

Non-destructive determination of humic acids in soils by near infrared reflectance spectroscopy

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Introduction

It has been estimated that 65% to 75% of the organic matter in predominantly inorganic soils consists of humic materials, that is, humic acid (HA), fulvic acid (FA) and humin.¹ Humic materials, especially HA and FA, perform many useful functions in soils.² A major goal in the study of soil organic matter is to know the characteristics and contents of humic and FAs. However, no completely satisfactory method exists for determination of the HA or FA in soils.

The conventional analytical method for determining HAs and FAs includes chemical extraction, separation, purification, quantitation and other complicated procedures.³ It not only takes a lot of time but also uses a large number of reagents. Near infrared (NIR) reflectance spectroscopy is a non-destructive, rapid and accurate analytical method. The use of NIR to evaluate soil organic matter and other soil properties has started to receive more attention in recent years.^{4–8} Dalal *et al.*⁴ found that the wavelengths at 1744, 1870 and 2052 nm contributed significantly to the prediction of organic matter in soils. It may be reflectance from > CH₂, –CN, –CONH, –CONH₂ and > NH aliphatic amines in the organic matter. The groups may come from HAs. Other researchers also concluded that the NIR technique could be used to determine soil organic matter, although their selected wavelengths were not all the same.^{4,7,8} The NIR spectral region is dominated by weak overtones and combinations of vibrational bands of light atoms that have strong molecular bonds, for example, chemical bond that contain H attached to atoms such as N, O or C. The spectral features of soil materials in the NIR (1000–2500 nm) spectral region are associated with vibration modes of functional groups. It is possible to measure humic and FAs in soil by using NIR, because they contain many functional groups in their structure, such as carboxyl, phenolic OH and amines. Therefore, predicting humic and FAs by NIR techniques may be more effective and accurate than evaluating organic matter. Our objective was to determine the uses of NIR to predict HA, FA and its total soil content.

Materials and methods

Soils

Nearly five hundred top and subsoil samples from paddy, upland, orchard and others were selected to cover the Youngnam and Honam regions of Korea. The soil samples represented different amount of organic matter and soil fertility levels.⁸ The soils were air-dried and ground to pass through a 0.5 mm sieve for chemical and spectroscopic determinations.

Determination of HA and FA

Soil humic and FAs were extracted with 0.1 N NaOH for 24 h under a blanket of N₂ gas. The dark-coloured supernatant liquor was separated from the residual soil by centrifugation, the pH of the solution was adjusted to about 1.0 with 2.0 N HCl, allowed to stand at room temperature for 24 h, then the soluble material (FA) was separated from the coagulate (HA) by centrifugation. The coagulate and centrifuge were washed with 0.05 N HCl. Total the supernatant liquor was collected and mixed with 0.05 N HCl for determination of FA. The precipitate was dissolved and mixed with 0.05 N NaHCO₃ (pH 8.0–8.2) to determine HA. HA and FA were measured by spectrophotometry, partly modified by the authors. Total HAs were calculated by the sum of FA and HA. All results were expressed on an oven dry weight basis.

Spectral analysis

NIR spectra were obtained from both the ground and air dried soil samples using a Technicon InfraAlyzer 500 fitted with an IBM compatible computer, scanning from 1100 to 2500 nm. For samples the spectral data were recorded as log 1/R (*R* = reflectance) at 2 nm intervals. A set of 269 samples was selected for calibration process and the other 179 samples were used for validation. Calibration equations for soil HA, FA and total HAs were based on the selection of the lowest standard error of estimation in a multiple regression analysis from the best combination of 2–9 wavelengths using IDAS software (InfraAlyzer Data Analysis System, Technicon Instrument Corporation, NY, USA). Equations were produced for the prediction of each soil constituent. The multiple linear regression (MLR) or partial least square regression (PLSR) were used from the first or second derivatives of the NIR reflectance spectrum after baseline and slope correction. The best predictive equation was chosen as the equation with the lowest standard error of calibration (*SEC*) and fewest wavelengths. The *SEC* and standard error of prediction (*SEP*) were calculated according to the following equations:

$$SEC = (\text{mean square error})^{1/2}$$

$$SEP = [\Sigma(Y-X)^2/n-1]^{1/2}$$

Where *X* is the value measured by the chemical analysis, *Y* is the predicted value on the basis of the spectral analysis and *n* is the number of samples.

Results and discussion

The content of HA, FA and its total HAs of soil samples determined by chemical method are showed in Table 1. The contents of the three constituents for validation sets ranged from 0.011 to 1.235%, 0.017 to 0.571% and 0.031 to 1.777%, respectively and were just within the content ranges for calibration sets. The mean values of HA, FA and total HAs were 0.3969, 0.2232 and 0.6101%, respectively, for the validation sets, close to the mean values for the calibration sets.

