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**EVALUATION OF DRY-SOIL INFRARED TECHNIQUES FOR SOIL  
ORGANIC CARBON CHARACTERIZATION**

A Thesis in

Agronomy

by

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## ABSTRACT

Soil organic carbon (SOC) is one of the most important soil quality parameter. It is a proxy for soil organic matter, which influences the physical, chemical and biological properties of the soil. Soil carbon also plays an important role in global C cycle. Understanding the dynamics of SOC is therefore, very important for monitoring soil quality. High density sampling is usually required to capture the spatial variability in SOC of an area which makes costly standard methods highly impractical. The standard procedures for SOC measurement are time consuming and expensive and thus discourage the detailed study of crop-soil management systems. Rapid and relatively in-expensive methods of SOC quantification are needed to improve our understanding of its dynamics and spatial and temporal variability in soils.

Infrared spectroscopy in combination with multivariate analysis is a technique for rapid and in-expensive quantification of SOC. This technique is non-destructive and preserves the basic integrity of the soil system. It does not require expensive and time consuming sample pre-processing or the use of expensive and environmentally hazardous chemical extractants. In addition, the method has the potential to be used for determining carbon quality and simultaneously measuring several other soil properties. This ability of spectroscopic technique can be particularly useful in site- specific agriculture (precision agriculture) which requires extensive information on soil properties and conventional laboratory systems are too costly and labor intensive to generate the necessary data.

Despite an increased interest in infrared spectroscopy especially MIR for measuring soil properties, its utility is not completely understood. There are some inconsistencies in the data and differences in opinions of various researchers about the

performance of this technique and its applicability in management systems. The objective of this study is to evaluate the ability of different IR techniques to characterize SOC, identify the factors that limit the performance and develop ways to overcome those limitations. Specifically we compared the ability of two infrared regions (near-infrared (NIR) and mid-infrared (MIR)) and also two MIR optical sampling options (diffuse reflectance (DR) and attenuated total reflectance (ATR)). We used samples from three management studies which were very different in terms of diversity to develop SOC calibration models, using partial least squares regression (PLSR) analysis.

In the first study we compared the ability of MIR and NIR spectroscopy to determine SOC. The accuracy of two methods as judged by root mean squares error of prediction (RMSEP) was similar for the two less diverse study sets (RMSEP = 0.12 - 0.14). For the more diverse study set error was smaller and correlation higher with the MIR region (RMSEP = 0.27) compared to NIR region (RMSEP = 0.31). SOC calibration models did not perform as well for diverse study compared to the other two which indicates that at the errors associated with calibration models depend on the sample set structure. There is a potential to improve the model accuracy in the MIR region by selecting the most relevant spectral regions. Although we could get slight improvement in model accuracy for all sets by eliminating part of the spectrum that was not associated with SOC, we could not get good performing models based just on the organic bands alone. For very diverse data sets, calibration performance can also be improved by using more than one calibration model. This was proved using samples from one study set where better calibration was achieved by using separate models for samples from upper and lower depths. Samples can be divided for separate calibrations either on the basis of

known sample information (for example depth or site of sampling) or by performing unsupervised clustering.

In general when dealing with a single set of soils, MIR proved to be more efficient than NIR when expressed as the percentage of study samples that are not needed in calibration, and the efficiency of both ranges were better with less diverse data sets. For adapting an existing calibration model to a new site, some samples from a new site have to be included in the updated calibration. We found that if the sample set for which the model was originally developed is diverse or is similar in mineralogy to the new study, lesser number of samples from the new set need to be included in calibration. However, better predictions can be obtained by building calibration model on some samples from that site instead of trying to extend an existing model especially in cases where the new site is more diverse or else is very different from the old site.

The second study we evaluated a new optical sampling approach called ATR for SOC quantity and molecular functional group quality determination and compared it with DR. Most of the MIR spectroscopic studies reported in the literature used diffuse reflectance (DR) sampling technique. Although the technique is useful in mineral soils, the high absorbance could be a problem in case of soils very rich in organic matter. ATR-FTIR technique also has a higher potential to be used in situ. In contrast to most DR instrumentation, the ATR technique involves a sealed optics and therefore is less influenced by the temperature, dust and humidity of the measuring environment.

Using two different types of soil collections we could show that ATR-FTIR calibrations perform nearly as well as DR-FTIR calibrations. The usable MIR spectral range for SOC determination is different for DR and ATR-FTIR. There are still

unresolved issues about the best spectral range for quantifications and qualitative analysis of SOC. However two ranges were found to perform well for SOC quantitative analysis (3900-1240, 1750-540  $\text{cm}^{-1}$ ). The better quality MIR spectral signature in the fingerprint region suggests that ATR may perform better than DR for SOC qualitative analyses.

Although our diamond-ATR studies utilized a lab based FTIR, the same instrumental components are found in portable instruments. Since ATR optics is easily sealed we can expect similar so on-site performance as we achieved in the lab.

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**CHAPTER 1**  
**REVIEW OF LITERATURE AND RESEARCH OBJECTIVES**

## **Introduction**

Since soil properties like clay and organic matter play important role in soil fertility, a detailed understanding of these properties is very important to make economic and environmental advantages of precision agriculture (Sorensen and Dalsgaard, 2005). Precision agriculture requires the judicious use of inputs like fertilizers to minimize costs and environmental impacts. This kind of site specific agriculture demands the estimation of several soil properties and hence collection and analysis of large sample sets. The cost of analytical methods severely limits the need to understand the spatio-temporal variability (Rossel and McBratney, 1998). Environmental monitoring and precision agriculture requires the use of good quality and in-expensive soil analysis methods (Rossel et al., 2006). Also the soil properties are neither static nor homogeneous (Udelhoven et al., 2003) and hence require monitoring in space and time. Inadequate sampling and analyses can lead to inefficient use of many valuable resources like water, nutrients and herbicides. The focus of many research studies is on developing scanning and screening techniques which can quantify and capture the variability in soil properties more efficiently and at minimum cost (Rossel and McBratney, 1998). Infrared (IR) is one such technique which has the ability to overcome the limitations of old standard procedures.

According to Schnug et al. (1998), the lack of physico-chemical soil maps is one of the major limitations for continuous soil monitoring at the farm level. Soil processes and mechanisms are complex and difficult to understand. The standard procedures of soil analyses are often used to analyze relationship between physical and chemical properties and individual soil components ignoring the complex interactions that are present in this



heterogeneous system. To understand and manage soil as a system and to meet the growing demands of food and fiber we need a large amount of accurate soil data (Rossel and McBratney 1998).

Among the soil properties that influence the soil quality, soil carbon is probably one of the most important. It is directly or indirectly related with almost all physical, chemical and biological properties. Soil carbon influences soil characteristics like hydraulic conductivity and soil structure which affect the soil erosion processes. It is therefore also a parameter of interest for ecologists and land managers who are dealing with land degradation and conservation issues (Udelhoven et al. 2003).

With increasing interest in carbon sequestration and its effect on global carbon flux, methods for determining soil carbon have gained even more importance. Carbon management at regional and global levels require efficient methods of C assessment. Trading of carbon credits and C-sequestration incentive programs will be reliant on methods that can quantify C in a relatively in-expensive way for a large area (Gehl and Rice, 2007).

Traditional methods of soil carbon determination have been associated with several limitations. Malley et. al, 1999 reported that many standard laboratory methods besides being costly and time consuming are irreproducible, un-reliable and laborious. The Walkley Black procedure (Walkley 1935) has been used as a standard for several years but the procedure requires the use of a correction factor due to incomplete digestion of all C in the samples and the correction factor might be different for different soils (Rosell et al. 2001). Reeves et al. (2001) reported that chromate oxidation method is time consuming, leaves chemical wastes and requires separate determination of organic and

inorganic carbon. The Walkley Black method also assumes that the oxidation state of organic matter is zero which could be a source of error (Nelson and Sommers, 1996). Dry combustion method (Nelson and Sommers, 1996) which is another standard method determines soil C by measurement of CO<sub>2</sub> released from the oxidation of organic C and thermo decomposition of carbonates. This method is associated with high costs. In some dry combustion methods, C determination is based on the weight change instead of measuring the CO<sub>2</sub> emitted, and hence can cause discrepancies in the results from different laboratories (Kimble et al., 2001). According to Reeves et al. (2002), dry combustion methods require separate determination of organic and inorganic carbon, are time consuming and costly and can not be used for in-situ determinations. According to Watson et al., 2000, combustion analysis for C determination is quite accurate but associated with high cost. Loss on ignition is another method that is used to estimate SOC. The method usually overestimates the SOC because the weight loss in the procedure is not only because of the organic matter but also clays and other inorganic constituents. The SOC values obtained are affected by sample size and temperature (Cambardella et al., 2001).

Infrared methods have several advantages over the standard methods and hence are a possible alternative with the potential to replace or enhance the current methods of soil analysis (Janik et al. 1998). Infrared analysis needs very little sample preparation, is rapid, in-expensive, non-destructive and non-hazardous and has the ability to predict many soil properties simultaneously (Batten, 1998; Barthes et al., 2006). Depending on sample condition, type of spectroscopic method and instrument, sample preparation for IR methods ranges from grinding and drying to none at all (Reeves et al., 2001). It also

minimizes reagent waste, offers high precision and cost savings increase multiplicatively with each analyte analyzed (Cohen et al., 2007). According to Rossel et al. (2006), this technique is sometimes more accurate than the standard methods. Rossel et al. (2001) reported that MIR gives more precise predictions of soil pH and lime requirement than conventional methods. IR technique being non-destructive also preserves the basic integrity of the soil samples in contrast with the chemical extraction methods which alter the equilibrium between various soil phases and hence complicate the interpretation of results. The chemical procedures done on a complex non-ideal soil system can complicate the interpretation of analyses conducted to understand the link among measured soil properties (Islam et al., 2003).

The infrared region of spectrum includes radiations with wavelengths ranging from 0.78 to 1000  $\mu\text{m}$ . Infrared spectroscopy utilizes the interaction of electromagnetic radiations with specimens. Absorbance peaks result due to transition of molecular vibrational states. The spectral features which result are thus related with the molecular structure of the specimen. The spectral signature of a material is its reflectance or absorbance expressed as a function of wavelength in the infrared region of the electromagnetic spectrum. From instrument and application point of view, the infrared spectrum is conventionally divided into near (NIR), mid (MIR) and far-infrared. The spectral features related to organic matter and other soil properties occur in mid to thermal – infrared range (2500 - 25,000 nm) with their overtones in near infrared region (1100 – 2500 nm) (Mutuo et al., 2006).

Infrared techniques are sensitive to both organic and inorganic phases of the soil. NIR and MIR ranges of infrared spectrum have been used for soil carbon quality and also

quantification studies (Sudduth and Hummel, 1993; Ben-Dor et al., 1997; Janik et al., 1998; Reeves et al., 1999; McCarty and Reeves, 2006; Islam et al., 2003). Both MIR and NIR spectroscopic determinations require development of calibration models that relate the spectral information to the soil property of interest (soil carbon) using multivariate data analysis techniques such as partial least squares regression (PLSR) (Workman, 1992).

### **NIR and MIR spectral ranges**

NIR region of the spectrum consists of weak overtones from the fundamental vibration that exist in the MIR region due to the stretching and bending of NH, OH and CH groups. Weak overtones make it very difficult to interpret the NIR spectra directly (Wetzel, 1983, Chang et al., 2001). Besides the chemistry NIR is also influenced by the physical structure of a material including size, shape and arrangement of particles and voids between the particles which affect the length of light passing through a sample (Wetzel, 1983). Murray and Williams (1987) reported that NIRS is limited in its application for qualitative analysis or spectral interpretation because of weak overlapping bands mainly associated with OH, NH and CH. But later on it was demonstrated that although the untransformed NIR spectra has very few features, with the use of the right chemometric approach, it can be used to correctly analyze many agricultural products (Roberts et al., 2004). Chang et al. (2001) reported that NIRS can also be used to determine soil properties (for example CEC) which have a primary response in NIR region. This can be done if those soil properties are correlated with other attributes like organic matter and clay contents which have a direct response in the NIR region.

Clays and to some extent organic matter have well recognized features in VNIR (Visible Near Infrared) range. Although the fundamental vibrations of most soil materials are found in MIR region, their weaker overtones and combinations are found in the NIR region (Brown, 2007). For example overtone of C-H stretch fundamental vibration at 3.4  $\mu\text{m}$  in the MIR spectrum can be found at 1.7, 1.15 and 0.85  $\mu\text{m}$  in the NIR spectrum (Workman and Springsteen, 1998; Weyer and Lo, 2002). Clay minerals have identifiable absorption features at 1.4  $\mu\text{m}$  (O-H stretch first overtone), 1.9  $\mu\text{m}$  (O-H stretch, water bend combination), 2.2-2.3  $\mu\text{m}$  (O-H stretch, metal-OH bend combination and many other small features (Hunt, 1977; Clark, 1999). Hematite and goethite also have signature in the NIR region at 0.7-1  $\mu\text{m}$  (Scheinost et al., 1998).

MIR region is dominated by intense fundamental vibrations related to soil components. Chemical groups of SOM are almost all infrared active and the IR technique can be used to characterize SOM (Skjemstad and Dalal, 1987). The overlapping of the peaks however makes the analyses difficult (Janik et al., 1998). Use of MIR was limited to qualitative analysis such as spectral interpretation (Reeves et al. 2001). This was mainly because of the belief that due to strong absorptions present in the mid-infrared region, the spectra of soils and other materials get distorted if they are not diluted with KBr (McCarty et al., 2002). Dilution with KBr increases the time and cost of analysis and hence makes the technique look less attractive. Later studies however revealed that MIR technique can be used on pure soils to determine various properties including total carbon, organic carbon, particle size, total sulfur, extractable Mn and exchangeable cations (Janik et al., 1998; Bertrand et al. 2002; McCarty et al., 2002). Compared to the NIR region which has absorption features due to combinations and overtones in MIR

region, the MIR region consists of fundamental vibrations. In addition absorption bands for soil constituents like inorganic carbon, and minerals such as phosphates only exist in MIR region (Pirie et al., 2005).

MIR spectrum of soil has several well identified features. High peak around 1200-1900  $\text{cm}^{-1}$  denotes stretching of Si-O-Si bonds and Al-O-Si bonds in clay minerals. Peaks between 900-500  $\text{cm}^{-1}$  are also associated with clay minerals (Haberhauer and Gerzabek, 1999; Haberhauer et al., 1998; Orlov, 1986). Peaks between 3600 and 3300  $\text{cm}^{-1}$  and near 1600  $\text{cm}^{-1}$  are associated with stretching and bending of O-H bonds of water (Johnston and Aochi, 1996). Peaks around 2900 are due to aliphatic C-H stretching. Peaks between 3500-3000  $\text{cm}^{-1}$  are due to O-H, N-H and C-H stretching (Baes and Bloom, 1989). Region between 1650-950  $\text{cm}^{-1}$  is associated with stretching and bending of C-O, COO and CH bonds (Stevenson, 1994). Peaks near 1600  $\text{cm}^{-1}$  are due to C=C bonds of aromatic rings and aromatic C-H bonds.

### **Chemometrics**

In ideal systems, responses of bonds to molecular stretching, vibration and rotation are well known, however, in the complex non-ideal soil system, spectral response of constituents overlap making the interpretations rather difficult. Thus the curve matching approach used to identify soil constituents is difficult especially in the NIR region which is dominated by weak overtones and combinations. Infrared technique is based on the calibration approach. A calibration equation is obtained using chemical data from conventional techniques and spectral data from MIR and NIR. Regression analysis used get this calibration equation extracts spectral information that is most related with the property of interest (SOC). The calibration equation thus obtained can be

used along with the spectral data to determine the property of interest without the need for laboratory determinations.

Univariate data analysis is not helpful when dealing with the spectral data because infrared peaks for soils are often too complex and the spectral data has enormous information about the property of interest. Sieielec et al. (2004) reported that chemometrics does not have to rely on physical relationship between spectral data and chemical functional groups. It is based on mathematical relationship between spectral and reference data. Although the spectral understanding might be challenging for some researchers but chemometrics for practical application is very successful. Speculating on the features that a multivariate model uses for successful predictions could be challenging and risky.

Quantitative analysis using the infrared method requires the use of sophisticated statistical techniques that can relate spectral information with the property of interest. Ben-Dor and Banin (1995) used multiple linear regression to relate soil properties with NIR data. Shibusawa et al. (2001) used stepwise multiple linear regression (SMLR) for the estimation of soil properties with NIR spectral peaks. Similarly techniques like multivariate adaptive regression splines (MARS) (Shepherd and Walsh, 2002), radial basis function networks (Fidencio et al., 2002) and artificial neural networks (Daniel et al., 2003) have been used to relate spectral data with soil properties. However, the most common techniques that have been used in soil spectral studies are PCR (principal component regression) (Chang et al., 2001, Islam et al., 2003) and partial least squares regression (McCarty et al., 2002). Compared to PCR, which decomposes the spectra into a set of eigenvectors and scores and performs regression with soil attributes in a separate

step, PLSR uses soil information during decomposition process (Rossel et al., 2006). Spectral vectors with PLSR analysis are directly related with soil property of interest because this technique takes advantage of the correlation that exists between spectra and the soil. PLSR handles multicollinearity better and is more robust to data noise and missing values. The calibrations and predictions with PLSR are more robust.

Partial least squares is a soft modeling technique used for quantitative analyses. Like PCA, it is a data reduction technique because it deals with many predictor variables that are collinear. In PLSR analysis, spectral and soil property information is used to divide the calibration spectra into smaller set of eigenvectors called loadings and scaling vectors called scores which maximize the covariance between spectral intensities and the soil property of interest (Haaland and Thomas 1988). PLSR is based on linear transition from a large number of original descriptors to a small number of orthogonal factors (latent variables). Successive latent variables are selected that maximize the covariance between predictor and response variable (Rossel et al., 2006). The first few factors explain most of the variation in X and Y. The remaining factors are mostly due to noise and probably should not be included in the model. SIMPLS (de Jong 1993, de Jong et al., 2001) is a PLSR algorithm where covariance matrix is deflated in every step to maximize covariance between scores.

