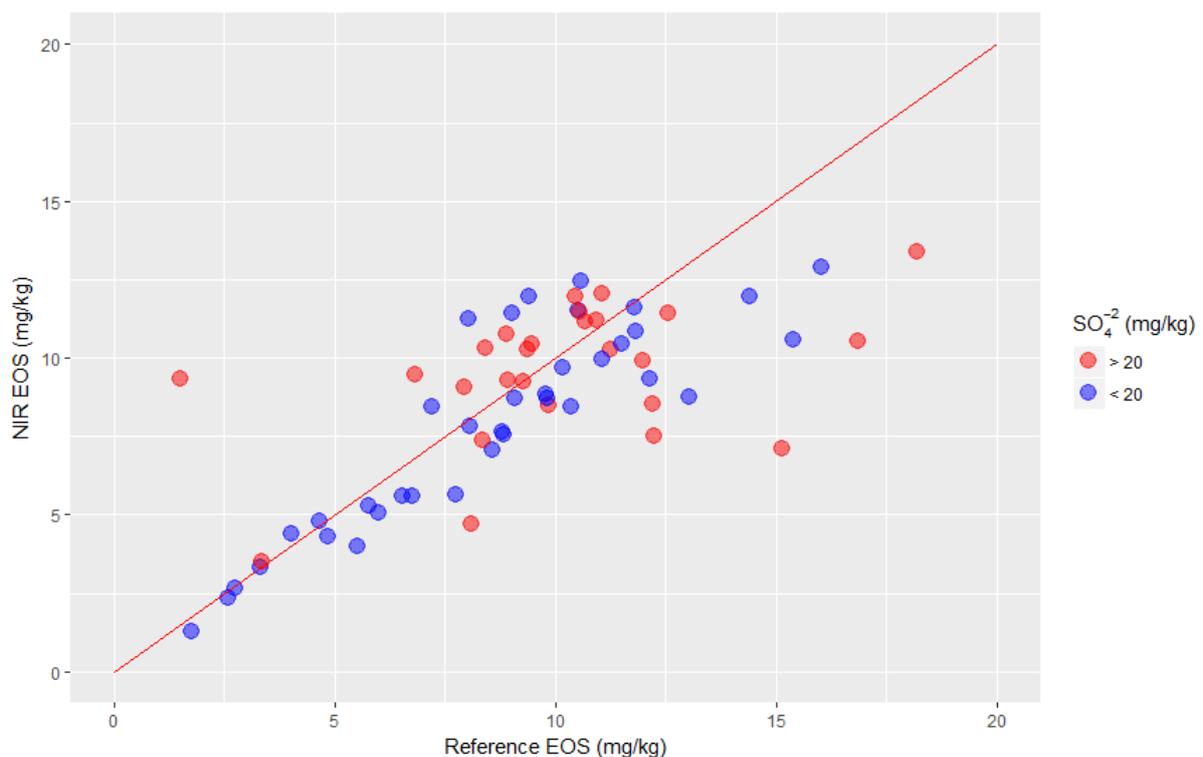


Figure 1. Comparison of NIR and Reference results for a subset of validation data.

Uncertainty Estimates

To understand the error in the NIR method measurements the error in the reference method first needs to be understood and estimated.

Two estimates of the reference method error were generated using the reference method results; a reproducibility and a repeatability estimate. These data sets were analysed using a Maximum Likelihood Estimate (MLE) calculation (Devey, 2014), which finds parameter values that maximise the probability for a given data set, i.e. it determines the most likely values for each error component. The error in the reference method was attributed to three components: an independent error value, a component that was directly related to the sulfate concentration in the sample and a value related to TES and all assuming a normal distribution (Eq. 2).

$$U_{Ref} = \sqrt{S_0^2 + ([SO_4^{2-}] \times S_{SO_4})^2 + ([TES] \times S_{TES})^2} \quad (\text{Eq. 2})$$

Where U_{Ref} is the uncertainty in the reference result;

$[SO_4^{2-}]$ is the concentration of sulphate in mg/kg;

S_{SO_4} is the uncertainty component associated with sulfate;

$[TES]$ is the concentration of total extractable sulfur in mg/kg;

S_{TES} is the uncertainty component associated with TES.

The reproducibility estimate (U_{refR}) was calculated by analysing ILCP data, where Hill Laboratories reference results were compared to the ILCP means (excluding the Hill Laboratories results). This data is expected to provide the most robust estimate of error in the reference method as it compared the values to an estimate of the “true value” (Eq. 3). Whereas the repeatability level error estimate (U_{refr}) was calculated using the duplicate data, this equates to the precision of the method, but not necessarily the error to the “true value” and so produces a lower error estimate (Eq. 4).

$$U_{refR} = \sqrt{1.1^2 + ([SO_4^{2-}] \times 0.067)^2 + ([TES] \times 0.067)^2} \quad (\text{Eq. 3})$$

$$U_{refr} = \sqrt{0.49^2 + ([SO_4^{2-}] \times 0.030)^2 + ([TES] \times 0.039)^2} \quad (\text{Eq. 4})$$

As the NIR method measured the EOS concentration directly from the sample (with no calculation) the error associated with an NIR result is expected to contain an independent error component (S_0) and an EOS concentration dependent component (S_{EOS}) (Eq. 5).

$$U_{NIR} = \sqrt{S_0^2 + ([EOS] \times S_{EOS})^2} \quad (\text{Eq. 5})$$

The comparison between the NIR results and the reference results contains the uncertainty of both (Eq. 6).

$$U_{MC} = \sqrt{U_{NIR}^2 + U_{Ref}^2} \quad (\text{Eq. 6})$$

Where U_{MC} is the uncertainty in the comparison, the expected error between the NIR and reference results.

