

This is a postprint (final draft post-refereeing) of an article published in
Communications in Soil Science and Plant Analysis
(ISSN: 0010-3624)

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DOI: 10.1080/00103629509369379

Vázquez de Aldana B.R., García Criado B., García Ciudad A., Pérez Corona M.E.
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Communications in Soil Science and Plant Analysis, 26: 1383-1396 (1995)

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ESTIMATION OF MINERAL CONTENT IN NATURAL GRASSLAND BY NEAR INFRARED REFLECTANCE SPECTROSCOPY

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ABSTRACT

Near infrared reflectance spectroscopy (NIRS) was tested to predict nitrogen and mineral concentration (N, P, K, Ca, Mg, Na, Mn, Fe, Cu and Zn) in natural grassland samples. The samples were taken from different community types according to the topographic gradient, at different maturation stages and during a period of four consecutive years. A subset of 95 samples was selected on the basis of the spectral variation. Chemical values from the calibration sample set were regressed on the corresponding spectral data using a stepwise multiple regression analysis. Another subset of 75 samples was used as the validation set. Standard errors of prediction and correlation coefficients, respectively, were: 0.71 and 0.97 (N), 0.22 and 0.73 (P), 1.83 and 0.84 (K), 0.83 and 0.92 (Ca), 0.15 and 0.92 (Mg), 3.94 and 0.66 (Na), 44 and 0.84 (Mn), 19 and 0.75 (Fe), 1.01 and 0.77 (Cu), 3.9 and 0.79 (Zn).

INTRODUCTION

New technologies have always aroused interest from several research fields. This is especially the case when the new technology offers the possibility of measurement of parameters with acceptable accuracy, being one of the most important qualities of any technique. Near Infrared Reflectance Spectroscopy (NIRS) shows great potential for rapid, non destructive analysis which environmentally friendly.

NIRS technology has been developed for evaluation of quality components

in forages and foodstuffs, being an alternative method for analysis of specific materials. However, it is not quite clear which mineral elements can be determined using this technique. Research papers concerned with mineral measurement by NIRS are not numerous, and mainly relate to macronutrients. The prediction of trace elements by NIRS has been reported even less frequently (Clark et al., 1987, 1989; Rodríguez Vázquez de Aldana, 1993). Minerals in agricultural and ecological products can probably be associated with certain organic acids (Clark et al., 1987). These bonds between metallic atoms and organic molecules may provide the basis for their detection by NIRS. However information about these molecules in living systems is scarce.

The aim of the present work was to assess the potential of NIRS to predict the mineral content: N, P, K, Ca, Mg, Na, Mn, Fe Cu and Zn, in semiarid natural grassland samples. These samples have a complex botanical composition that increases the difficulty of calibration development. Several investigations have been carried out in this type of samples for the determination of protein (García-Ciudad et al., 1992), quality parameters (García-Ciudad et al., 1993) and botanical composition (García-Criado et al., 1991) by means of NIRS with excellent results.

MATERIALS AND METHODS

Grassland Samples

Samples were taken on several sites in the Dehesa zone (province of Salamanca) located in the central-western Spain. A total of 170 samples was collected in seminatural grassland communities over a period of four consecutive years (1986-1989).

The samples were taken from different community types according to variations in the topographic gradient (Corona et al., 1991). The samples were only collected on the lower part of the slope in 1986. During 1987 and 1988 samples were taken in both upper and lower zones. During this period (1986-1988) the samples were collected annually in June. The phenologic stages in both zones of the slope were different: the samples from the lower parts were in the transition phase between the flowering and fruiting stages, and those from the upper parts were in the fruiting phase. In the last sampling year, 1989, the collection of the vegetation samples was different: plots protected by fences were used to prevent

grazing. In this case, plant material was collected fortnightly during May and June in three zones, also differentiated topographically (upper, medium and lower). The aim of the change in the procedure was to increase the variability of the samples as a result of the effect of maturity and topographic gradients, and botanical composition.