Techniques like PLSR are considerably better than the others like multiple linear regression where specific peak intensities have to be selected which are then related with the soil properties. With PLSR there is no need to isolate specific peaks and the technique is able to use even small spectral variations to model soil properties of interest. PLS can also find application in the studies where discrimination of soils from a large set is



required. In the factor loading scores plots, samples with similar properties are grouped together.

For the IR calibrations the use of multivariate algorithm such as PCA or PLS is essential to compress the data and reduce multi-collinearity. Before the multivariate analysis the data is pre-processed to remove the spectral features which are not related to the chemical absorption of light. Usually several types of transformations are tried out and the best transformation is determined from the trials. For example the difference in particle size causes spectral baseline shift called multiplicative scatter. This can be corrected by a pre-treatment called multiplicative scatter correction (Beebe et al., 1998). Derivatives such as those using Savitzky-Goaly filter are known to minimize the variation in data brought about by grinding and optical setup (Martens and Naes, 1989). Noise brought about by light scattering can be corrected using multiplicative scatter correction (Wise et al., 2003).

Two different approaches can be used to build calibration models. One involves the use of separate samples for building (calibration set) and validating the calibration models (test set or validation set). Performance of models in this case is checked by coefficient of correlation and root mean square error of prediction (RMSEP) which is an estimate of average difference between the predicted and measured response. In the second approach an internal validation system is used and there is no separate test set. The original samples are partitioned into  $K$  subsets and from these a single subset is retained as the validation set for testing the model, and the remaining  $K - 1$  subsets are used as calibration set. The cross-validation process is then repeated  $K$  times with each of the  $K$  subsets used once as the validation data. The results from  $K$  subsets can be

averaged to get root mean square error of cross validation (RMSECV) which is equivalent to RMSEP computed in the test-set approach. Results of cross validation approach are usually over-optimistic and hence a test-set approach is more appropriate.

Use of a separate validation set is important to get more reliable results instead of using all samples for calibration (Dardenne et al., 2000; Islam et al. 2003). Cross validation results are usually over-optimistic. When independent samples are predicted with calibration developed on another set of samples, SEP is usually larger than SECV (Dardenne et al., 2000).

### **Model performance and figures of merit**

The performance of calibration models is judged by several parameters. Coefficient of determination ( $r^2$ ) is probably most commonly used. It is a measure of total variance accounted for by the model. SEP or the standard error of prediction expresses the infrared results corrected for bias (Sorensen and Dalsgaard , 2005)

$$SEP = \sqrt{\frac{1}{N-1} \sum (x_i - y_i - bias)^2}$$

where  $x_i - y_i$  = difference between results obtained by infrared method ( $x_i$ ) and the reference method ( $y_i$ ) for sample  $i$  and bias is

$$Bias = \frac{1}{N} \sum (x_i - y_i) = \bar{x} - \bar{y}$$

where N = number of samples

RMSEP (root mean squared error of prediction) includes SEP and bias in a single term. When bias is small, RMSEP and SEP are almost the same values. RMSEP gives more reliable estimate of the predictive ability of the model.

$$\text{RMSEP} = \sqrt{\frac{1}{N} \sum (x_i - y_i)^2}$$

When the models are not based on separate validation set, figures of merits like RMSECV (root mean square error of cross validation) and SECV (standard error of cross validation) are used which are comparable to RMSEP and SEP respectively.

Various authors have found that RPD is more useful than  $r^2$  or RMSEP and RMSEC when comparing different calibration models (Batten 1998; Islam et al. 2003). In most agricultural studies RPD value of  $> 3$  is considered acceptable (Pirie et al. 2005). Although acceptable values of RPD depend on intended applications of the predicted values (Dunn et al. 2002), Chang et al., (2001) reported that calibrations with  $\text{RPD} > 2.0$  are excellent, those with  $\text{RPD} 1.4\text{-}2.0$  are acceptable and those with  $\text{RPD} < 2$  are unacceptable.

Selecting optimum number of factors is also an important part of the calibration process. If the calibration model uses too few factors, it is said to be under-fitted and may not capture the full variability in the data. If model is based on too many factors it is said to be over-fitted and may be modeling redundant information from the data. A calibration model that uses fewer factors is more likely to yield accurate predictions for newer samples (Madari et al. 2006). If the samples are very diverse, calibration is likely to use more factors. Reeves et al. (2001) while developing separate models for samples from no-tillage field and conventional tilled field observed that for C and N, no-till calibration used twice as many factors as plow-till calibrations. Fidencio et al. (2002) reported that number of factors in the calibration model depend on variability in the samples caused by OM content and other components.

Outliers can sometimes affect the calibration performance significantly. Outliers in some studies have been defined as the samples for which the difference between measured and predicted values is greater than threefold the RMSEP or RMSEC (Chang et al. 2001, Islam et al., 2003).

While looking at the figure of merits reported in a study, careful attention should be given to the number of samples and diversity of samples. Calibration built on limited number of samples or on samples from limited number of sites can only be used for certain type of soil or area studied (Islam et al. 2003).

For meaningful interpretation of spectral prediction error it is important to quantify the within-laboratory uncertainty. Judging the suitability of spectral methods based on spectral accuracy alone ignores the laboratory error (Cohen et al., 2007).

### **NIR application in soils**

NIRS technique was first developed for rapid moisture determination of grains about three decades ago (Ben-Gera and Norris, 1968). It is now being used as a routine procedure for grain and forage quality assessment. Several studies have focused on soil analysis using NIRS. Bowers and Hanks (1965) reported that soil moisture, organic matter and particle size affect the reflectance spectra of soil. Al-Abbas et al. (1972) reported that there was a negative relationship light reflectance and organic matter content of soil. Krishnan et al. (1980) predicted organic matter of 12 soils from Illinois with  $r^2$  of 0.87. Similarly Dalal and Henry (1986) predicted OC of 144 soils with  $r^2$  of 0.86 and Sudduth and Hummel (1993) predicted OM with  $r^2 > 0.85$ . Ben-dor and Banin (1995) used NIRS to determine several soil properties including organic matter. Using a separate validation set they obtained an  $r^2$  of 0.55 for organic matter. Chang et al (2001)

used NIRS-PCR technique to estimate several soil properties. Using 802 soil samples from United States they could determine SOC with an  $r^2$  of 0.87 and RMSECV of 7.86.

NIR has also been used to determine soil properties besides OC. Vagen et al. (2006) were able to develop stable calibration models for several key soil properties including SOC, total N, CEC and clay content for Madagascar soils using vis-NIR data. They concluded that this rapid and cost effective method could be very promising in assessing changes in soil quality as a result of deforestation or other land use changes. Cozzolino and Moron (2003) determined several soil properties including sand, silt, clay, K, Ca Mg, Cu and Fe with an  $r^2$  between 0.80-0.95.

Some studies have used NIRS for qualitative analysis of soils as well. NIRS has been used to study the soil OM components like humic acids and lignin contents and various fractions of OM (McLellan et al., 1991 and Henderson et al., 1992). NIRS has also been used to study the decomposition of organic matter (Ben-Dor et al. 1997 and Terhoeven-Urselmans et al., 2006). Islam et al. (2005) used biplot of PC1 and PC2 based on soil UV VIS and NIR diffuse reflectance spectra to capture the similarity and dissimilarity among soil samples from different regions. The location of samples in the biplots represented the degree of variation among the samples. Odlare et al. (2005) used NIR-PCA approach to capture the variation in different soils. They concluded that since the NIR spectra has information on either the actual content of a soil component or other properties that are related to the soil component, PCA of the NIR data will always capture the spectral information that represents the largest variation in soils. Such an approach although not useful for the exact quantification purposes, can be used to determine whether there is significant variability within a soil field.

In some studies NIRS has been successfully used to study the mineralization rates in soils. Fystrob (2002) used visible and NIR spectroscopy to predict OC, total nitrogen and potential mineralization using grassland samples. For the variables investigated, the  $r^2$  value was between 0.65- 0.87. They reported that some of the error in the method could be attributed to error in reference values (By running samples in duplicates they found that standard error of laboratory determinations using LOI methods was 1 g/kg). They concluded that thawed, moist samples were more accurately estimated compared to dried samples, but grinding of 4 mm screened samples did not improve predictions. Mutuo et al. (2006) used NIRS to predict C mineralization rates in physically fractionated soil aggregates and free organic matter in aerobically incubated samples from a clayey soil and a sandy soil. Predictions for C respired in aggregate fractions and C mineralized organic matter fractions had an  $r^2$  of 0.82 and 0.71 respectively. Authors were also able to discriminate major soil fractions using a 50% random holdout validation sample.

There are some reports in literature which say that NIRS performance is not satisfactory for routine soil analyses. Sorensen and Dalsgaard (2005) while studying the feasibility of NIR (400-2500 nm) to determine SOC of soils from all regions of Denmark using PLS regression reported an RMSECV of 0.40 with SD/RMSECV ratio of 2.4. They observed a non-linear trend in the data and increased prediction errors with higher C-contents and hence concluded that NIR maybe not be very useful for C-calibration especially when a universal calibration model is needed for very diverse soils. The performance of calibration model was found to be poor for sample sets low in clay content. Ludwig and Khanna (2001) while reviewing use of NIRS to estimate organic and inorganic carbon in soils concluded that compared to the traditional methods of C-

estimation including combustion analysis and Walkley Black procedure, NIRS technique does not bring great improvement in terms of easiness of operation or cost. NIRS is probably more attractive than the conventional methods because of its ability to simultaneously measure several soil properties and also because of the availability of portable NIR instruments and hence the chance for in-situ measurement of soil C.

Islam et al. (2003) studied the ability of UV, VIS and NIR reflectance spectroscopy to predict various soil properties simultaneously. They studied 161 (121 in calibration and 40 in validation) surface and sub-surface soil samples and could predict pH, gravimetric water content, organic carbon, clay, cation exchange capacity and exchangeable calcium and magnesium with good accuracy but got poor predictions for electrical conductivity, free ion, sand and silt contents and exchangeable potassium and sodium. OC was predicted with an  $r^2$  of 0.76 and SEP of 0.44 using the UV-VIS-NIR range.  $r^2$  and SEP values were 0.81 and 0.35 with VIS-NIR range and 0.68 and 0.45 with NIR range. They concluded that with the reflectance technique sample preparation is simple and highly skilled personnel are not needed to acquire spectral data. The technique is less precise than the chemical methods but is fast, less expensive and environmental friendly. Spectroscopic method has the potential to analyze a large number of samples at finer sampling intervals and using this technique will outweigh the loss in precision.

In-situ performance of NIRS has been tested in some studies. Udelhoven et al (2003) evaluated the usefulness of Vis-NIR spectroscopy to estimate soil properties of samples representing various locations in Germany using PLS regression. In general they found that lab spectrometry performed better than the field spectrometry because of the strong effect of soil structure. While authors could get good estimations of several other

soil properties, the soil organic carbon was poorly predicted. Authors concluded that Vis-NIR can not be used as a diagnosis tool of short or medium-term changes in soil properties but it can be used as a quick screening method in studies involving soil development and soil degradation monitoring and when time or laboratory costs are critical factors. Waiser et al. (2007) used NIRS for in-situ characterization of soil clay content. They concluded that it is an acceptable method for measuring clay content in situ for various water contents and parent materials. However soils from all parent materials have to be represented in the calibration set.

Various authors have tried to identify and address the factors that affect the performance of NIR calibration models including range of analyte being calibrated, sample heterogeneity and diversity, sampling handling and error in reference methods. Barthes et al. (2006) studied the effect of drying, grinding and replication on the performance of calibration models to predict total C and N using NIRS. NIR predictions improved with oven drying of samples compared to air-drying and with fine ground samples (0.2 mm) compared to coarse ground (2 mm). Replication improved predictions for 2 mm sieved samples but not as much for 0.2 mm sieved samples. Thus to make the procedure less tedious, good results can be obtained by replacing grinding with replication. Authors reported that the effect of grinding could influence the calibration model performance differently depending on the texture of soil. In clayey soils the grinding will destruct aggregates and thus reduce heterogeneity and improve predictions. For coarse textured soils, quartz particles are not easily broken with grinding and hence there are more chances of increased heterogeneity. Also the peeling of coatings with grinding might result in more heterogeneous particle size distribution and hence poor



predictions. Drying of samples improves the predictions because strong water absorption bands can mask the organic functional group peaks (Barthes et al., 2006). Madari et al. (2005) also reported that homogeneity of samples affects the calibration performance for both MIR and NIR but NIR is more sensitive to particle size effects

Cohen et al. (2007) used VISNIR spectroscopy to predict organic matter, soil pH, Mehlich-1-extractable P, K, Ca, Mg, Cu, Mn and Zn and saturated hydraulic conductivity using 1933 samples representing major soil orders in Florida. They could predict organic matter and Al with good accuracy ( $RPD > 2$ ), pH, P, Ca and Ksat with medium accuracy ( $1.5 < RPD < 2$ ) and K, Cu, Mg, Mn, Zn and Fe with low efficiency ( $RPD < 1.5$ ). Out of the two techniques used for analysis, they found that PLSR was generally superior to GBT (gradient boosted tree) regression. For most of the analytes studied, the authors could not get predictions as good as reported in the literature. Some of the factors they thought were responsible were the less variability in the mineralogy of soils studied and the error introduced by laboratory methods. They also compared error rates of spectral models with error rates for duplicate measurements using the standard method. Standard error between OM measurements using LOI method was 0.79 compared to the standard error of 0.47 between OM values predicted using spectral method and observed LOI values. Even after removing the systematic bias for the duplicate measurements, standard error was 0.39 which is still comparable to spectral error. They suggest that in order to address the uncertainty issue, calibration models should be developed using mean of multiple laboratory observations for any given soil property. This will minimize the effect of laboratory error and help realizing the true potential of spectroscopic techniques.

Malley et al. (2000) studied two groups of Manitoba soils which had different range of OC. For the first set ( $n = 108$ ) which had samples with C range of 0.9-111.3 mg/g, they obtained an  $r^2$  of 0.90 and SEP of 6.2 mg/g. For the second set ( $n = 940$ ) which had a wider range of OC (0.1-593.4 mg/g), they obtained an  $r^2$  of 0.78 and SEP of 29.5 mg/g. Similarly Reeves et al. (2002) got different performance results for different groups of soils they studied. For the first set of soils ( $n=136$ , OC range 6.8-29.2 mg/g),  $r^2$  of 0.78 and RMSD of 1.66 mg/g; for the second set ( $n = 64$ , OC range 5.3-28 mg/g),  $r^2$  of 0.93 and RMSD of 1.18 mg/g and for the third set ( $n = 179$ , OC range 6.1-33.9 mg/g),  $r^2$  of 0.97 and RMSD of 0.82 mg/g. Martin et al. (2002) tried to determine the OC content of soils using NIRS. They found good correlation between NIRS predicted OC values and OC values obtained with combustion analysis. Over the range of 0-40 mg/g OC, correlation ranged from 0.75-0.78 and it improved to 0.80 when a narrower range of 0-20 mg/g was considered.

Cohen et al. (2007) point out that the biggest advantage in using VISNIR spectroscopy is the cost saving. They estimated that the per sample cost using the spectral technique is \$ 0.83 (including instrument capital costs during 8 years of instrument life and operating as routine analysis tool running ~30,000 samples per year). Authors compared this cost against \$12 that is charged by Extension Soil Testing Laboratory for a producer test which does not include OM and K sat determination.

### **MIR application in soils**

Most of the early studies reported that MIR could not be used for quantitative analysis. Niemeyer et al. (1992) reported that DRIFT-MIRS is not suitable for quantification studies because the equipment is very sensitive to homogeneity of the soil material used in the analysis. Later studies however have shown the application of MIRS for quantitative analyses.

Masserschmidt et al. (1999) determined SOM using PLS-MIR technique. Using reflectance data they obtained an  $r^2$  of 0.98 and RMSEP of 0.63%. Reeves et al. (2001) based on 180 samples from two locations and two tillage practices reported that MIR calibrations for properties including total C, total N, pH and many measures of biological activity, were at least as accurate as NIR and sometimes significantly better. Janik and Skjemstad (1995) while using MIR-PLS to determine SOC observed a significant curvature in regression and high SOC samples were underestimated. These authors reported that variation in the type of C might be responsible for this. When the models were developed for samples with 0-2.5% C (instead of 0-23%)  $r^2$  increased to 0.99.

Madari et al. (2006) studied selected MIR spectral ranges to develop calibration models. They reported that information exists throughout the spectral range. Using whole spectra always yielded more accurate results than using only parts. Removing even those regions which seem to have no apparent bands yielded less accurate results.

Recently some authors have utilized MIRS for the study of soil fractions. Zimmermann et al. (2007) reported that PLS-MIR approach can be used as a fast tool to determine OC contents in different fractions. They separated 111 samples into fractions of dissolved OC, particulate organic matter (POM), sand and stable aggregates, silt and

clay particles, and oxidation resistant OC. Using MIR data, correlation coefficient between measures and predicted values for different fractions ranged from 0.89 – 0.97.

Similarly Janik et al. (2007) used MIR and PLS analysis to predict the concentration of OC fractions. Soils were analyzed for total organic carbon, particulate organic carbon, and charcoal carbon. Using the MIR calibrations  $r^2$  for these fractions ranged between 0.73 – 0.9. Sarkhot et al. (2007) used DRIFTS to examine the chemical composition of the size fractions. The DRIFT spectra showed that the recently added organic matter was present in the largest sized fraction while the more decomposed organic matter was present in smaller size fraction (<53 $\mu\text{m}$ ).

For MIR determination of soil carbon most studies have used diffuse reflectance optical sampling (Janik et al., 1998; Reeves et al., 2001; McCarty et al., 2002; Reeves et al., 2002). Another sampling option called ‘Attenuated total reflectance’ (ATR) can conceptually overcome the limitations posed by other sampling accessories used in the infrared region. ATR has been used for the study of materials in which intense scattering of absorption makes it hard to use the transmission spectroscopy. The spectra of bulk soils can be obtained just by bringing the sample in contact with the surface of ATR crystal. The spectrum acquisition by this method is independent of the thickness of the sample which makes it useful for studying solids, solutions or suspensions. Also, since the light needs to penetrate just few microns in the sample, ATR technique requires smaller sample than DR just enough to cover the crystal. In case of soils, the biggest attraction of ATR is its potential use in in-situ studies.