The calibration statistics and wavelengths selected for predicting the content of HA, FA and total HAs are showed in Table 2. The mathematic treatment used to develop the equations was log 1/R (raw spectra). An MLR procedure between the content of a given constituent and the spectral response of several bands was combined until the highest coefficient of correction was achieved. Calibrations were evaluated by using the equations to predict HA, FA and total HAs of soil samples.

HA *SEC* was 0.176 and *SEP* was 0.185. The plot of the NIR-predicted HA data v. the chemical data of the validation set revealed a good correlation (Figure 1). Dalal *et al.*⁶ and Ryu *et al.*⁸ used three and eight wavelengths, respectively, to predict organic matter content in different soil samples and obtained similar results. The higher number of wavelengths required in our calibration is due to the greater heterogeneity of the samples. The wavelength data supported part of the chemical information (Table 2).

Table 1. Statistical data for HA, FA and HA+FA determined by chemical method.

Set	Type	<i>N</i> ^a	Range	Mean	SD
HA	Calibration	269	0.008 - 1.296	0.3933	0.2776
	Validation	179	0.011 -1.235	0.3969	0.2804
FA	Calibration	269	0.014 - 0.878	0.2219	0.1155
	Validation	179	0.017 - 0.571	0.2232	0.1129
HA + FA	Calibration	269	0.018 - 2.174	0.6088	0.3755
	Validation	179	0.031 - 1.777	0.6101	0.3741

N^a : number of soil samples

Table 2. NIRS calibration statistics for predicting HA, FA HA+FA contents in soils (MLR).

Set	<i>N</i> ^a	<i>R</i>	<i>SEC</i>	<i>SEP</i>	Wavelength (nm)
HA	269	0.776	0.176	0.185	1888,2272, 2400, 2476
FA	269	0.893	0.052	0.056	1884,2488,22562472,2272, 2404
HA + FA	269	0.868	0.190	0.244	1916, 2272, 2476, 1140, 1460, 1196, 1220, 2338, 2304

N^a : number of soil samples

The groups contribution to vibrations at these four common wavelengths could be: P-OH(1880 nm), CHO and OH (2272 nm), COO- (2400 nm) and C=N, C-H, C-C (2476 nm).^{9,10} Consequently, this may explain why the HA content is predicted by NIR.

The *SEC* and *SEP* were all lower for FA content than for HA (Table 2). The *SEC* and *SEP* for FA were 0.052 and 0.056, respectively. The F-value was much higher than that of HA. This showed that the prediction for FA content by NIR reflectance spectrophotometry accurate than the prediction of HA. There is very close correlation relationship between the NIR predicted data and chemical data for

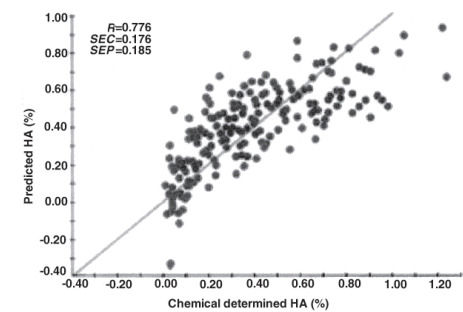


Figure 1. HA content by chemical method and predicted by NIR spectra (MLR).

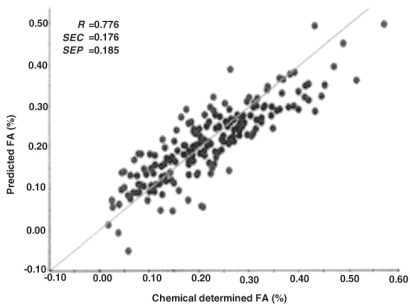


Figure 2. FA content by chemical method and predicted by NIR spectra (MLR).

FA content. The coefficient (R) was 0.893 (Figure 2). Six wavelengths were used for reflectance values to predict the FA content. The selected wavelengths and group contributions to reflectance were mainly similar to HA predictions. It illustrated that FA determined by UV-spectrophotometry might be similar to the functional groups determined by NIR.