In all the cases three sampling squares (0.25 m²) of biomass were collected. The plant material was dried at 70°C in a forced air oven to constant weight. It was then ground through a 0.5 mm sieve in a Retsch mill and stored in polyethylene containers before analysis.

Chemical Analysis

Combustion of subsamples preceded analysis for P, K, Ca, Mg, Na, Mn, Fe, Cu and Zn (Duque, 1970). Phosphorus concentration was measured colorimetrically as molybdovanado-phosphoric acid. Potassium, Ca, Mg, Na, Mn, Fe, Cu and Zn were determined by atomic absorption spectrophotometry (AAS). Nitrogen concentration was determined separately by Kjeldahl digestion.

Near Infrared Reflectance Spectroscopy Analysis

All the samples were scanned with monochromatic radiation from 1100 to 2500 nm, at 4 nm intervals, using a Technicon InfraAlyzer 500. The spectral data were recorded as $\log(1/\text{reflectance})$. Of the total samples (170), a set of 95 samples were selected by means of PICKS program (InfraAlyzer Data Analysis System, Technicon Inst corp, Tarrytown, NY, USA) for the calibration process, on the basis of the spectral data alone. The chemical analysis data for each element were regressed sequentially (using modified stepwise multiple linear regression) against the corresponding spectral data to develop calibration equations. This mathematical procedure is also used to determinate the optimum wavelengths. The best equations were selected using statistical criteria related to equation accuracy and performance (García-Criado et al., 1991).

The remaining samples (75) were utilized as the validation set. Each equation chosen as the best for a particular mineral element was used to predict the mineral content of the samples in the validation set. The validation of the calibration equation was carried out by means of a simple regression between NIRS-predicted values and those obtained by the reference method. The accuracy of the prediction process was assessed by the standard error of performance (SEP)

(square root of the residual mean square), coefficient of simple correlation (r) and bias (deviation of NIRS mean from chemistry laboratory mean). Coefficients of variation (CV) were calculated $\{CV = [(SEP/mean) \times 100]\}$ for each mineral. These are used as a tool for comparing performance for different elements since CV values are affected by the mean from the chemical procedure and SEP.

RESULTS

Macronutrients

The statistical data for the population sets used in calibration and validation processes, for the macronutrients analyzed are presented in Table 1. The characteristics of both sample sets were similar for all the elements studied. The wide concentration range showed that the samples taken were representative of semiarid grassland communities (phenology, botanical composition, etc.), and factors which influence the mineral contents (Vázquez de Aldana et al., 1993).

The results of multiple regression analysis are presented in Table 2 which shows the coefficient of multiple determination (R^2), standard error of calibration (SEC) and the wavelengths selected.

There were no big differences in the number of wavelengths used for the elements, being six for N, P and K, and seven for Ca, Mg and Na. By comparing the number of wavelengths selected from several reports, it is observed that the more complex the sample population, in terms of heterogeneity, the greater the number of wavelengths necessary to explain the chemical parameter. Saiga et al. (1989) used three wavelengths to predict the mineral content in samples of a single species (*Dactylis glomerata* L.) while Clark et al., (1987) selected two wavelengths for Ca and P content in crested wheatgrass samples. On the other hand, Shenk and Westerhaus (1985) used equations with eight wavelengths to predict Mg and Ca content in haylage samples and Redshaw et al., (1986) selected seven wavelengths for predicting Ca concentration in mixed samples of grass and legumes.