In soils, most ATR studies have focused on adsorption studies involving the surface analysis of samples (Dobson and Mcquillan, 1999; Kang and Xing, 2007).

Recently ATR-FTIR has also been used for some quantification studies. ATR-FTIR has been used for determination of nitrate concentration in soils (Shaviv et al., 2003 and Linker et al., 2004). Linker et al. (2005) studied FTIR-ATR to determine nitrate concentration of soil pastes using spectral region around the nitrate band ( $1300-1550\text{ cm}^{-1}$ ) to build the calibration models. They reported that the main hindrance in predicting nitrate levels accurately was the presence of the interfering carbonate band around  $1370\text{ cm}^{-1}$ . ATR-FTIR technique has also been used to identify types of agricultural soil based on absorbance bands associated with characteristic soil components including calcium carbonates, clay minerals and organic constituents (Linker et al., 2005, 2006). Linker et al (2005) used wavelength region ( $800-1200\text{ cm}^{-1}$ ) to classify soils using ATR sampling accessory and reported that each soil type has a distinctive fingerprint in this region and hence the region can be used for soil identification.

### **NIR and MIR comparison studies**

Most of the NIR and MIR comparison studies in soils have revealed that MIR is better than NIR. McCarty et al. (2006) compared MIR and NIR in their ability for field scale measurement of soil properties including C, N, texture, pH, Mehlich I extractable Ca, K, Mg, and P. They sampled 272 locations at 0-10 cm, 10-30 cm, with total of 544 samples. MIR consistently outperformed NIR and the effect was even stronger for properties which were weakly calibrated with IR methods. McCarty et al. (2002) got more accurate predictions for total carbon, organic carbon and inorganic carbon using MIR ( $r^2 = 0.94-0.98$ ) than NIR ( $r^2 = 0.82-0.87$ ). They hypothesized that the possible reason for this was the presence of well defined organic molecules absorption features in the MIR range compared to the NIR range. Reeves et al. (2002) reported that both MIR

and NIR can be used to determine SOC. MIR was more accurate than NIR especially for diverse soil set in the study.  $r^2$  of 0.84 was achieved with NIR compared to 0.95 for MIR.

Reeves et al. (2006) compared MIR and NIR spectroscopy to measure soil carbon and other key SOC fractions based on a diverse sample set consisting of 230 samples from 14 sites in 10 states. With one-out cross validation, and  $r^2$  and RMSD values of 0.975 and 0.219 were obtained with MIR data and 0.89 and 0.455 with NIR data respectively. With the independent sample approach too, MIR ( $r^2 = 0.96$ , RMSD = 0.354) performed better than NIR ( $r^2 = 0.784$ , RMSD = 0.831).

Siebielec et al. (2004) developed PLS calibrations for metals including Fe, Cd, Cu, Ni, Pb and Zn using NIR and MIR data. Except for Pb, all the metals were predicted well. NIRS was outperformed by MIR. They reported that better performance of MIR was probably because of more information in the MIR region. They also concluded that MIR can be used for determining metal contents of diverse soils if the calibration is developed on soil samples that are representative. For successful calibration the proper and full representation of soil types and properties is important. In general, the larger sets of samples could improve the calibration but if the samples are very diverse, it might be advantageous to have two or more separate calibrations.

There are other reports in literature which state that NIR performs better than MIR. Van Groenigen et al. (2003) assessed the potential of NIR and MIR for predicting crop and soil parameters in a flooded California rice field. They could get good predictions of eCEC, Ca and Mg with NIR ( $r^2 = 0.83-0.90$ ) predictions being better than MIR ( $r^2 = 0.56-0.61$ ). They however did not find any significant correlations for total C or N. They concluded that the lower value for the correlation coefficient in their case was

because of the too low variability of organic carbon in their samples. They were able to get a low SEP value of 1.6 g/kg with NIR data which suggests that the strong predictive ability of the model. They concluded that NIR has better predictive ability than MIR. Madari et al. (2006) used NIR and MIR to determine total C and N, sand, silt, clay and other measures of soil aggregation of bulk soils (BS) and their 8 aggregate size fractions (ASF). For BS, C was predicted with an  $r^2$  of 0.99 using NIR range and 0.93 using MIR. For ASF samples,  $r^2$  of 0.98 and 0.96 was obtained for C using MIR and NIR respectively.

Recently, studies have been conducted to see the performance of combined NIR and MIR ranges in predicting soil attributes. Pirie et al. (2005) compared UV-VIS-NIR and MIR spectroscopic techniques to predict several soil properties using 415 surface and sub-surface soil samples from Australia. They found that the prediction efficiency in terms of RPD values was higher for the models using the MIR spectral data compared to other regions. Using a separate validation set approach they obtained an  $r^2$  of 0.85 and SEP of 0.55 and RPD of 2.6 with the MIR spectra compared to the UV-NIR-VIS model for which the  $r^2$ , SEP and RPD values were 0.76, 0.50 and 2.0 respectively. For the combined models the performance was slightly better than UV-NIR-VIS model, with  $r^2$ , SEP and RPD values were 0.79, 0.62 and 2.2 respectively.

Rossel et al. (2006) compared Vis, near and mid-infrared regions in their ability to provide qualitative and quantitative information on various soil properties including soil  $\text{pH}_{\text{ca}}$ ,  $\text{pH}_{\text{w}}$ , lime requirement, organic carbon, clay, silt, sand, CEC, exchangeable Ca, exchangeable Al, nitrate nitrogen, available phosphorus, exchangeable potassium and electrical conductivity. They found that MIR was better than NIR and vis in providing

qualitative soil interpretation using loading weight factors from PLSR decomposition. For quantitative analysis, accuracy of three regions varied for different soil properties. MIR gave more accurate predictions for pH, lime requirement, OC, clay, silt and sand content while NIR provided more accurate predictions for exchangeable Al and K. Combining the three ranges brought only slight improvement in the predictions of clay, silt and sand contents.

Besides the accuracy, NIR and MIR have been compared with respect to instrumentation and cost etc. Reeves et al. (2002) concluded that MIR calibrations are more robust and better handle the sample diversity. However there is very less information on the effect of moisture and particle size on the MIR calibrations. This information is important to assess the ability of MIR for in-situ SOC determinations. NIRS is better understood than MIR regarding the effect of these variables. Instruments for in-situ use are available for both ranges. The relatively cheap instruments however cover only part of the spectral range. Similarly Van Groenigen et al. (2003) reported that considering the less complicated sample preparation and availability of cheaper instruments for NIRS, it should be preferred over MIR.

Rossel et al. (2006) provided a review of various soil calibration studies done using different infrared regions. They reported that average  $r^2$  value for prediction of soil organic carbon and organic matter has been reported to be 0.96 with MIR data, 0.81 with NIR data and 0.78 with visible range. They however report that since MIR technology is more complex and expensive than NIR and visible range, there might be a trade off between accuracy of predictions and cost.



Most authors are of the view that IR calibration models are limited in their application to a new area which is different from the one for which the calibration was developed. Ingley and Crowe (2000) tried to predict SOC of 5 Saskatchewan fields and reported that for each field the best model was different in terms of number of regressors and reflectance wavelengths required which suggests that the models have to site-specific.

Van Groenigen et al. (2003) concluded that PLSR models are unique to the calibration site. PLSR models need to be calibrated for the site on which they will be used because of the effect of texture, mineralogical composition, organic matter content and other properties on model parameters. They however reported that once a calibration model is developed for a site, it can be used over different growing seasons.

Recently Brown (2007) tried to address the issue of model adaptation for a new study area. He built models constructed using stochastic gradient boosted regression trees (BRT) to predict soil organic carbon by augmenting global spectral library (4148 samples) with local calibration (418 samples). They found that SOC predictions improved with increasing number of local calibration samples added to the global spectral library. For the upland soils, with addition of just 20 local samples, RMSD dropped from 6.3 g/kg to 3 g/kg maybe due to the elimination of prediction bias. Addition of all local 206 samples dropped RMDS value to 2 g/kg. The author hypothesized that this could be due to the absence of highly weather soils in the global library. For dambo soils, with the addition of 40 local samples, RMSD dropped from 6.4 to 5.7 g/kg. With the addition of al local 206 samples, RMSD dropped to 5.3 g/kg.

When the author used just the local 206 samples for prediction, RMSD decreased to 1.9 g/kg for upland soils and increased to 5.8 g/kg for dambo soils. But for both sets a measure of ‘lack of accuracy due to bias’ and ‘non-unity regression line’ increased. These results made the authors believe that addition of diverse soil-spectral library to the local calibration can help in building robust models and minimize overfitting.

### **Research Objectives**

The main objective of this study is to assess the ability IR methods to determine SOC, identify the limitations of the methods and develop ways to overcome those limitations.

The specific objectives are:

- 1) To compare NIR and MIR techniques for SOC determination to
  - I. determine if there is an accuracy difference between MIR and NIR instruments,
  - II. evaluate if MIR calibration accuracy can be improved by eliminating some of the spectral range,
  - III. better understand and manage the impact of sample-set diversity on MIR multivariate calibration accuracy and efficiency, and
  - IV. determine if there is an efficiency difference between MIR and NIR instruments while building a calibration or updating a calibration.
- 2) To compare ATR and DR optical sampling techniques in the MIR region to determine SOC with respect to
  - I. SOC calibration performance
  - II. spectral basis of SOC calibration
  - III. potential for differentiation of SOM types and

IV. to evaluate potential advantages of ATR configuration.

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**CHAPTER 2**  
**PERFORMANCE OF LABORATORY-BASED DRY-SAMPLE INFRARED SOIL**  
**CARBON CALIBRATIONS IN LAND MANAGEMENT STUDIES**

## ABSTRACT

Previous studies have shown that both near (NIR) and mid infrared (MIR) spectroscopy in combination with multi-variate data analysis can be used to estimate soil carbon and other properties. However there are some inconsistent reports on the accuracy and efficiency of the two regions. In the present study calibrations were developed for three different types of soil management studies: single site single depth (SSSD), single site multi depth (SSMD) and multi-site multi depth (MSMD). Diffuse reflectance Fourier transform infrared (DR-FTIR) and diffuse reflectance-near infrared (DR-NIR) spectroscopy were used to determine SOC using partial least squares regression (PLSR). The accuracy of two methods as judged by root mean squares error of prediction (RMSEP) was similar for the less diverse SSSD and SSMD sets (RMSEP = 0.12 - 0.14). For the more diverse MSMD set error was smaller and correlation higher with the MIR region (RMSEP = 0.27) compared to NIR region (RMSEP = 0.31). SOC of samples from MSMD set were predicted with higher errors than those from SSSD and SSMD, which suggests that the errors associated with calibration models depend on the sample set structure. There is a potential to improve the model accuracy in the MIR region by selecting the most relevant spectral regions. Although we could get slight improvement in model accuracy for all sets by eliminating part of the spectrum that was not associated with SOC, we could not get good performing models based just on the organic bands alone. Calibration performance can also be improved by using more than one calibration model for a data set that had considerable diversity. Samples can be divided for separate calibrations either on the basis of known sample information (for example depth or site of sampling) or by performing un-supervised clustering. For the SSMD samples, we found



that developing separate models for upper and lower depths reduced error and number of factors needed for calibration.

In general when dealing with a single set of soils, MIR proved to be more efficient than NIR when expressed as the percentage of study samples that are not needed in calibration, and the efficiency of both ranges were better with less diverse data sets. Efficiencies of MIR models for SSSD, SSMD and MSMD were 79%, 69% and 60% respectively while that of NIR were 68%, 65% and 49% respectively. For extending an existing calibration to a new site, some samples from a new site need to be included in the updated calibration. For model adaptation we found that if the sample set for which the model was originally developed is diverse or is similar in mineralogy to the new study, fewer samples from the new set need to be included in calibration. Better predictions can be obtained by building a calibration model on some samples from that site instead of trying to extend an existing model especially in cases where the new site is more diverse or else is very different from the old site.

## **INTRODUCTION**

Infrared (IR) techniques have gained importance as rapid and inexpensive tool to characterize soils (Ben-Dor and Banin, 1995; Janik et al., 1998, Reeves et al., 1999, Reeves and McCarty, 2001; Shepherd and Walsh, 2002; Islam et al., 2003, Pirie et al., 2005). With increasing emphasis on site-specific agriculture and interest in global soil carbon monitoring, we need to have spatially intensive information on soil properties based on the analysis of a large number of samples, rather than a few composite samples which are unable to fully capture the variation due to soil heterogeneity. Many standard laboratory methods besides being costly and time consuming are irreproducible, un-

reliable and laborious (Malley et al., 1999). The cost of analytical methods usually limits the need to understand the spatio-temporal variability in soil properties which change with time and in space (Rossel and McBratney, 1998).

Infrared methods have several advantages over the standard methods and hence are a possible alternative. Infrared analysis needs very little sample preparation, is rapid, in-expensive, non-destructive and non-hazardous and has the ability to predict many soil properties simultaneously (Ben-dor and Banin, 1995). These advantages and a good predictive ability suggest that the IR technique might replace the standard laboratory methods (Rossel et al., 2006). The biggest advantage in using infrared spectroscopy is that the cost saving increases multiplicatively with each attribute analyzed (Cohen et al. 2007).

Being non-destructive, the technique also preserves the basic integrity of the soil sample in contrast with the chemical extraction methods which alter the equilibrium between various soil phases and hence complicate the interpretation of results. The chemical procedures done on complex non-ideal soil system can complicate the interpretation of analyses done to understand the link between measured soil properties (Islam et al., 2003).

Many of the IR studies have focused on determination of soil organic carbon (SOC) because it is an important soil attribute from both agricultural productivity and environmental quality perspectives. By virtue of the central role of SOC in many soil properties, good calibration performance with SOC may well translate to good performance for other soil parameters. Traditional methods of soil carbon determination have been associated with several limitations. The Walkley Black procedure (Walkley

1935) has been used as a standard for several years but it requires the use of a correction factor due to incomplete digestion of all C in the samples and the correction factor might be different for different soils (Rosell et al. 2001). Dry combustion method (Nelson and Sommers, 1996) is another standard method that determines soil C by measurement of CO<sub>2</sub> released from the oxidation of organic C and thermo decomposition of carbonates. This method is associated with high cost and in some cases, C determination is based on the weight change instead of measuring the CO<sub>2</sub> emitted which causes discrepancies in the results from different laboratories (Kimble et al., 2001). Loss on ignition is another method that is used to estimate SOC. The method usually overestimates the SOC because the weight loss in the procedure is not only because of the organic matter but also clays and other inorganic constituents. LOI values obtained are affected by sample size and temperature (Cambardella et al., 2001). Total carbon measured by some methods will be equal to the organic carbon if there is negligible carbon in inorganic form. However if the soil has limestone, dolomite or other carbonate containing minerals, inorganic forms might be in significant amount. In that case distinction has to be made between organic and inorganic carbon by burning off inorganic carbon by acid treatment to measure total OC. Inorganic carbon is then determined by subtraction of OC from total carbon.

With increasing interest in carbon sequestration and its effect on global carbon flux, methods for determining soil carbon have gained importance. Trading of carbon credits and C-sequestration incentive programs will be reliant on methods that can quantify C at relatively in-expensive way for a large area (Gehl and Rice, 2007). Carbon management at regional and global level requires efficient methods of C assessment. For

this reason an increasing number of studies are exploring infrared spectroscopy as an alternative method.

### **Utilization of NIR and MIR for SOC analysis**

The infrared region of the spectrum includes radiations with wavelengths ranging from 0.78 to 1000  $\mu\text{m}$ . From an instrument and application point of view, the infrared spectrum is conventionally divided into near (NIR), mid (MIR) and far-infrared (Table 2.1). Infrared techniques are sensitive to both organic and inorganic phases of the soil. NIR and MIR ranges of infrared spectrum have been used for soil carbon quality and also quantification studies (Sudduth and Hummel, 1993; Ben-Dor et al., 1997; Janik et al., 1998; Reeves et al., 1999; McCarty and Reeves, 2006; Islam et al., 2003). Both MIR and NIR spectroscopic determinations require development of calibration models that relate the spectral information to the soil property of interest (soil carbon) using multivariate data analysis techniques such as partial least squares regression (PLSR) (Workman, 1992).

Region	Wavelength ( $\mu\text{m}$ )	Wavenumber ( $\text{cm}^{-1}$ )
Near	0.78-2.5	12800-4000
Middle	2.5-50	4000-200
Far	50-1000	200-10

Table 2.1: Different infrared spectral regions

There are some technical differences between MIR and NIR instrumentation and spectral phenomena which can translate to differences in the performance of these two techniques. The MIR range has more spectral features and probably better quality information than NIR range. NIR is popular due to simple sample preparation procedures.

MIR requires much less sample than NIR (few mg versus a few grams). This is because of the differences in the way in which the light interacts with the sample and also because of the instrumentation. In studies at small scale level (for example: next to roots) where it is difficult to get large amount of sample, this characteristic gives MIR an advantage over NIR. Generally the NIR technique is less complex and expensive than MIR and also there is an availability of portable NIR spectrometers for “on the go” field use (Rossel et al., 2006).

While NIR has been used for quantitative analysis of organic matter, organic carbon or total carbon for decades (Baumgardner et al. 1970, Krishanan et al. 1980, Dalal and Henry 1986), early work on MIR of soil focused on qualitative studies because of the belief that salt dilution is required to avoid spectral distortions resulting from strong absorptions (Culler 1993), a problem not faced in NIRS analysis because of weak absorbances (Reeves 2003). Niemeyer et al. (1992) reported that DRIFT-MIR technique is not suitable for quantification studies because of its high sensitivity to homogeneity of the soil material used in the analysis. More recent studies however showed that MIR can be used for the quantitative analysis of neat soil samples (Janik et al., 1998; Reeves et al., 2001; McCarty et al., 2002; Reeves et al., 2002).