Total HAs (HA + FA) estimated by NIR also had better results. SEC was 0.190 and SEP was 0.244 for total HAs (Table 2). Figure 3 shows that a close relationship exists between the NIR predicted total HAs and the chemical data. The correlation coefficient was 0.868 and matched the 0.01 significance level. The selected wavelengths and its group contributions to reflectance were in some cases, similar to those for predicting HA and FA. But the overtones and combination vibrations at 1140, 1460, 1916 and 2304 nm wavelengths (Table 2) could possibly be: $^{\circ}\text{CH}$ aromatic groups (1140 nm), $-\text{CONH}_2$ (1460 nm), $-\text{CONH}$ (1916 nm) and $=\text{NH}$, $>\text{CO}$ amino acid (2304 nm)^{9,10}. All these functional properties are more likely to be similar to humic substances. They also support part of chemical analysis further.

To find the optimum calibration equations for HA and FA, the PLSR calibration method was adopted from the first or second derivative of NIR reflectance spectrum after baseline and slope correction. The results showed that the first derivative obtained better calibration equations for HA and FA, but the second derivative was not effective (Table 3). Table 3 shows that the SEC and SEP for HA content obtained by the first derivative were lower than those by the raw spectra (Table 2) and the correlation coefficient was higher in the first derivative treatment than raw spectra treatment. There was a close correlation relationship between the predicted data and actual data for HA (Figure 4). The coefficient was highly significant. The SEC and SEP for FA content by using first derivative were 0.055 and 0.058 separately, close to the raw spectra treatment (Table 2) and the correlation coefficient also reached 0.01 significance level. This showed that the PLSR calibration equation by the first derivative treatment could be used for predicting FA content in soils (Figure 5). The accuracy and precision of prediction for FA were very high, close to the calibration equation by raw spectra (Table 2).

Conclusions

The NIR method has higher accuracy and precision to predict HAs in soils. Simultaneous, rapid and nondestructive determination of HA, FA and its total contents in soils is possible by using the NIR

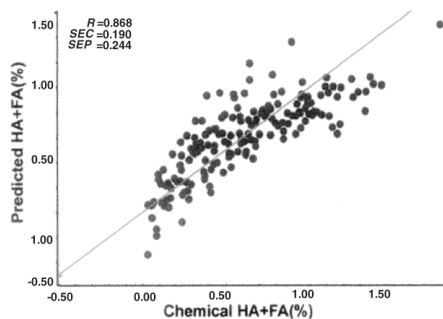


Figure 3. HAs + FA by chemical methods and predicted by NIR spectra (MLR).

Table 3. Equation calibration statistics for HA and FA contents in soils (PLSR).

Set	N^a	Mean	Range	R	SEC	SEP	Math treatment
HA	269	0.3933	0.008 - 1.296	0.805	0.167	0.178	1st derivative
	269	0.3933	0.008 - 1.296	0.605	0.222	0.221	2nd derivative
FA	269	0.2219	0.014 - 0.878	0.883	0.055	0.058	1st derivative
	269	0.2219	0.014 - 0.878	0.743	0.078	0.088	2nd derivative

N^a : number of soil samples

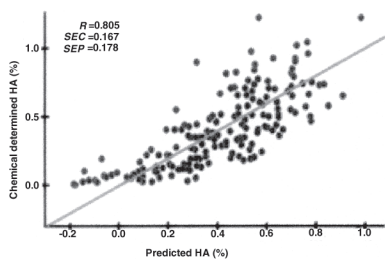


Figure 4. HA content by chemical method and predicted by NIR spectra (PLSR).

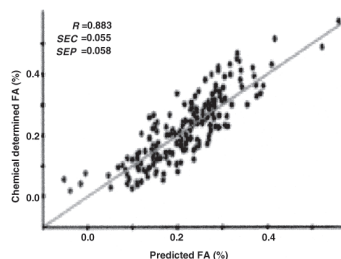


Figure 5. FA content by chemical method and predicted by NIR spectra (PLSR).

technique. The raw spectral data ($\log 1/R$) can be used for estimating HA, FA and its total contents in soil by the MLR procedure between the content of a given constituent and the spectral response of several bands. The predicted results for FA are the best in all constituents, because the chemical data for FA may be accurate. The new spectral data that are converted from the raw spectra by PLSR, such as the first derivative of each spectrum can be used for predicting HA and FA contents of soil samples too, in which the predicted data for HA content seems better than those estimated by the raw spectra. The estimated results for FA content by the new spectral data are basically the same as those determined by the raw spectra. Many possibilities should be examined to select the optimal calibration equations. A low *SEC*, *SEP* and a high coefficient of correlation in the calibration and validation stages give the best selection.

Generally, we should select a simple calibration and prediction method for determining HA, FA and its total contents in soil under similar accuracy and precision of prediction. It should be remembered that the NIR approach would never replace the traditional methods. However, NIR techniques may be an effective method for rapid and non-destructive measurement of a large number of soil samples.

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