The best performance in the calibration equation was for N content with R^2 of 0.98 and SEC of 0.77. Standard errors of calibration were 0.27 for P, 1.89 for K, 0.92 for Ca, 0.16 for Mg and 0.62 for Na. Clark et al. (1987) reported SEC, depending on the forage species considered, between 0.2-0.3 for P, 1.4-2.6 for K,

0.6-1.1 for Ca, 0.2-0.6 for Mg and 6.0-160 for Na. Our results for P and Ca are also comparable to those obtained by Redshaw et al. (1986): 0.71 (R^2) and 0.2 (SEC) for P, and 0.87 (R^2) and 1.8 (SEC) for Ca, in samples including several grass and legume species. Noteworthy was the high SEC obtained for Na (Table 2) and the wide range in Na concentration (Table 1), which reflects the variability of this element in the herbage. The calibration obtained by Clark et al. (1987) for Na also show large errors, even within single species, due to the extreme range in Na values and perhaps a lack of correlation between Na and organic functional groups sensed by NIRS.

The equations were tested on the validation set (Table 3). Bias and slope of the simple regression equation are measures of the performance of the prediction equations. Ideally there should be no bias and the slope should be 1.0. The highest coefficient of simple regression and lowest coefficient of variation was obtained for N, followed by Ca and Mg. The results for P and K were similar to those reported by Shenk et al. (1981) in forage samples. The performance obtained for Mg is comparable with the range of 0.14-0.7 (SEP) and 0.75-0.94 (r) reported by Clark et al. (1987), Saiga et al. (1989) and Smith et al. (1991). Potassium and Na had the largest bias of all the macronutrients. The performance for Na prediction was significantly inferior to all other elements as shown by the low correlation coefficient (r), high coefficient of variation and also the high bias. These results agree with those of Convertini et al. (1991) and Clark et al. (1987) and suggest that NIRS cannot determine the Na concentration.

Micronutrients

Means and standard deviations for Mn, Fe, Cu and Zn are listed in Table 4 to show the concentration variation in the grassland samples selected. There were no large differences between the mean contents of the calibration and validation set samples. The means are considered to be within the normal ranges in herbage (Rodríguez Vázquez de Aldana, 1993). The Mn concentration range was wider in the calibration set than in the validation set. For Fe, attempts were made to avoid the selection of samples with high Fe contents, since it is a symptom of soil contamination. Soil contamination is also observed in the sample reflectance spectra, showing a pronounced effect on the baseline and being particularly evident in the lowest wavelength zone.

The statistical for calibration showed lower accuracy for trace elements (Table 5) than for macronutrients (Table 2). For all the micronutrients analyzed, seven wavelengths were used to obtain the equation with the best fit to the spectral data. The number of terms in each calibration equation was larger than those used by Clark et al. (1987) for the same elements. The equation developed for Cu achieved the highest R^2 (0.82). The R^2 values were 0.74 for Mn, 0.74 for Fe and 0.72 for Zn. Those results seem high compared with the R^2 ranges of 0.24-0.50 for Mn, 0.45-0.72 for Fe and 0.44-0.61 for Zn reported by Clark et al. (1987) in different forage species. The SEC value for Mn was lower than the range 6-19 quoted by Clark et al. (1987), and the Fe value was higher than the range 69-200 reported.

The statistics of validation, which demonstrate equation performance, are listed in Table 6. The highest correlation coefficient was obtained for Mn, however the SEP value was high; therefore CV values are useful to compare the errors in the four analyzed elements, of which Mn had the highest CV. The performance for this element was also reflected in the bias value. The results obtained for Mn and Fe were similar, and for Cu and Zn were better than those quoted by Clark et al. (1987) for single forage species.

DISCUSSION

Calibration development is a long process involving sequential steps in order to obtain the most acceptable statistics. The results are influenced by factors like the type of sample and the size of the population set. For instance, the analysis of Mg of single grass species is reported with greater precision by Saiga et al. (1989) in a population smaller than that chosen by Clark et al. (1987). On the other hand, the variation inherent in the population studied could be increased when the sample set consists of a larger number of samples. The difficulty increases with size and heterogeneity of the sample set. Some authors have proposed the establishment of an universal calibration to be used in a wide range of samples. But this would involve many complications and conflicting interests. The calibration sample set must be representative of all samples to be tested in future. There is a trade-off between the diversity of the calibration and its ability to discriminate among samples that are of very similar composition.