### **Inconsistencies in NIR and MIR SOC calibration performances**

There are some inconsistent reports about the accuracy of the two infrared techniques and whether the performance is affected by the diversity to sample set. In most calibration studies the figures of merits used are (1)  $r^2$  (coefficient of determination) which is a measure of total variance accounted for by the model, (2) SEP/SEC (standard error of prediction/calibration) which is the standard deviation of the difference between

measured and estimated values for samples from validation/calibration sets and (3) RPD (residual prediction deviation) which is the ratio of standard deviation of measured values of the soil attribute in the prediction set to the SEP. McCarty et al (2002) compared NIRS and MIRS with respect to their ability to quantify total, organic and inorganic C in samples representing 14 soil series collected over a large region in west central US, and found that MIR was more accurate than NIR. They also concluded that MIRS is more robust for developing calibration for widely diverse soils. McCarty and Reeves (2006) reported that MIR was superior to NIR in the estimation of various soil properties including organic carbon, total N and texture. Pirie et al. (2005) compared UV-VIS-NIR and MIR spectroscopic techniques to predict OC of 415 surface and sub-surface soil samples from Australia. They found that the relative prediction accuracy in terms of RPD values was higher for the models using the MIR spectral data compared to other regions. Using a separate validation set approach they obtained an  $r^2$  of 0.85 and SEP of 0.55 and RPD of 2.6 with the MIR spectra compared to the UV-NIR-VIS model for which the  $r^2$ , SEP and RPD values were 0.76, 0.50 and 2.0 respectively. McCarty et al. (2002) hypothesized that the possible reason for better MIR accuracy was the presence of well defined organic molecules absorption features in the MIR range compared to the NIR range. On the other hand, Madari et al. (2006) reported a good correlation ( $r^2 = 0.99$ ) for determination of C using NIRS which was higher than obtained with MIR ( $r^2 = 0.93$ ). They however reported that these results were not easily explainable. Van Groenigen et al. (2003) while assessing the potential of NIR and MIR for predicting crop and soil parameters in a flooded California rice field concluded that NIR has better predictive ability than MIR.

There is less data on the relative efficiency of the two infrared techniques probably because figures of merits and experiments for assessing calibration efficiency have not been clearly defined. We envision two basic cases of efficiency (1) the minimum size of an effective calibration set for a study and (2) the minimum number of samples that have to be included in the calibration to effectively adapt an existing model to a new set of soils. Poor efficiencies in spectral instrumentation or calibration algorithms may underline inadequate observed performance. More efficient technique is the one which is less costly and easier to apply to the soil management studies. The efficient technique will also have lower risk of erroneous results due to overfitting. Udelhoven et al (2003) were not able to get reliable C estimations using NIRS technique with PLS regression and concluded that development of statistical prediction models should be limited to geologically homogeneous areas. Van Groenigen et al. (2003) reported that the calibration models are unique for specific agro-ecosystems and a new site might require a new calibration. Madari et al. (2006) tried to adapt a calibration model developed from un-fractionated bulk samples (BS) for prediction of the carbon content of aggregate stable fractions (ASF) of soils. Using only BS to predict C of ASF resulted in poor  $r^2$  because of the additional variability brought in when the samples were fractionated. Adding just 35 samples from ASF resulted in improvement of  $r^2$  from 0.74 to 0.90 using MIRS and from 0.58 to 0.87 using NIRS.

**Objectives of present study:**

The main focus of this investigation is to evaluate the ability of DR-IR spectroscopy to characterize SOC in three different types of soil management studies. More specifically the objectives are: 1) to determine if there is an accuracy difference

between MIR and NIR instruments, 2) to evaluate if MIR calibration accuracy can be improved by eliminating some of the spectral range, 3) to better understand and manage the impact of sample-set specimen diversity on MIR multivariate calibration accuracy and efficiency, and 4) to determine if there is an efficiency difference between mid- and NIR instruments while building a calibration or updating a calibration.

## **MATERIAL AND METHODS**

### **Site description and soil sampling**

In order to make the conclusions more generally applicable, we utilized soil specimens from three representative types of agricultural land management studies (Table 2.2). Tables 2.3 and 2.4 provide information on soil series and parent materials of soils from the three study sites. The single site single depth study (SSSD) samples were collected from a single trial site under a long-term crop rotation by fertility trial and from a single depth of 0-20 cm. The trial also called the Hunter Rotation Experiment (HRE) is located at The Pennsylvania State University R.E. Larson Agronomy Research Center in Rock Springs, PA (Figure 2.1 (a)). The soil type for this experiment started in 1969 is Hagerstown silt loam which is characterized as well drained and agriculturally productive soil (Braker, 1981). The experiment consisted of four crop rotations and three fertility types in 4 replicated blocks for a total of 192 plots of 5.76 m width and 12.8 m length (Mirsky, 2008). The experiment is randomized complete block split plot design with fertility treatments as main plots, cropping systems as subplots. Every crop of a rotation is grown every year and the whole system is under conventional moldboard plow tillage. For SSSD study, 192 samples were collected in spring 2004. Eight cores were taken from each plot to a depth of 20 cm and mixed together to form a representative set.



Study	n	Sampling depth (cm)	Sampling year	Reference method
SSSD	192	20	2004	Loss on ignition*
SSMD	240	0-5,5-20,20-100	2004	Combustion
MSMD	178	0-10,10-20,20-40	1998,1999,2000	Combustion

Table 2.2: Description of three sample sets used for the study. (\*Organic matter values were converted to organic carbon by the equation described in method section)

For the second soil management study, called the single-site multi-depth (SSMD), samples were collected from a single trial site under a long-term reduced tillage trial and from three depths of 0-5, 5-20 and 20-100 cm. The trial is also located at Fry-K in Agronomy Research Farm at Rock Springs, PA (Figure 2.1 (b)). The experiment was initiated in 1978 on 7.7 hectares of the soil that has been characterized as Hagerstown-Hublersburg-Opequon complex (Braker, 1981). The experiment has been in corn cultivation since 1978 except for the years 1986 when it was under oats, 1993 when it was under half corn and half soybeans and 1994 when it was under soybeans. The experiment is laid out in a randomized complete block design with four replications and 6 types of tillage as the main treatment. For the SSMD, 240 samples were collected in summer of 2003 from the four tillage treatments from depths of 0-5 cm, 5-20 cm and 20-100 cm and three landscape positions: shoulder, sideslope and deposition. Five sampling positions were selected in each plot to represent tillage treatment and landscape positions. For the five sampling points selected in each plot, two 3.4 cm diameter cores were taken to a meter depth within half meter of each other. Cores were split at 0-5, 5-20 and 20-100 cm depth and both cores were composited to form one sample per position per depth.

Study	Soil Series	Parent material	Taxonomic classification
SSSD	Hagerstown	Limestone residuum	Fine, mixed, semiactive, mesic Typic Hapludalfs
SSMD	Hagerstown	Limestone residuum	Fine, mixed, semiactive, mesic Typic Hapludalfs
	Hublersburg	Limestone residuum	Clayey, illitic, mesic Typic Hapludults
	Opequan	Limestone or dolomite residuum	Clayey, mixed, active, mesic Lithic Hapludalfs

Table 2.3: Soil series, parent material and taxonomic classification of soils sampled in SSSD and SSMD studies. (Soil Survey Staff)

For the third set, called the multi-site multi depth study (MSMD), samples were collected from a biosolid treatment study from 20 different sites in 18 counties of Pennsylvania and three different depths of 0-10, 10-20 and 20-40 cm (Figure 2.2) (Shober et al., 2003). Agricultural soils with a history of biosolids application were identified. Also at every site, similar agricultural fields were selected which received no biosolids application. For the MSMD, samples were collected in the fall of 1998, 1999 and 2000. Five locations were sampled in each field at a depth of 0-10, 10-20 and 20-40 cm. These five samples were then composited for each depth to produce one sample per field per depth.

For this particular study we had 192 samples from SSSD, 232 samples for SSMD and 274 samples from MSMD for our final analysis.

Site	Soil Series	Parent material	Taxonomic classification
1	Wharton	Shale, siltstone & sandstone residuum	Fine-loamy, mixed, active, mesic Aquic Hapludults
2	Gilpin	Shale, siltstone, and sandstone residuum	Fine-loamy, mixed, active, mesic Typic Hapludults
	Athol	Triassic conglomerate or breccia residuum	Fine-loamy, mixed, active, mesic Ultic Hapludalfs
3	Hublersburg	Limestone residuum	Clayey, illitic, mesic Typic Hapludults
4	Hagerstown	Limestone residuum	Fine, mixed, semiactive, mesic Typic Hapludalfs
5	Allenwood	Pre-Wisconsinan acid sandstone & shale glacial till	Fine-loamy, mixed, semiactive, mesic Typic Hapludults
6	Duffield	Limestone residuum	Fine-loamy, mixed, active, mesic Ultic Hapludalfs
7	Hagerstown	Limestone residuum	Fine, mixed, semiactive, mesic Typic Hapludalfs
8	Weikert	Shale, siltstone & sandstone residuum	Loamy-skeletal, mixed, active, mesic Lithic Dystrudepts
9	Bedington	Siltstone, shale, sandstone residuum	Fine-loamy, mixed, active, mesic Typic Hapludults
	Hagerstown	Limestone residuum	Fine, mixed, semiactive, mesic Typic Hapludalfs
10	Edom	Limestone & shale residuum	Fine, illitic, mesic Typic Hapludalfs
11	Bedington	Siltstone, shale, sandstone residuum	Fine-loamy, mixed, active, mesic Typic Hapludults
12	Manor	Micaceous schist residuum	Coarse-loamy, micaceous, mesic Typic Dystrudepts
13	Berks	Shale, siltstone & sandstone residuum	Loamy-skeletal, mixed, active, mesic Typic Dystrudepts
14	Washington	Limestone & granitic gneiss colluvium	Fine-loamy, mixed, semiactive, mesic Ultic Hapludalfs
15	Berks	Shale, siltstone & sandstone residuum	Loamy-skeletal, mixed, active, mesic Typic Dystrudepts
16	Watson	Sandstone, siltstone & shale glacial till	Fine-loamy, mixed, active, mesic Typic Fragiudults
	Shelmadine	Pre-Wisconsinan acid sandstone & shale glacial till	Fine-loamy, mixed, semiactive, mesic Typic Fragiaquults
	Volusia	Siltstone, sandstone & shale or slate till	Fine-loamy, mixed, active, mesic Aeric Fragiaquepts
17	Hagerstown	Limestone residuum	Fine, mixed, semiactive, mesic Typic Hapludalfs
18	Elliber	Shale, siltstone, chert & limestone residuum	Loamy-skeletal, mixed, semiactive, mesic Typic Hapludults
	Glenelg	Micaceous schist residuum	Fine-loamy, mixed, semiactive, mesic Typic Hapludults

Table 2.4: Soil series, parent material and taxonomic classification of soils sampled at different sites in MSMD study (Shober et. al, 2003 and Soil Survey Staff)

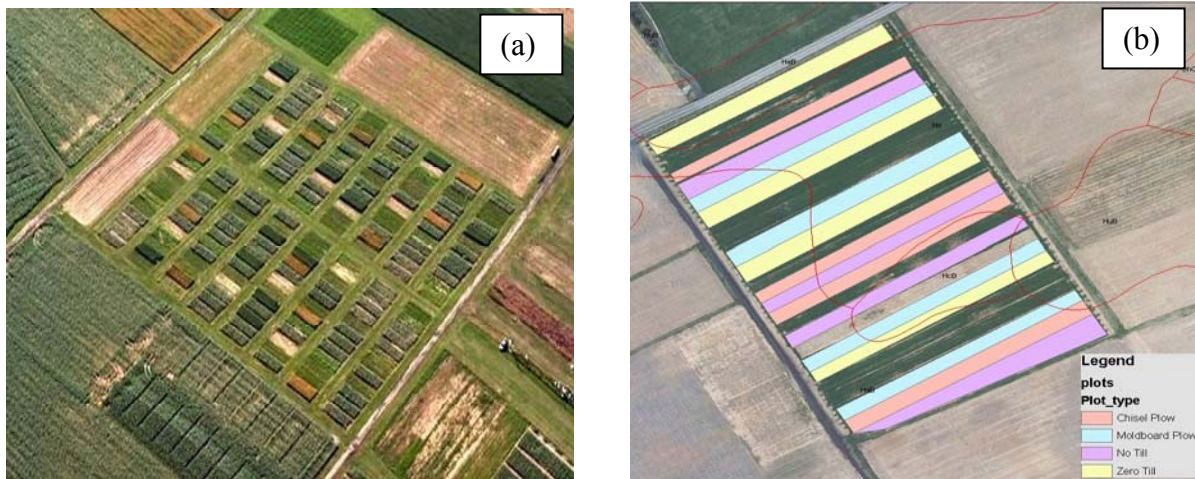


Figure 2.1: Aerial picture of (a) SSSD study trial (Hunter Rotation Experiment) (b) SSMD study trial (tillage trial experiment)

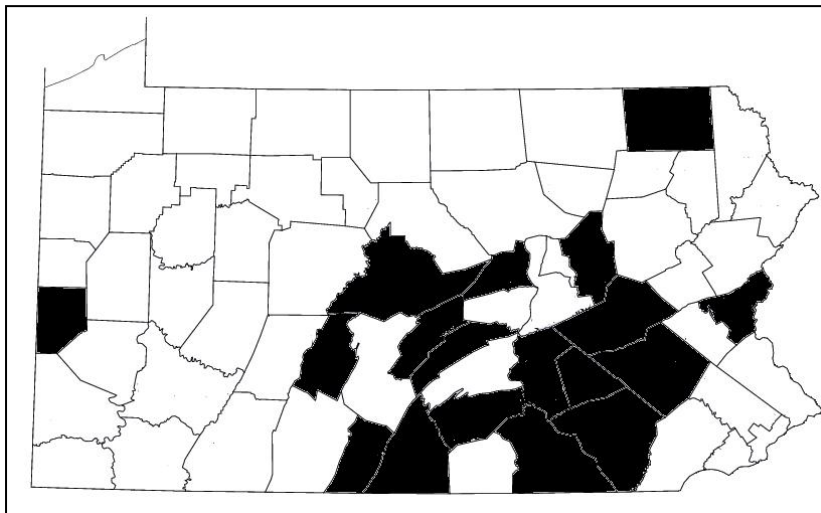


Figure 2.2: Location of sampling sites in 18 counties of Pennsylvania of MSMD study

### Chemical Analyses

For the SSSD soils, soil organic matter (SOM) was determined by weight loss on ignition method (Schulte, 1995). The procedure involved oven drying 5-10 g of 2 mm sieved samples at 105°C followed by heating at 360°C. To make units of SSSD comparable with other sets SOM values were converted to SOC values by using a

regression equation developed on a sub-set of SSSD soils ( $n = 10$ ,  $r^2 = 0.96$ ,  $SE = 0.07$ ) (APPENDIX A). For SSMD, SOC was determined by dry combustion method using Carlo-Erba C-N Analyzer (Nelson and Sommers, 1996). For MSMD study, total carbon and inorganic carbon values were obtained using a Shimadzu TOC-5000A Total Organic Carbon Analyzer with the SSM-5000A Solid Sample Module. Organic carbon was determined by subtracting acid dissolvable inorganic carbon from the total carbon.

#### **MIR peaks identification experiment:**

To identify which MIR peaks are associated with organic and inorganic components, a small study was conducted using samples from SSSD data set (APPENDIX C). Some organic and inorganic compounds (including humic acids, pepsin, agar, silica gel, gibbsite) that are known to be found in soils were mixed with the soil sample at levels found in natural soils and the spectra thus obtained were compared with the spectrum of neat soil. Enhancement of any neat soil peak when a particular compound is added would suggest an association between that peak and the compound.

#### **Spectral data acquisition**

Air dried samples (< 2 mm) were scanned at 2 nm intervals in 1100-2498 nm range to obtain the NIR-reflectance measurements. The soil samples were packed in quartz windowed 2 inch minicups and scanned manually using a NIR Systems 6500 with Unitran sampling accessory and ceramic reference. The reference material used in this case was alumina. For mid-infrared analysis, dry screened samples (< 2 mm) were passed through a 250  $\mu\text{m}$  screen and were scanned from 6998 – 299  $\text{cm}^{-1}$  at 6  $\text{cm}^{-1}$  interval using a Brukers FTIR with La-digs detector and Pike Technologies XY-autosampler for 96-

well plate. Aluminum mirror was used as a reference material. Duplicate measurements were taken in the MIR range but single measurements in NIR.

### **Statistical Analyses**

The multivariate calibrations were calculated using MATLAB version 7.5 (The Mathworks, Inc., Natick, MA) or Unscrambler software version 7.6 (CAMO, U.S.A). Our chemometric approach involves the use of numeric factor analysis Partial Least Squares Regression (PLSR) to extract information from spectral data that relates to a property measured within a population of training samples. PLSR is a multivariate calibration method that can decompose spectral data (X) and soil property data (Y) into a new smaller set of latent variables and their scores that best describe all the variance in the data (Haaland and Thomas, 1988 and Wold et al. 2001). Spectral data pretreatment was optimized by testing the performance of various transformation methods like normalization, baseline correction, multiplicative scatter correction, derivatives, standard normal variate correction and various combinations of these transformations to correct undesirable spectral distortion due to scattering and other environmental factors. Two different approaches can be used to build calibration models. One involves the use of separate samples for building (calibration set) and validating the calibration models (test set or validation set). Performance of models in this case is checked by coefficient of correlation and Root Mean Square Error of Prediction (RMSEP) which is an estimate of average difference between predicted and measured response. In the second approach an internal validation system is used and there is no separate test set. The original samples are partitioned into K subsets and from these a single subset is retained as the validation set for testing the model, and the remaining K – 1 subsets are used as calibration set. The

cross-validation process is then repeated K times with each of the K subsets used once as the validation data. The results from K subsets can be averaged to get root mean square error of cross validation (RMSECV) which is equivalent to RMSEP computed in test-set approach. Results of cross validation approach are usually over-optimistic and hence a test-set approach is more appropriate. For all the three studies, a test set approach was used in which 1/3 samples selected randomly were used in the validation set and the remaining two thirds for calibration (Table 2.5). Cross validation models were developed using calibration set samples which is composed of duplicate samples in DR-MIR range and singles in case of DR-NIR range. For deciding on the best transformation and number of factors, we compared RMSECV of different models developed using different transformations and different number of factors. To avoid the overfitting of data by using too many factors, we selected a higher factor model only when the RMSECV improvement was more than 0.005% (which is probably less than 1/10 the standard error of repeatability of the SOC reference method). After deciding on the optimum transformation and number of factors, a calibration model was developed using the calibration samples, to predict SOC values of averaged (in cases where duplicates were available) or un-averaged (when no duplicates were available) validation samples. Regression vectors obtained using the chosen transformation and number of factors was screened for excessive noise. The RMSEP,  $r^2$  and slope values for the validation set were reported. The best calibration is the one which attains highest  $r^2$  and lowest RMSEP. For same value of RMSEP, we preferred a simple transformation and regression vector that seemed to be based on spectral signature for SOC rather than random noise. A soil sample was considered to be an outlier if the difference between its predicted and

reference value was 3 times larger than the RMSEP for rest of the samples (Chang et al. 2001, Islam et al., 2003).