Using an MLE approach on the method comparison data, the reference error values can be included as a known parameters in the calculation, providing an estimate related only to the NIR measurements. Two different estimates for the NIR uncertainty can be created, depending on whether the reproducibility (Eq. 3) or the repeatability (Eq. 4) estimates are used for the reference method error (U_{ref} in Eq. 6), thus giving the following two NIR error functions (Eq. 7 and Eq. 8).

$$U_{NIR[RefR]} = \sqrt{0.81^2 + ([EOS] \times 0.21)^2} \quad (\text{Eq. 7})$$

$$U_{NIR[RefR]} = \sqrt{([EOS] \times 0.12)^2} \quad (\text{Eq. 8})$$

The reproducibility level uncertainty is expected to provide a better estimate of the NIR method error as it better reflects the true error in the reference method. This particular estimate calculated no S_0 value meaning that all error was associated directly with the concentration of EOS in the sample.

The average concentration of EOS in soil samples was approximately 7.6 mg/kg within the validation data set. Figure 2 shows the uncertainty estimates for both methods and at both levels of precision across a range of Sulfate and TES values (keeping a constant value of 7.6 mg/kg), to highlight the calculated differences between the methods.

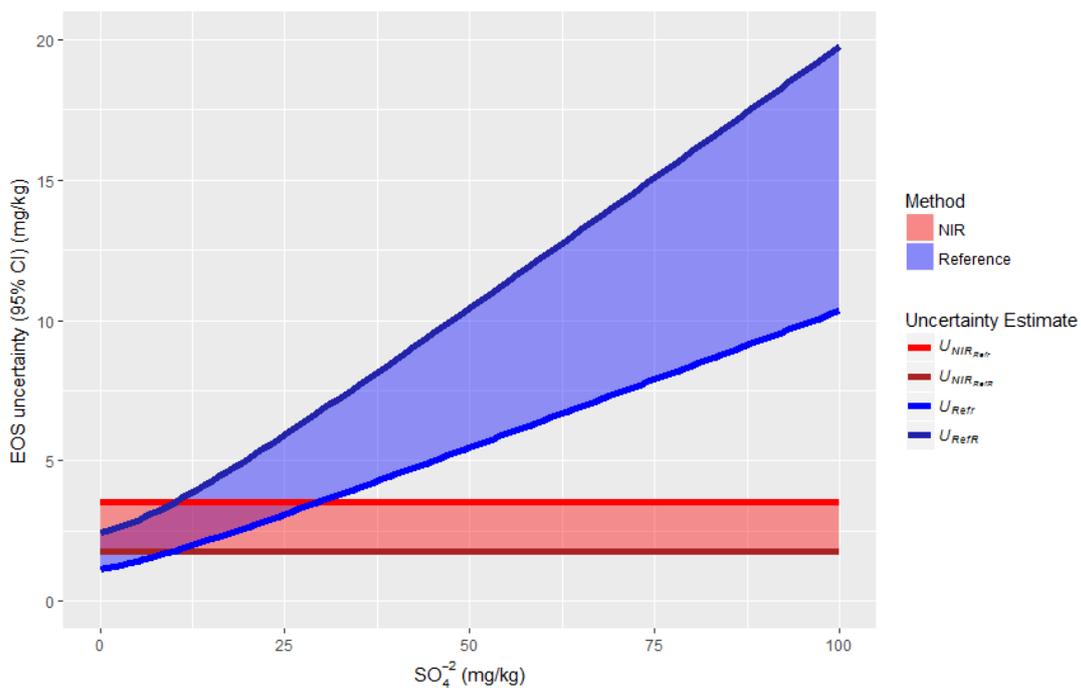


Figure 2. Uncertainty estimates for the Reference and NIR methods over a range of sulfate concentrations, assuming an average EOS result of 7.6 mg/kg.

As the sulfate concentration increased the uncertainty associated with the EOS results for the reference method increased dramatically. This was not the case for the NIR method which has no uncertainty associated with the sulfate result. The NIR method is expected to produce results with lower uncertainty than the reference method for samples with a concentration of sulfate less than 30 mg/kg irrespective of the uncertainty estimates used. More importantly, using the reproducibility error estimates of the reference method (Eq. 3 and Eq. 7) the NIR method is expected to produce results with less uncertainty across all sulfate concentrations.

Conclusions

Extractable Organic Sulfur is an important sulfur status test for New Zealand soils. The concentration of EOS in soil samples needs to be determined in the most accurate way possible to ensure that well informed management decisions can be made.

This study has shown that an NIR method for measuring EOS is expected to be more accurate than the current KH_2PO_4 extraction method, especially at higher sulfate concentrations.

References

- Devey, K. (2014). Productivity of multivariate analysis in analytical chemistry. *PhD thesis*. University of Waikato.
- Edmeades, D. C., Thorrold, B. S., & Roberts, A. H. (2005). The diagnosis and correction of sulfur deficiency and the management of sulfur requirements in New Zealand pastures: a review. *Australian Journal of Experimental Agriculture*(45), 1205 - 1223.
- Watkinson, J. H., & Kear, M. J. (1996a). Sulfate and mineralisable organic sulfur in pastoral soils of New Zealand. I. A quasi equilibrium between sulfate and mineralisable organic sulfur. *Australian Journal of Soil Research*(34), 385 - 403.
- Watkinson, J. H., & Kear, M. J. (1996b). Sulfate and mineralisable organic sulfur in pastoral soils of New Zealand. II. A soil test for mineralisable organic sulfur. *Australian Journal of Soil Research*(34), 405 - 412.
- Watkinson, J. H., & Perrot, K. W. (1990). A new soil test for sulfate and mineralisable organic sulfur. *New Zealand Fertiliser Manufacturers Research Association*, (pp. 189 - 198).