The wavelengths selected are not expected to be identical to those obtained by other authors, because only some of the wavelengths have chemical significance. However for N content, the 2152 and 2484 nm selected in this study are close to those used by Marten et al. (1983) and García-Ciudad et al. (1992). The 2172 nm band, reported here, is related to N or protein content, and it is close to those quoted by many authors in several types of plant samples (Wessman et al., 1987; McLellan et al., 1991; García-Ciudad et al., 1992). In such a range is included the wavelength absorption characteristic of protein (2180 nm). The information supplied by this wavelength is so well correlated to N, that Saiga et al. (1991) have developed a calibration equation with only one term to predict N content in *Dactylis glomerata* with acceptable results.

Mineral elements are measured indirectly by the instrument in the NIR region. If NIRS can be used for determining mineral concentrations it is probably due to associations between minerals and organic functional groups (Shenk et al., 1979; Clark et al., 1987). Ca and Mg are associated with components of the cell wall. For example, calcium pectate may bind plant cell walls and has a reflectance spectrum in the near infrared region. The chlorophyll bands in the NIR region (Clark et al., 1987) are close to the wavelength used for the calibration of Mg content (2076 nm) in grassland samples. These associations may explain the use of NIRS for determining element concentrations.

Clark et al. (1987) found some similarities between bands in the potassium malate spectrum and the wavelengths used for the determination of K in grass samples (2360, 2420 nm). Organic acids are important plant metabolites and accumulate in grass to 2 to 8 % (of DM). Malic acid has been identified in several forage species (Boland et al., 1976).

As expected, the results obtained for the prediction of mineral elements were not as good as for organic compounds, due to the lack of a direct relation between element and spectra. Standard errors were higher because mineral elements are found in diverse forms in different plant types. Coefficients of determination were smaller, probably due to the narrow concentration range of minerals in herbaceous samples and, low concentration of the associated organic compounds sensed by NIRS (Shenk and Westerhaus, 1985; Valdes et al., 1985; Smith et al., 1991).

CONCLUSIONS

On the basis of the results obtained, NIRS is considered suitable for estimating nitrogen concentration in grassland samples. The results predicted by NIRS for Ca and Mg, in natural grassland samples, have acceptable performance. For Cu, Mn and K, the estimates provided by NIRS, have only limited utility. For P, Na, Fe and Zn the model developed by NIRS was not successful for prediction of these parameters. In this case, the model does not include all the variation which would be needed to explain the concentration of those elements in the samples studied. Nevertheless, NIRS may provide an acceptable monitoring technique for certain minerals in natural grassland samples.

ACKNOWLEDGEMENTS

This work was supported by the European Union (Contract N 8001-CT90-0021). Thanks are due to L.G. Criado, C. Estévez and M. Hernández for their assistance in the field work.

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TABLE 1. Statistical data for macronutrient concentrations determined by wet chemical analysis.

Element	Set	N(a)	Range(b)	Mean(b)	SD(c)
N	<i>Calibration</i>	92	6.8-29.0	16.4	3.9
	<i>Validation</i>	73	10.3-25.5	16.1	3.1
P	<i>Calibration</i>	90	0.9-3.2	2.1	0.49
	<i>Validation</i>	69	1.2-3.7	2.1	0.47
K	<i>Calibration</i>	92	4.8-24.7	14.0	3.88
	<i>Validation</i>	66	7.2-24.5	13.7	3.42
Ca	<i>Calibration</i>	92	1.6-13.0	7.6	2.49
	<i>Validation</i>	70	3.1-17.3	7.4	2.10
Mg	<i>Calibration</i>	91	0.8-2.6	1.5	0.40
	<i>Validation</i>	72	0.7-2.3	1.4	0.34
Na	<i>Calibration</i>	88	0.05-7.3	0.7	1.04
	<i>Validation</i>	74	0.05-7.3	0.9	1.27

(a): number of samples

(b): element concentration (g kg^{-1}) determined by wet chemistry

(c): standard deviation

TABLE 2. Statistical data for NIRS calibration of macronutrients in grassland samples.