Clustering algorithm available in MATLAB was used to group samples from the three data sets using the MIR and NIR data to understand how different the samples were for each study based on the spectral features in the two regions (Figure 2.7 and 2.8). To accomplish this we applied a relatively standard form of data-preprocessing that reduces the weighting of baseline offset and broad peaks. MIR data between 3900-1240  $\text{cm}^{-1}$  and NIR data from 1100-2498 nm was transformed using standard normal variate (SNV) followed by a SG first derivative transformation (11 points, quadratic fit). We used average distance and correlation as linkage method in our clustering algorithm.

### **Model Efficiency**

To determine the efficiency of a model, we determined the number of calibration samples needed to predict the remaining samples with prediction error within 125% of the minimum error that could be achieved with the best calibration model for the set. Using our preferred preprocessing we started with 10 random samples in calibration to predict the remaining samples of a set and kept adding samples in the calibration at an increment of 10 to observe the effect on prediction error.

### **Model adaptability**

To evaluate the efficiency of the models in terms of adaptability, for all the three data sets and two infrared ranges, we tried to use models developed on one study to predict SOC of samples from other study. We started with no samples in calibration from the new study and kept on adding samples from the new set at an increment of 10 to observe how many samples are needed from the new set to predict the remaining samples



with prediction error within 125% of the minimum error that could be achieved with the best calibration model for this set.

## **RESULTS AND DISCUSSION**

### **MIR and NIR spectra**

Although soil carbon calibration models can be developed on infrared data without much knowledge of spectroscopy, understanding the spectral basis of calibration should enhance efforts to achieve efficient and robust calibrations. There is widespread expectation that a calibration model which isolates the purest spectral signature of the property of interest will be more robust compared to the one which includes spurious sources of variation. By using the most important and informative spectral regions or excising uninformative range, calibration models may stand better chance of being successfully applied to new samples.

NIR and MIR are the two ranges of infrared spectrum which have been used for quantification of SOC. There are some distinct differences in the NIR and MIR spectra of the soil. Figure 2.3 shows a typical spectrum of soil in the NIR and MIR regions. Soil spectrum in the MIR region contains more spectral features due to both organic and inorganic components with higher absorption values than the NIR spectrum (Pirie et al., 2005; McCarty et al. 2002). Neat soil spectra often get distorted in some frequency regions of mid-infrared range especially at  $1100\text{ cm}^{-1}$  (Brimmer and Griffiths, 1987). For this reason, a true absorption spectrum was obtained by salt dilution technique (4% soil in powdered KCl, reference = powdered KCl) (APPENDIX B) and is shown in Figure 2.3 (a). Except for the peak distortion near  $1100\text{ cm}^{-1}$ , rest of the neat soil spectrum has same features as the soil spectrum diluted with salt. For SOC calibration purpose we used neat

undiluted soils because it is more convenient. The peak assignments for the NIR and MIR regions are provided in the tables 2.6 and 2.7.

Study	OC (%)							
	Calibration set				Validation set			
	n	Mean	SD	Range	n	Mean	SD	Range
SSSD	144	1.55	0.22	0.97-2.48	48	1.55	0.23	1.22-2.39
SSMD	174	1.22	0.72	0.12-3.08	58	1.25	0.71	0.19-3.10
MSMD	204	1.58	0.85	0.31-6.24	68	1.55	0.85	0.28-5.17

Table 2.5: Descriptive statistics on organic carbon values obtained by the reference method for calibration and validation sets of three studies (n = number of sample, SD = standard deviation)

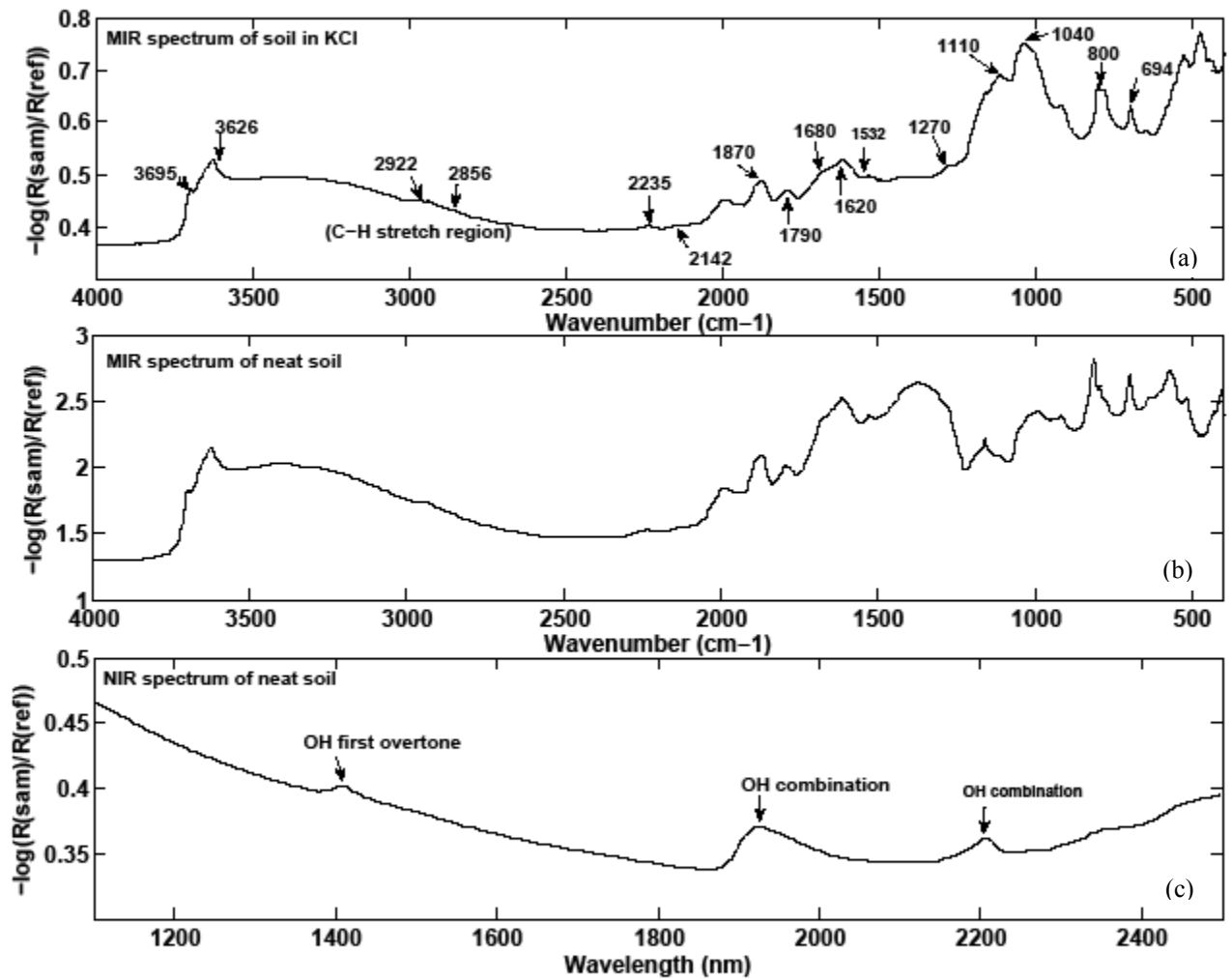


Figure 2.3: Salt diluted (a) and undiluted (b) DR-MIR soil spectrum are dominated by several organic and inorganic peaks while DR-NIR soil spectrum (c) has fewer broad bands

Wavenumber (cm <sup>-1</sup> )	Group Assignment	Source
3695	Kaolinites	Nguyen et al., 1991
3626	Smectites and Illites	Nguyen et al., 1991
1010	Alumino-silicate	Nguyen et al., 1991
694, 800	Clay and Quartz	Nguyen et al., 1991
1870, 1790	Quartz	Nguyen et al., 1991
1680 and 1532	Amides	Rumpel et al., 2001
1790	Aromatic lignites	Rumpel et al., 2001
1530 and 1270	Phenols	Baes and Bloom, 1989
1270 and 1620	Carboxylic acids	Stevenson, 1994
2235 and 2142	Carbohydrates	Rumpel et al., 2001
2922 and 2856	Aliphatics	Madari et al., 2006

Table 2.6: Peak assignment for MIR soil spectrum

Wavelength (nm)	Group Assignment
1400	O-H stretch first overtone
1900	O-H stretch, water bend combination
2200,2300	O-H stretch, metal-OH bend combination

Table 2.7: Peak assignment for NIR soil spectrum (Hunt, 1977; Clark, 1999)

Regarding the quality of organic signal for SOC in the MIR region, there are distinct OM bands (e.g. the C-H stretch region) (Figure 2.4 a). There are clear differences in the spectra of low and high OC samples. Thus the MIR soil spectrum contains some

identifiable peaks which can be used for the spectral interpretation and at least distinguishing samples which are high from those which are low in carbon content.

The soil spectrum in the NIR region is relatively featureless showing three or four low intensity bands which most likely reflect the hydroxyl functional groups (Siebielec et al., 2004). NIR spectra of high and low organic carbon soils look almost the same (figure not shown). However the derivatized NIR spectra of high and low carbon soil show some significant differences in spectral intensity (Figure 2.4 b). The NIR soil spectrum makes any kind of spectral interpretation very difficult in the absence of some kind of transformation and multi-variate analysis. The O-H signal from clays and silicon become stronger with more SOM. So the differences in intensity are more due to optical effect rather than absorption effect. Thus due to change in mineral O-H bands caused by optical effects and serious overlap of mineral and OM bands, spectral interpretation becomes a difficult task in the NIR range.

Based on these differences a reasonable approach for MIR region is to identify the organic peaks and use them for the calibration model development. Identifying the organic peaks in the NIR region and using them for calibration would be more challenging.

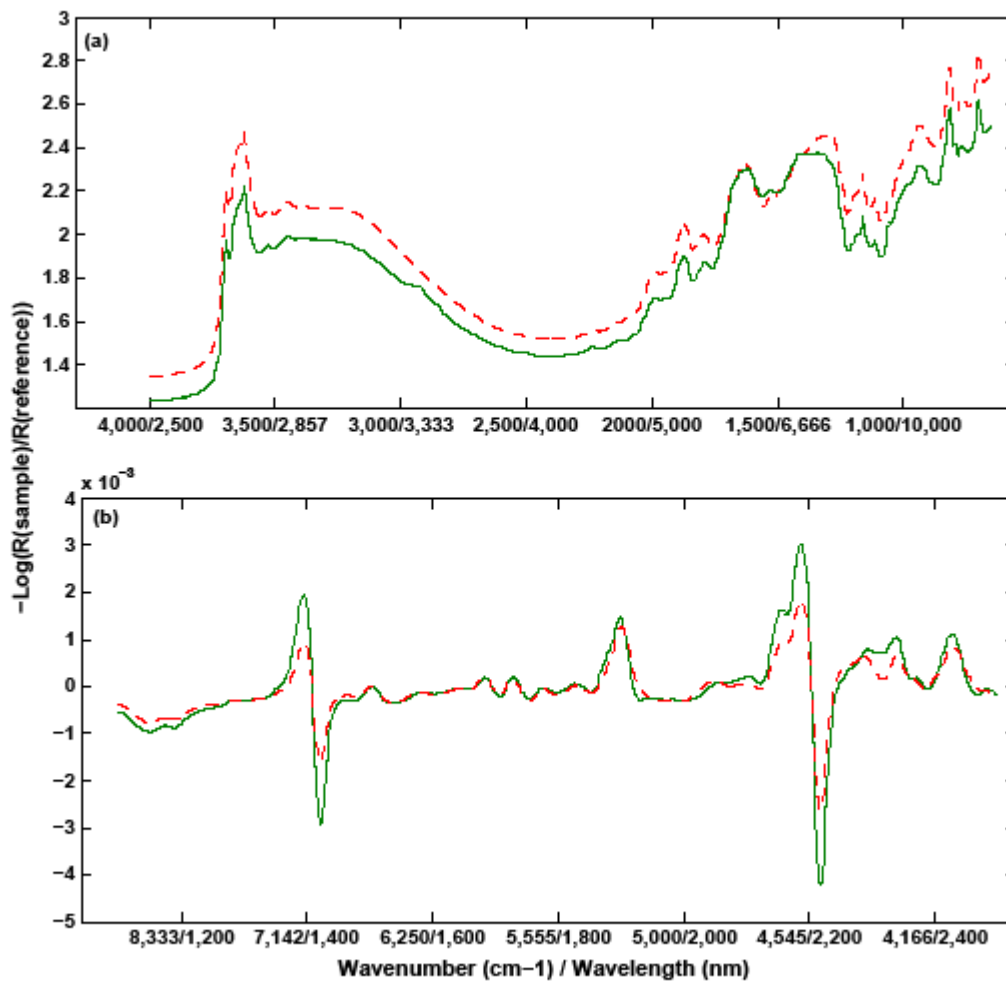


Figure 2.4: (a) MIR spectra of two soils high in OC (red) and low in OC (green) and (b) derivatized spectra of same samples in the NIR region

The results of the experiment done to identify MIR peaks (APPENDIX C) showed that organic peaks are present in the regions  $2938, 2853 \text{ cm}^{-1}$  (C-H stretch region) and also in the region  $1620 \text{ cm}^{-1}$  which has been reported to be associated with C=O stretching (Figure. 2.5 a and b). Broad bands around  $3400\text{-}3200$  and  $2800\text{-}2500 \text{ cm}^{-1}$  which are associated with water of hydration and acid groups respectively were also enhanced by addition of organic compounds. The organic bands in the range  $2500\text{-}1800 \text{ cm}^{-1}$  were weak or non-existent. Part of the spectral range was lost due to strong

absorption of neat soil (1300-1000, 750-300  $\text{cm}^{-1}$ ). Figure 2.5 (c) shows that the organic and inorganic peaks are not very easy to separate in the complex and non-ideal soil matrix. Also in these soils containing  $< 5\%$  SOC, the natural level of SOM will yield only a weak organic signature compared to the inorganic signature.

This experiment suggests that while it might be advantageous to remove some parts of the spectra which are featureless or are due to instrument artifacts, it will be very difficult if not impossible to base the calibration just on the organic region of the spectra. The way in which the inorganic and organic components of the soil interact with each other will be different in soils bringing in the variation in the calibration basis. We tested different MIR spectral ranges (4000-400, 3900-400, 3900-640, 3900-1080, 3900-1284, 3900-1240) to find out which range is most suitable for building C-calibration models.

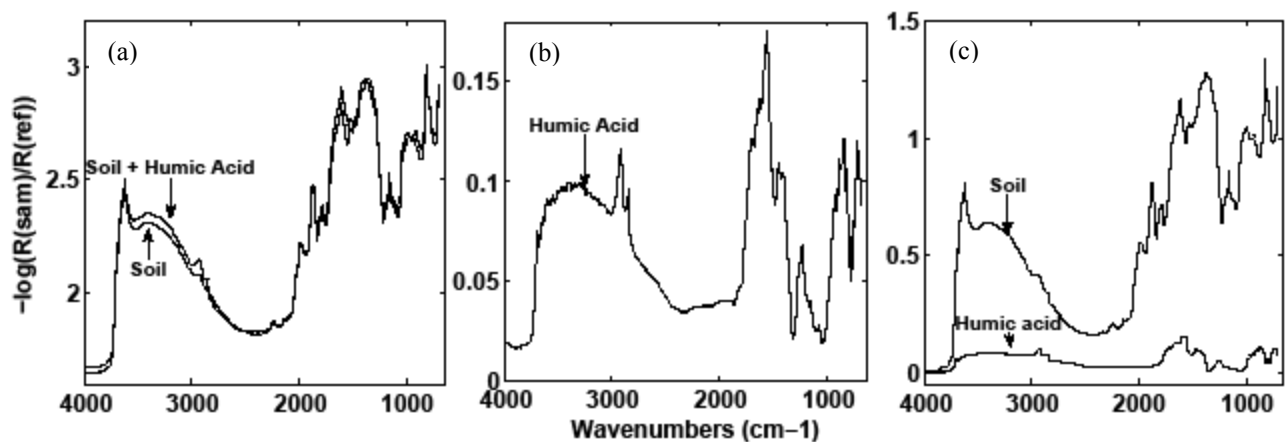


Figure 2.5: (a) Soil and Soil + HA spectra (b) Humic acid spectrum obtained by subtracting soil spectrum from soil + humic acid spectrum (c) Offset corrected soil and Humic acid spectra.

### **Structure of data-sets**

The structure of spectral data-sets generated by three soil management studies is another factor that plays an important role in developing calibration models and affects their performance. In soil-crop management studies, samples are collected with different objectives in mind. In the present study SSSD samples were collected from a long-term crop rotation by fertilization trial with an objective to study how soil quality is altered by these practices. Samples were collected from single site and single depth (20 cm). For SSMD study, the main objective was to observe differences in the soil properties particularly the pattern of carbon storage due to one area versus landscape position that are brought by the application of reduced tillage treatments. Samples were taken from a single site but three different depths (0-5 cm, 5-20 cm and 20-100 cm). The MSMD study used paired sites to see the effects of land application of biosolids on soil properties at various tillage zones of agricultural soils throughout PA. Samples were taken from 20 different sites across Pennsylvania and from three different depths (0-5 cm, 5-10 cm and 10-20 cm). The design of these studied data sets is different in terms of diversity of samples. The samples from SSSD study are more likely to be similar in mineralogy than the SSMD samples with samples from MSMD being most diverse as supported by information in Tables 2.3 and 2.4 and APPENDIX D. Also the ranges of SOC in the three data sets are different with MSMD having a wider range of OC and SSSD the narrowest (Table 2.5 and Figure 2.6).

Histograms showing the distribution of OC suggest that it is unimodal for the samples from SSSD but increasingly bimodal going from MSMD to samples from SSSD. This is primarily due to very different OC contents at the top depths compared to the



lower depths. Considering the variation in sample sets brought about by sites, depth of sampling and organic C-ranges, it was expected that the infrared spectral sets would also differ and impact the calibration model performance.

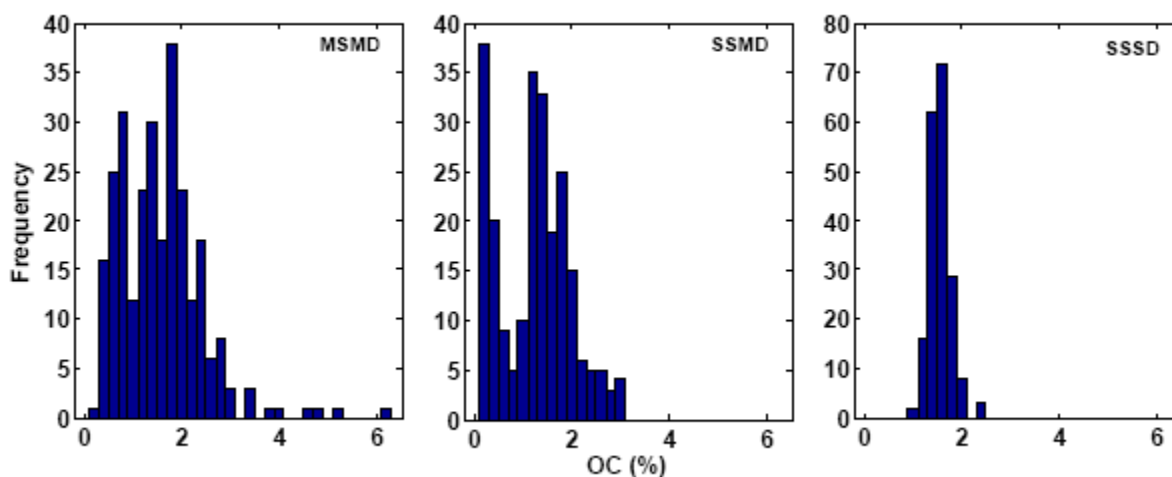


Figure 2.6: Distribution of OC content of the specimens from the three studies

Results of the cluster analysis revealed that the largest branch clustering of MSMD with both MIR and NIR spectral data was the site of sampling. For the MSMD samples, there were three main clusters in the data based on DR-MIR data. Cluster 1 had samples from just 2 sites, cluster 2 had samples from 5 sites and cluster 3 contained samples from 10 sites (Figure 2.7 (a)). Samples from the remaining three sites shared membership in cluster 2 and 3. Further analysis of averaged spectra of samples belonging to each of the clusters revealed that the most obvious difference is in the average spectrum of cluster 1 and cluster 2 and 3 in the region of  $3695\text{ cm}^{-1}$  which is mainly associated with the mineral hydroxyl peak (Figure 2.9). With the NIR data on MSMD study, samples were divided into four clusters. The main branch clustering still seemed to be by the site of sampling but not as clearly as for the MIR data. Cluster 1 had samples

from 2 sites, cluster 2 from 1 site, cluster 3 from 5 sites and cluster 4 consisted of samples from 5 sites. The remaining 7 sites shared membership among two or more clusters.

In case of SSMD samples, the main branch clustering was distinctly a result of the depth of sampling for both MIR and NIR data. Samples were divided into two main clusters with the first cluster containing samples from depth 3 and second cluster containing samples from depth 1 and depth 2. The averaged preprocessed MIR spectra of samples belonging to each of the cluster are shown in the figure 2.10. The spectral patterns were similar and the most obvious differences were the greater hydroxyl band intensities ( $3700\text{ cm}^{-1}$ ) in depth 3 (cluster 1) and small but important increase in C-H band intensities ( $2800\text{-}2900\text{ cm}^{-1}$ ) of cluster 2 (depth 1 and 2) is in the average spectrum of cluster 1 and 2 which is associated the C-H stretch. In the case of SSSD samples, we could not identify a single sample characteristic that explained the two main branches of the MIR dendrogram (Figure 2.7 (c)).

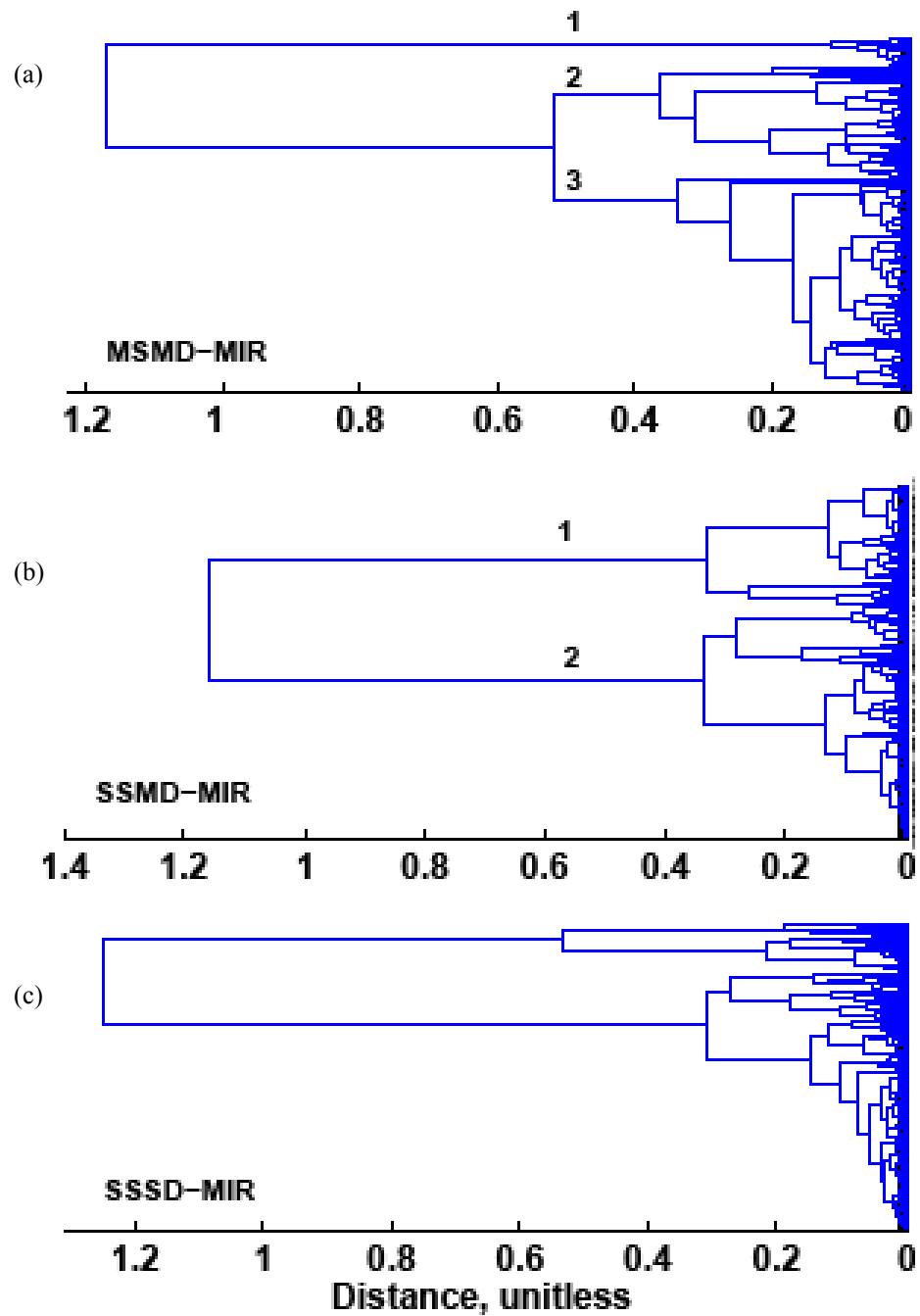


Figure 2.7: Cluster analysis performed on three data sets using MIR region

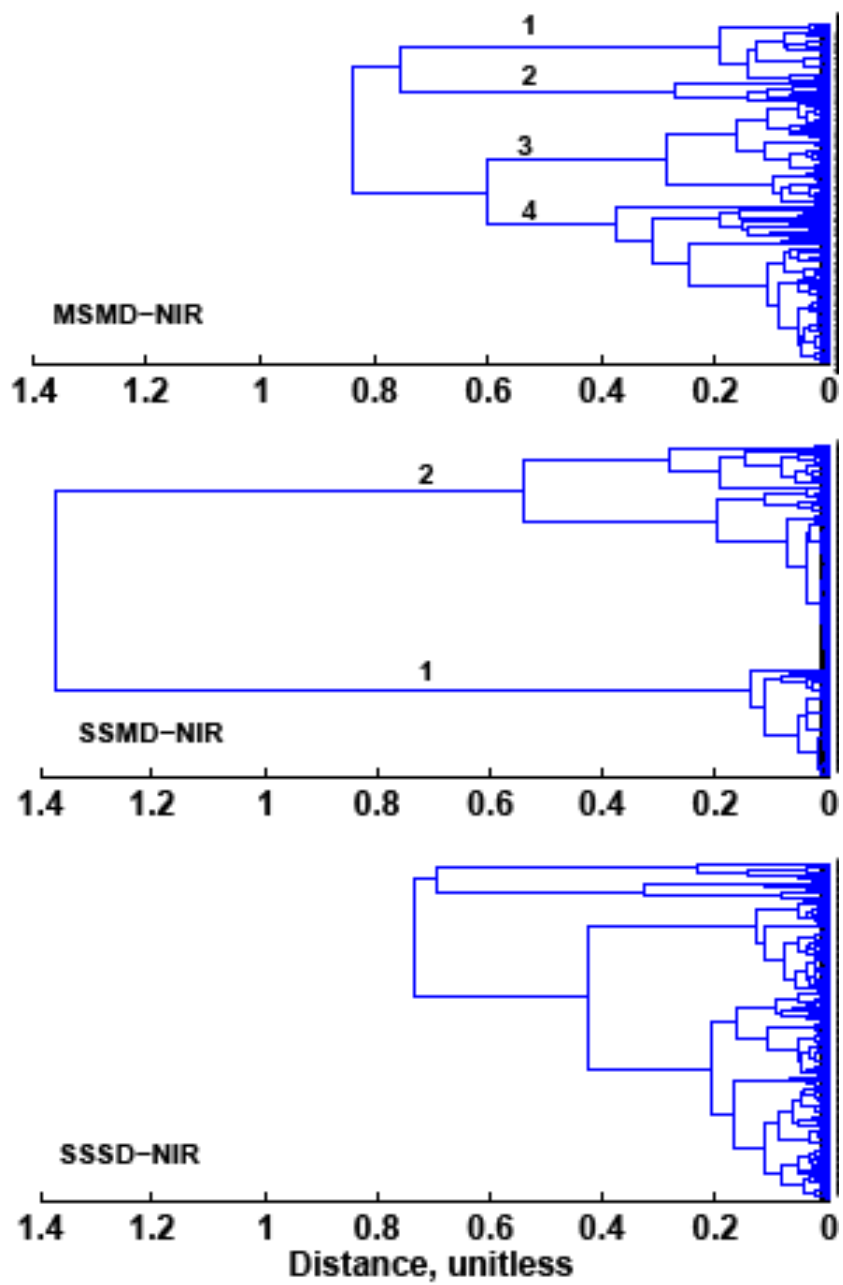


Figure 2.8: Cluster analysis performed on three data sets using NIR region

None of the other sample characteristics including level of SOC, landscape position, cropping system treatments could explain the clustering pattern. MIR and NIR data yielded similar cluster membership for SSMD. However this was not the case for MSMD samples. The sites that were clustered together based on NIR data were also put in different clusters using MIR data.

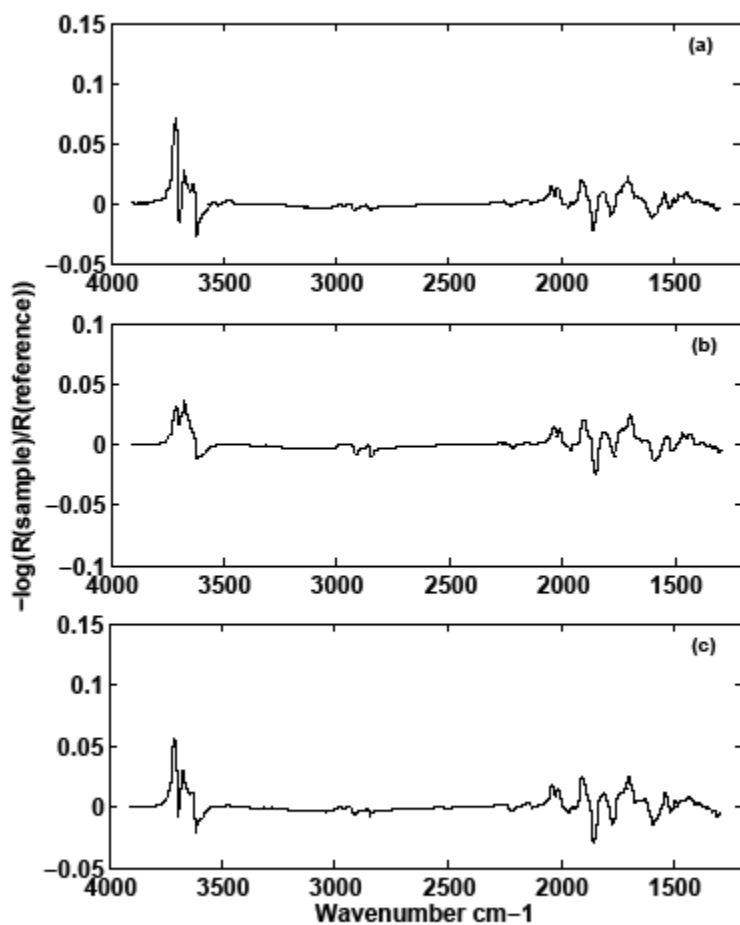


Figure 2.9: Average preprocessed spectra of samples from MSMD study belonging to (a) cluster 1 (b) cluster 2 (c) cluster 3 corresponding to dendrograms in figure 2.7(a)

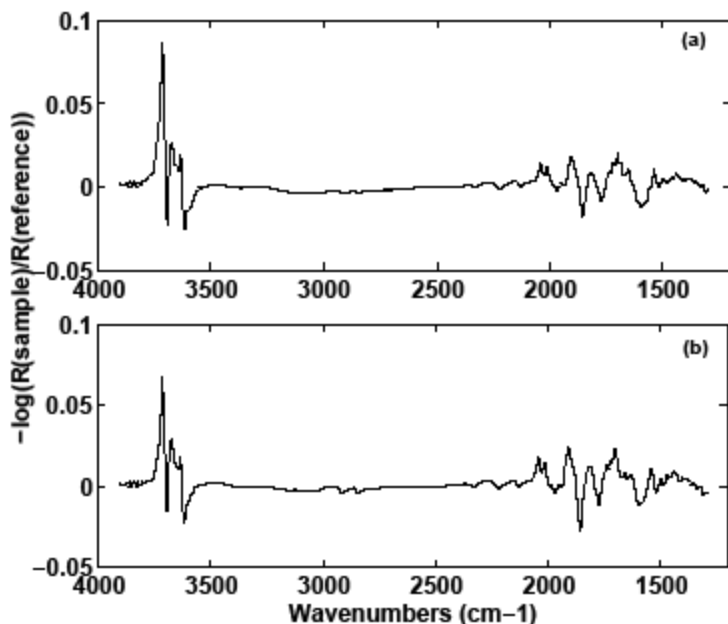


Figure 2.10: Average spectra of samples from MSMD study belonging to (a) cluster 1 and (b) cluster 2 corresponding to dendrograms in figure 2.7(b)

Clustering analysis can help better understand the structure of data sets and their challenges. If there are two or more main branches in the dendrogram and there is a reasonable explanation for those branches (for example different depths in SSMD), the branches could be split off for separate calibrations. We tried this approach for SSMD samples and it resulted in improvement in the calibration results (discussed in later sections). Careful attention should also be paid to the branch tips. If there are a lot of singleton clusters at larger distances or if the nearest neighbor distances are quite varied, the calibration models may not perform well as they have to interpolate between all spectra. Compared to other data sets the MSMD study had many small clusters separated at large

distances. This suggests that calibration model in case of MSMD may perform poorly compared to other data sets.

### Quantification of SOC by MIR and NIR

Results of the SOC calibration models are shown in Figure 2.11 and Table 2.8 using spectral region from 4000-400  $\text{cm}^{-1}$  for MIR the spectral region from 1100-2498 nm for NIR.

Study		Samples for validation	Pre-processing	Correlation	RMSEP	Number of factors
SSSD	NIR	48	SG 11pt after mean norm	0.85	0.12	8
	MIR	48	MSC	0.86	0.12	6
SSMD	NIR	56	MSC	0.98	0.14	9
	MIR	56	SG 11pt after SNV	0.98	0.14	12
MSMD	NIR	68	SG 9 pt.	0.93	0.31	13
	MIR	68	SG 11 pt.	0.94	0.30	7

Table 2.8: Carbon calibration model performance for the three studies using NIR and MIR data.