Element	R ² (a)	SEC(b)	Wavelengths (nm)
N	0.98	0.77	1692, 1864, 2152, 2172, 2216, 2484
P	0.70	0.27	2000, 2344, 2380, 2400, 2440, 2464
K	0.78	1.89	1732, 1752, 1828, 2016, 2360, 2420
Ca	0.88	0.92	1896, 2036, 2060, 2236, 2244, 2324, 2424
Mg	0.84	0.16	1896, 1940, 1964, 2080, 2148, 2196, 2500
Na	0.79	0.62	2036, 2128, 2164, 2340, 2412, 2440, 2472

(a): coefficient of multiple determination

(b): standard error of calibration

TABLE 3. Statistical data for correlations between chemical value of macronutrients and NIRS estimate for validation sample set.

Element	r ^(a)	SEP ^(b)	CV ^(c)	Regression equation	Bias
N	0.97	0.79	4.9	$Y = 0.35 + 0.98 X$	-0.071
P	0.73	0.31	14.8	$Y = 0.99 + 0.50 X$	0.005
K	0.84	1.95	14.2	$Y = 3.45 + 0.73 X$	0.158
Ca	0.92	0.97	13.1	$Y = 1.05 + 0.86 X$	0.022
Mg	0.92	0.18	12.9	$Y = 0.07 + 0.91 X$	0.055
Na	0.66	0.71	78.9	$Y = 4.09 + 0.48 X$	0.505

(a): coefficient of simple regression

(b): standard error of prediction

(c): coefficient of variation [$CV=(SEP/mean) \times 100$]

TABLE 4. Statistical data for micronutrient concentrations (expressed in mg Kg⁻¹) determined by wet chemical analysis.

Element	Set	N(a)	Range(b)	Mean(b)	SD(c)
Mn	<i>Calibration</i>	89	38-813	179	105
	<i>Validation</i>	70	38-385	185	81
Fe	<i>Calibration</i>	90	38-203	87	29
	<i>Validation</i>	72	48-165	95	28
Cu	<i>Calibration</i>	89	1.3-10.0	5.5	1.9
	<i>Validation</i>	69	2.0-10.0	5.0	1.6
Zn	Calibration	89	10-50	31	6.9
	Validation	71	18-43	29	6.4

(a): number of samples

(b): element concentration (mg Kg⁻¹) determined by wet chemistry

(c): standard deviation

TABLE 5. Statistical data for NIRS of calibration of micronutrients in grassland samples.

Element	R ² (a)	SEC(b)	Wavelengths (nm)
Mn	0.74	50	1168, 1196, 1372, 1940, 1960, 2192, 2204
Fe	0.74	15	1568, 1696, 1724, 2260, 2388, 2440, 2460
Cu	0.82	0.84	2188, 2212, 2228, 2248, 2264, 2448, 2500
Zn	0.72	3.8	1104, 1252, 1312, 1336, 1352, 1964, 2008

(a): coefficient of multiple determination

(b): standard error of calibration

TABLE 6. Statistical data for correlations between chemical value and NIRS estimate for validation sample set.

Element	r ^(a)	SEP ^(b)	CV ^(c)	Regression equation	Bias
Mn	0.84	54	29.2	$Y = 37 + 0.76 X$	6.81
Fe	0.75	16	16.8	$Y = 31 + 0.63 X$	3.39
Cu	0.77	0.89	17.8	$Y = 1.8 + 0.66X$	-0.18
Zn	0.79	3.4	11.7	$Y = 9.9 + 0.69 X$	-0.66

(a): coefficient of simple regression

(b): error standard of prediction.

(c): coefficient of variation [$CV=(SEP/mean) \times 100$]