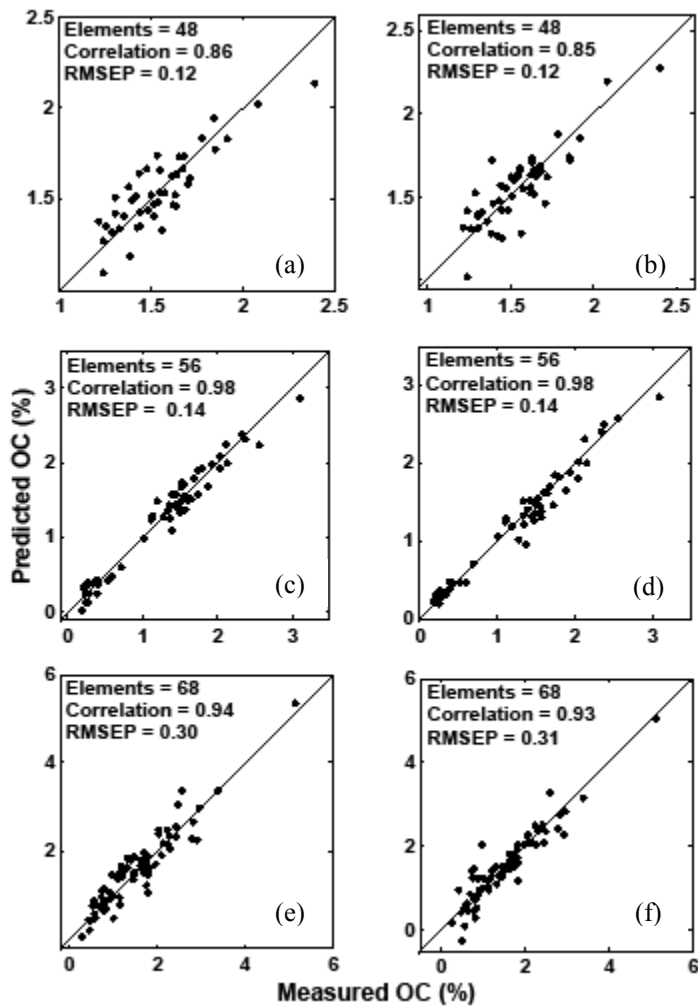


Figure 2.11: Validation set predicted versus measured plots from the preferred SOC calibration model developed on samples from (a) SSSD study with MIR data (b) SSSD study with NIR data (c) SSMD study with MIR data (d) SSMD study with NIR data (e) MSMD study with MIR data (f) MSMD study with NIR data (The lines shown in the graph are ideal)



For the SSSD study, two outliers were detected in both calibration sets and removed from further analysis. For the SSMD set, four outliers, 2 in calibration and 2 in validation set were detected and removed from further analysis. For the MSMD study, 1 outlier was detected and removed from the data set. Results of SOC-calibration models developed on samples from three different studies show that both MIR and NIR have the ability to predict soil organic carbon. Models performed equally well in terms of error values in both MIR and NIR regions for SSSD and SSMD. However the number of factors needed in the NIR range was lower than the MIR range for the SSMD set and higher in the case of the SSSD set. The most noticeable difference between the spectral ranges was for the MSMD set where MIR performed slightly better in terms of error value and also used many fewer factors than the NIR (7 versus 13). Regression vectors for the three MIR data sets had strong consistent weightage of 3000-2800 and 1950-1750  $\text{cm}^{-1}$  which contains peaks identified in the humic acid addition study (Figure 2.5) and are mostly relied on regions near 2900 and 1700  $\text{cm}^{-1}$  (Figure 2.12). However, except for SSMD where more factors yielded higher regression coefficients, mineral associated bands had very significant weights.

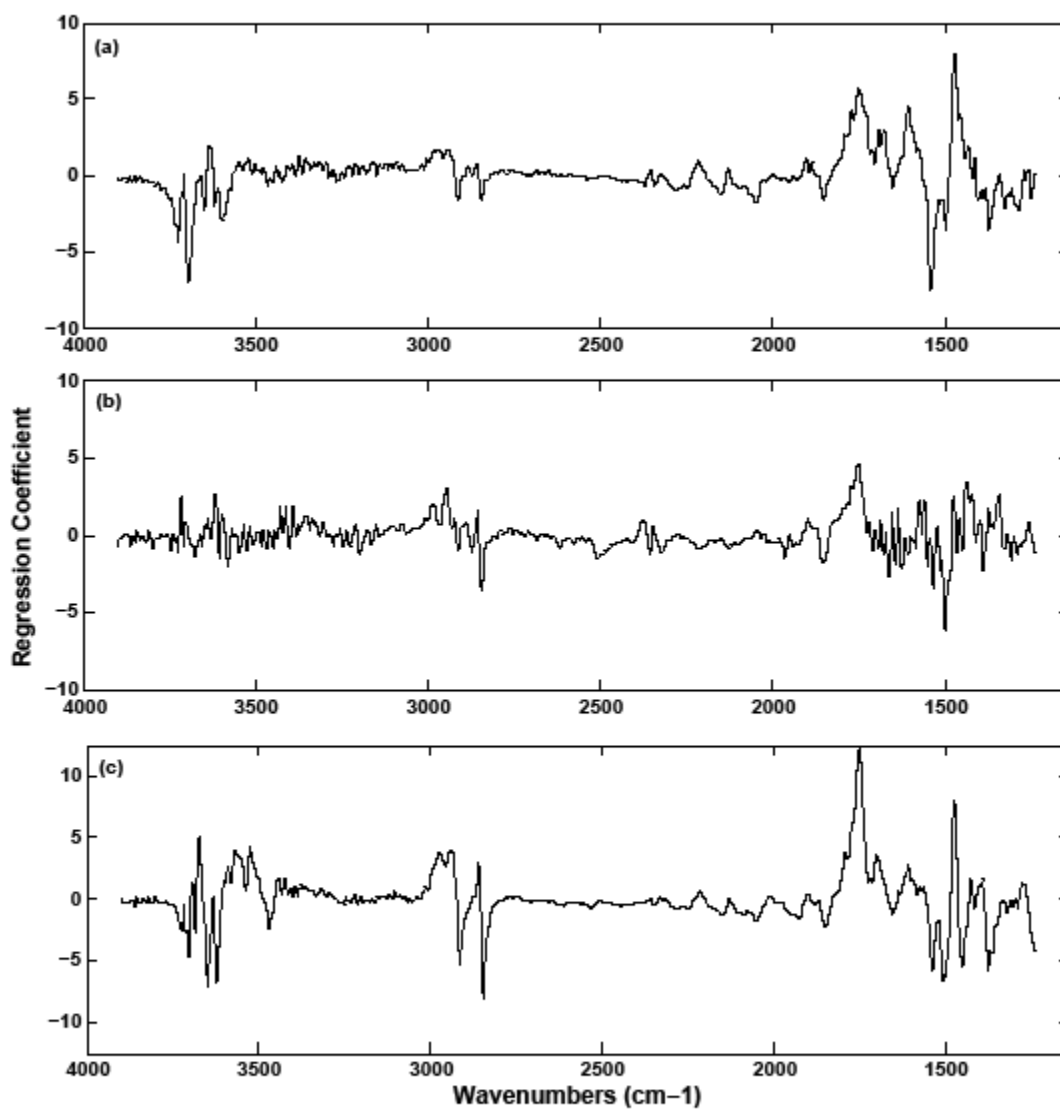


Figure 2.12: Regression vectors for the best SOC calibration model using MIR data for three studies

Coefficient of determination ( $r^2$ ) values obtained for the SSSD samples in both spectral ranges were lower than the other two sets, though this might simply be due to

narrower range of OC. In terms of RMSEP however the models built on SSSD samples were comparable to SSMD and quite a bit better than MSMD. Literature reports also support the assertion that the  $r^2$  figure of performance is related more to sample set rather than spectral ranges and typically there is a slight advantage of MIR over NIR. Chan et al. (2005) and Brown et al. (2006) reported an  $r^2$  value of 0.88 and 0.87 respectively while predicting the OC content of air-dried soils using NIRS data. Madari et al. (2006) while comparing the prediction abilities of NIR and MIR ranges reported that the correlation value for the NIRS data was 0.99 and that for the MIRS data it was 0.93 but the number of factors used in case of MIR range were 4 compared to 10 in the NIR range. McCarty et al. (2006), however reported a lower  $r^2$  value with NIR data (0.88) compared to MIR data (0.95) in predicting SOC. Results were based on 120 samples from tillage experiments and areas covered by secondary natural forests. The C-range for the samples included in the study was 7.9- 48 g/kg. Rossel et al. (2005) reported smaller  $r^2$  values using samples from a single field with C range of 8.1-19.8 g/kg, but still the MIR achieved higher  $r^2$  (0.73) than NIR (0.60). In a comparison study of MIR and NIR, Reeves et al (2001) based on their study on 180 samples from two locations on Maryland (C-range 6.1-33.9 g/kg) reported  $r^2$  and RMSD of 0.96 and 0.35 for MIR data and 0.78 and 0.83 for NIR data.

#### **Effect of mineralogy on OC calibration models:**

Calibration model results show that the performance of the models varied for the three sample sets (Table 2.8). Models performed best when developed on soils from a single site and a single depth. Model performance deteriorated slightly when developed using samples from a single site and multiple depths, and greatly when developed on

samples from multiple sites and multiple depths. Specimen sub-sampling error is probably not the source of the error because in all three sets, specimens were similarly uniform and fine.

The most likely cause was the variation in mineralogy of the samples that were collected from different depths and more significantly different sites. SSSD samples represented a single soil series while samples from SSMD were derived from a complex of three soil series derived from the same parent material (Table 2.3). Mineralogy of a soil varies not only with the site of sampling but also the depth of sampling (APPENDIX D). It is very likely that for SSMD samples too, the upper two depths (depth 1 and 2) varied from the lower depth not only in SOC content but also in the quantity of different minerals. The MSMD study, had samples from more than 20 soil series and 13 different parent materials (Table 2.4). Since the mineralogy of some soil series can be markedly different from the others, it is very likely that MSMD soils had wider range of mineralogy compared to the soils from other studies. This is supported by the MIR spectra of samples from the three studies. Spectra of samples from SSSD had very similar mineral bands compared to those from MSMD. Figure 2.13 shows mid-infrared spectra of 6 soil samples from two sites of multi-site sets. There is one sample from each site having high, medium and low OC levels. It is clear from the figure that the mineralogical peaks were large, variable and overlapped with the OM peaks. Preprocessing only partly corrected the matrix effects (Figure 2.13 (b)). Mineral peaks still remained more dominate than organic means. Also there is could be a possible non-linear response of peak intensities. OM spectral signature may also vary with mineralogy and affecting the calibration model performance.

The type of mineral matrix could affect the detection of SOC by either the primary method, combustion gas analysis, or the secondary spectral methods. On the one hand, the study provided no means of evaluating the accuracy of the SOC primary methods across mineralogy but on the other hand there are mechanistic reasons to suspect that spectroscopic calibrations can be biased by mineralogy.

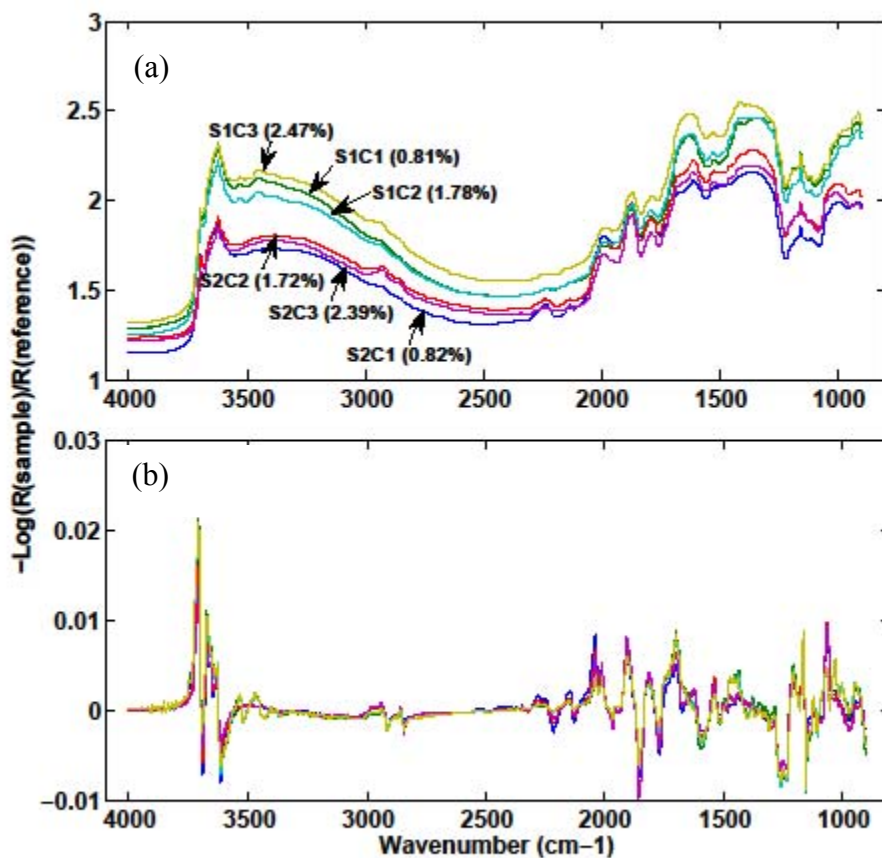


Figure 2.13: (a) Unprocessed MIR spectra of soil samples from two sites of MSMD that have similar range of SOC. S1 and S2 are the sites and C1, C2 and C3 are corresponding carbon levels (values given in parenthesis) and (b) same spectra after preferred transformation.

**Refining the calibration approach:**

Although the infrared-multivariate calibration approach has received recognition as a rapid and economical technology with potential to replace or enhance the existing methods used to measure OC and other related soil parameters, there are still uncertainties about how best to apply the technique. To this point in the study we have applied relatively standard protocols of multivariate calibration. Higher error for MSMD and SSMD sets, large number of factors used in calibration models and known subsets in study sets (depth in case of SSMD and sites in case of MSMD) suggest that there is an opportunity for improvement. This section focuses on some less often used techniques to improve MIR calibrations.

- Selecting the useful part of the spectrum that will contain information about the property of interest and discarding the spectral regions that are devoid of any useful peaks or that are completely redundant.
- Assessing the sample set and determining whether using more than one calibration models on the subsets of samples will be more appropriate

For SSSD MIR data set, excluding noisy spectral regions below  $1250\text{ cm}^{-1}$  did not change the RMSEP. However, selecting just the parts of the spectrum that are clearly related to organic carbon ( $3010\text{-}2800\text{ cm}^{-1}$ ) for calibration yielded higher error than using the spectral region from  $3900\text{-}750\text{ cm}^{-1}$ . It seems that some mostly mineral bands are necessary for good calibration accuracy which makes good sense when one realizes that SOC is carbon content as fraction of total soil dry mass. We found that the MIR range from  $3900\text{-}1240\text{ cm}^{-1}$  improved SOC calibration for all sample sets (Figure 2.14). For the SSSD set, the error dropped from 0.12 to 0.11 and the number of factors decreased from

6 to 3. For the SSMD set, the error was reduced from 0.14 to 0.13 and the number of factors dropped from 12 to 9. For the MSMD set, the error was reduced from 0.30 to 0.27 and number of factors dropped from 7 to 6. Leaving out the uninformative region of the MIR spectrum improves the calibration models in terms of error and the number of factors.

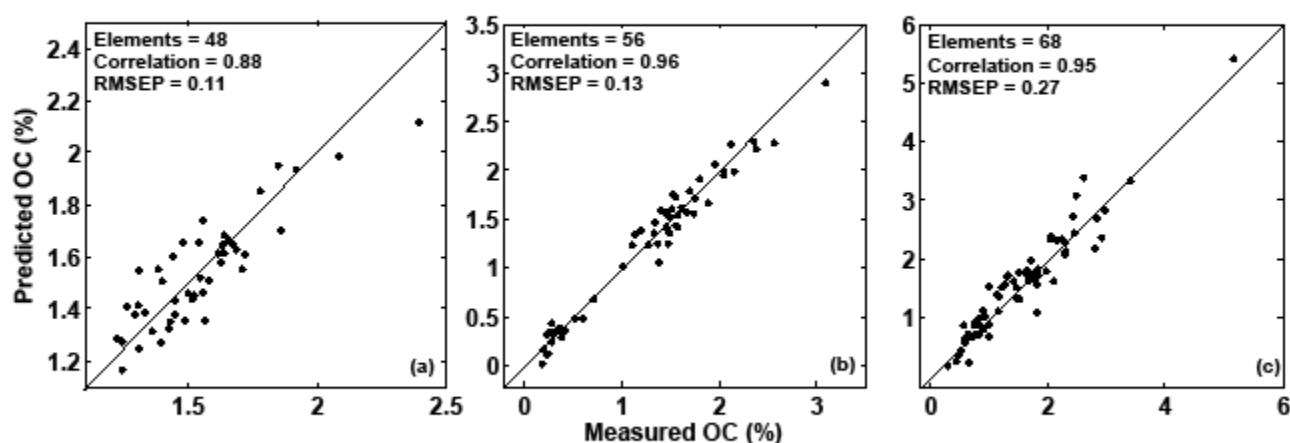


Figure 2.14: Validation set predicted versus measured organic carbon plot from the preferred calibration model developed using 3900-1250  $\text{cm}^{-1}$  range of MIR data for (a) SSSD (b) SSMD and (c) MSMD data (The lines shown in the graph are ideal).

For the next objective of assessing whether separate calibrations can improve model performance in very diverse sample studies, we hypothesize that if the sample set varies greatly by mineralogy or levels of SOC, it may be more appropriate to develop and use more than one calibration model. To explore this, we used the samples from SSMD set where samples came from three different depths. C-calibration model developed on samples ( $n=227$ ) from all three depths resulted in RMSEP value of 0.13. In figure 2.14

we suggest that the low SOC samples which are exclusively from depth 3 are less scattered and probably have a regression vectors response less than 1. Indeed when we looked at how well the samples from different depths were predicted using a single calibration versus two calibrations, it was found the RMSEP value for samples from depth 1 plus 2 was reduced from 0.15 to 0.13 with same number of factors and for depth three, a separate model did not reduce the error any further (below 0.08) but the number of factors were reduced from 9 to 6 (Figure 2.15). We conclude that separate calibration models are beneficial due to different SOC optical response in the low SOC range.



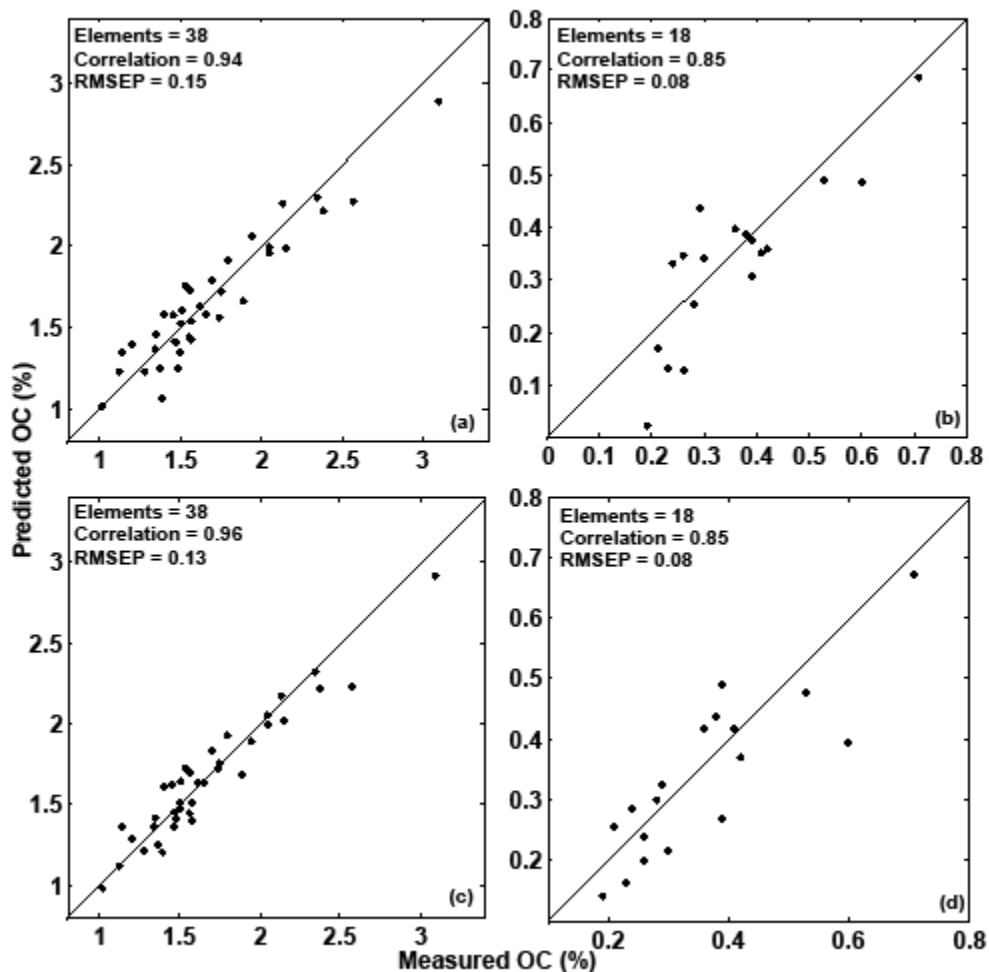


Figure 2.15: Validation set predicted versus measured SOC plots for samples from (a) depth 1 plus 2 and (b) depth 3 using model developed from all depths, and (c) depth 1 plus 2 and (d) depth 3 using samples from the depth 1 plus 2 versus depth 3 respectively. (The lines shown are ideal)

Another approach for dealing with sample diversity is to do unsupervised clustering of samples and then develop separate calibration models on samples belonging to the main clusters (Naes and Isaksson, 1991). The schematic (Figure 2.16) shows the

steps followed for this purpose. It involves performing cluster analysis on samples using the spectral data to group specimens that have similar spectral features and then develop calibration models on the clusters. This approach of unsupervised clustering was tested on sample from SSMD study. The unsupervised clustering approach resulted in a dendrogram with two main divisions that divided samples based on depth. Cluster one represented depth 1 and 2 and cluster 2 represented depth 3 (Figure 2.7 (b)). The result suggests that the unsupervised clustering could be helpful in grouping samples that are sufficiently different to warrant a separate calibration.

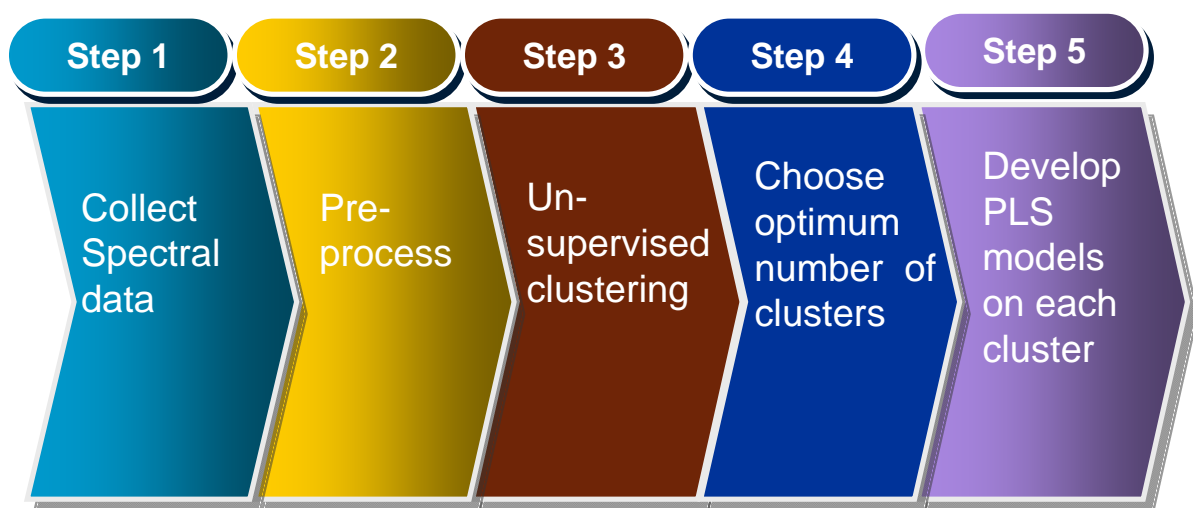


Figure 2.16: Schematic of the approach involving sample clustering before developing calibration models

We evaluated this approach on MSMD MIR data set. Clustering analysis revealed an isolated cluster consisting of samples from just two sites which mainly differed from other samples in mineralogy. When a calibration was developed using samples just from the remaining sites, RMSEP value dropped to 0.25 (from 0.27) with correlation of 0.93 and 9 factors. However the model built on second cluster containing samples from two sites had very high error of 0.59% even when we used full cross validation approach.

This is probably due to limited number of samples with very nontypical mineral spectra. Since the clustering analysis suggested that the main difference in the samples from these two sites and rest of the samples was in the region of 3750 and 3500  $\text{cm}^{-1}$ , we re-built our full-set calibration by eliminating that region from our selected range of 3900-1240. This did not reduce any error again suggesting that the mineral based matrix effect is not easy to eliminate. It appears that subset calibration based on soil MIR spectra, which is mainly a result of mineralogy can produce more accurate SOC determinations, but only if the clusters are large enough.

### **Efficiency of calibration models**

The main purpose of using IR calibrations for SOC determination in soil management studies is to reduce the need for the primary method of determining SOC by combustion gas analysis. To address the efficiency of the IR calibration techniques we evaluated the number of combustion analysis SOC values needed to either build a new calibration or update an existing calibration.

Efficiency results are shown in Table 2.9. Efficiency drops dramatically with increasing sample set diversity and MIR always gave more efficient models than NIR. The lower efficiencies observed with the MSMD sets suggest that insufficient numbers of calibration samples might account for the poor accuracy of the full-set models described in Table 2.8. The efficiency differences we observed are likely to be worse in real applications when small calibration sets are used to both optimize and build the calibrations.

Set	Range	Samples in calibration set	Samples in validation set	Efficiency
SSSD	MIR	40	150	79
	NIR	60	130	68
SSMD	MIR	70	158	69
	NIR	80	148	65
MSMD	MIR	110	163	60
	NIR	140	133	49

Table 2.9: Efficiency of C-calibration models for the three studies using NIR and MIR data

To assess the adaptability of calibration models, when we tried to predict SOC of SSSD samples using calibration model developed on SSMD samples using MIR data, 50 samples were needed from SSSD set. For all other data-sets, even the addition of up to 100 samples from the new study did not bring the errors within 125% of the minimum. We saw no bias in predicted values so bias does not seem to be the problem. Re-calibration was always more efficient. The error was high when we tried to predict more diverse site with a model developed on less diverse site and NIR predictions were poor compared to MIR (Figure 2.17)

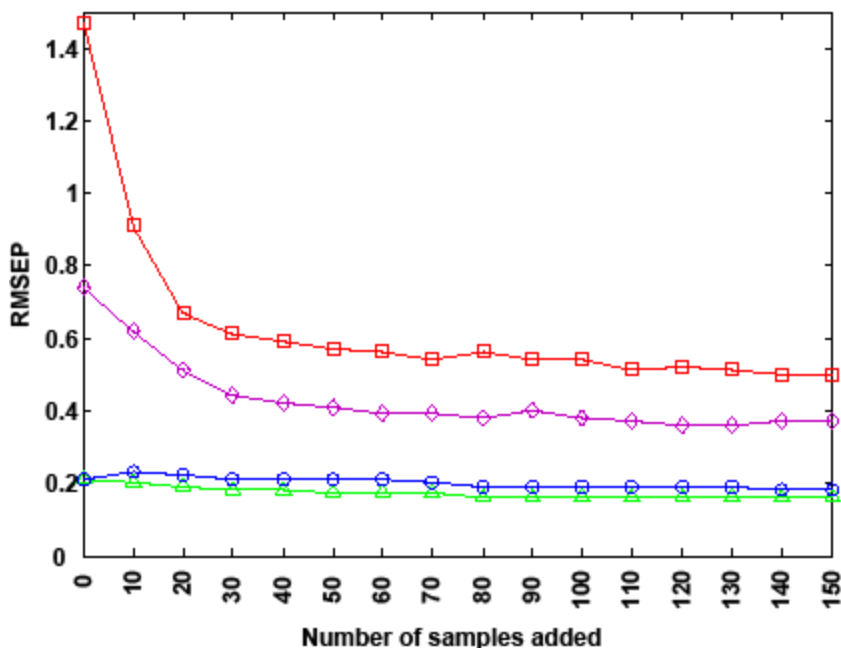


Figure 2.17: Effect of number of samples added to an existing calibration on RMSEP for the prediction of new sets of samples. MSMD samples were predicted by using model built on SSSD samples using MIR (red squares) and NIR (magenta diamonds) data. Similarly SSSD samples were predicted by using model built on MSMD samples using MIR (blue circles) and NIR (green triangles) data

In related work, Brown (2007) used models constructed using stochastic gradient boosted regression trees (BRT) to predict soil organic carbon by augmenting global spectral library (4148 samples) with local calibration (418 samples) and reported that predictions improved with increasing number of local calibration samples added to the global spectral library. But for one out of the two sample sets in the study, error with the addition of all local samples to global spectral library was higher than the error obtained by using local samples alone for calibration. Indiscriminant pooling of calibration sets is

risky. For all the three data soil sets studied here found that when building an IR SOC calibration for a new site, it is better to develop calibration model only using samples from that site rather than trying to extend an existing model, especially if the matrices of the new site are more diverse or are very different from the old site.

## CONCLUSIONS

New efficient approaches for soil characterization are needed for the sample-intensive studies in soil management, ecology and precision agriculture, especially when there is a need to map soil properties. In the present study we compared the ability of mid and near-infrared spectroscopy to determine soil organic carbon across three distinct types of soil management studies. In general MIR was slightly more accurate than NIR and required fewer multivariate factors in the calibration models. For the most diverse sample set composed of soils from several depths and many PA sites, the error values obtained with the two techniques were similar but the number of PLS factors used in the model were much less for MIR than NIR (5 compared to 12). We also found that NIR or MIR calibration models performed much poorer when the sample set was very diverse. For the MIR data slight improvement in calibration accuracy could be achieved by omitting uninformative regions of the spectrum. We found that although we can identify the organic regions in the MIR soil spectrum (which might be useful for the qualitative studies), basing calibration just on those regions reduced the calibration accuracy. When soil management sample sets are too diverse because of multiple depths or sites it can be slightly beneficial to calibrate on subsets generated by splitting or by removing unusual sites. Samples can be effectively divided into different calibration groups by unsupervised clustering of spectra and then separate PLS models can be used on samples

belonging to some clusters. We found that low SOC deep soils seem to have much different multivariate regression coefficients than the soils from upper horizons. The incompatibility reduces the overall accuracy. Another important effect is the variation in clay mineralogy that appears to interfere with OM diffuse reflectance bands and alters the spectral response per unit of OM. We found that poorly represented spectral types that are too small for a stand-alone calibration were predicted better by the full diverse set.

In terms of efficiency (minimum number of samples needed in calibration to predict remaining of the samples), we found that for all sets MIR calibrations proved to be slightly efficient than NIR. Also the efficiency was much better for sets that are less diverse ( $SSSD > SSMD > MSMD$ ). The study also revealed that the calibration models developed for one soil management study can be used for a new study by adding some samples from the new study to the calibration. If the study for which the model was originally developed is diverse or is similar in mineralogy to the new site, fewer samples from the new study are needed in the updated calibration. If the new study has more sample diversity than the old one, more samples from the new study are needed. However in all three studies we found that it is more efficient to build a calibration model using samples from that site instead of trying to extend an existing model.

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**CHAPTER 3****ATTENUATED TOTAL REFLECTION MID INFRARED SPECTROSCOPIC  
DETERMINATION OF ORGANIC CARBON CONTENT OF DRY SOILS.**

## ABSTRACT

Most of the infrared studies have utilized diffuse reflectance (DR) optical sampling to determine the OC contents of the soils. In this study the possibility of using attenuated total reflectance (ATR) sampling accessory has been explored which has some advantages over the DR technique. Soil samples were obtained from two contrasting studies with respect to the diversity of soils. For the first set, samples ( $n = 192$ ) were taken from a long-term crop rotation by fertility trial from a single depth of 0-8 inches. The second set samples ( $n=123$ ) represented a much diverse study with samples taken from 20 different sites across PA and also from 3 different depths. SOC was determined for these samples using both ATR and DR-FTIR spectroscopy using partial least squares regression. For the DR data, wavelength region of  $3900\text{-}1240\text{ cm}^{-1}$  was used for C-calibration. In the same range, ATR spectra had bands which were in agreement with those in DR spectra but much less intense. Instead ATR spectra are dominated by strong peaks found in the region  $1750\text{-}540\text{ cm}^{-1}$ . However this region with intense peaks does not seem to have any direct signature for OC and high and low C soil look very similar in this region. For the ATR data we therefore evaluated the usefulness of two wavelength regions of  $3900\text{-}1240\text{ cm}^{-1}$  and  $1750\text{-}540\text{ cm}^{-1}$ . For the less diverse site, the wavelength region of  $3900\text{-}1240\text{ cm}^{-1}$  resulted in better performing model (correlation = 0.80, RMSEP = 0.12 with 8 number of factors) compared to wavelength region of  $1750\text{-}540\text{ cm}^{-1}$  (correlation = 0.67, RMSEP = 0.13 with 9 number of factors). The performance of ATR models was comparable with the DR model (correlation = 0.82, RMSEP = 0.11 with 4 number of factors). For the more diverse study, for the ATR data, models developed using wavelength region of  $3900\text{-}1240\text{ cm}^{-1}$  performed slightly better



(correlation = 0.94, RMSEP = 0.38 with 9 number of factors) than 1750-540  $\text{cm}^{-1}$  (correlation = 0.93, RMSEP = 0.38 with 14 number of factors). Performance of calibration models using ATR data in this case also was similar to that of models developed using DR data (correlation = 0.93, RMSEP = 0.37 with 5 number of factors). In general calibration models developed for less diverse site performed better than those for more diverse site. Comparable performance of ATR instruments offers a new opportunity for at-site or in-situ carbon determination because of the availability of ATR spectrometers designed for field use. The better quality MIR spectral signature in the fingerprint region suggests that ATR may perform better than DR for SOC qualitative analyses.

## INTRODUCTION

Infrared spectroscopy probes the molecular vibrational signatures of materials and has been successfully used for non-destructive estimation of soil properties (Chang et al., 2001; Shepherd et al., 2002; Janik and Skjemstad 1995; McCarty et al. 2002). Although the use of near-infrared (NIR) range is still more common than mid-infrared (MIR) for soil analyses, the later is gaining interest due to greater specificity of absorbance bands (Stuart, 1997). Transmission IR has been used to study soils and other materials dispersed in halides salts and pressed into pellets or occasionally as films either free standing or supported by plates (Madejova, 2003, Nguyen et al, 1991., Haberhauer and Gerzabek, 1999). For MIR determination of soil carbon most studies have used diffuse reflectance (DR) optical sampling (Janik et al., 1998; Reeves et al., 2001; McCarty et al., 2002; Reeves et al., 2002). Attenuated total reflectance (ATR) is an alternative optical sampling technology with quite different optical and instrumental attributes. ATR has

been used for the study of materials in which intense MIR scattering and/or absorption makes it difficult to use the transmission or DR spectroscopy. ATR spectroscopy of abrasive materials including soils was not very practical until the diamond faced ATR was invented. Because solids must be brought in very close contact with the ATR sensor to achieve significant signal strength, the spectra of soils can be obtained just by pressing the sample on the surface of ATR sensor which can be 1-mm diameter. The spectrum acquisition by this method is independent of the thickness of the sample beyond certain  $\mu\text{m}$  making the tool useful for microscopy. There are some optical differences between DR and ATR that can be advantageous for portable MIR instruments. ATR optics are sealed which protects them from dirty environments and reduces baseline variation due to absorption of atmospheric gases especially water.

In ATR spectroscopy, the source of light makes one or more total internal reflections inside an optical waveguide and at each reflection an evanescent wave penetrates a fraction of wavelength beyond the waveguide surface; molecular absorbances of specimens can be studied by placing them in contact with the evanescent waves.

In soils most ATR studies have focused on adsorption studies involving the surface analysis of samples (Dobson and Mcquillan, 1999; Kang and Xing, 2007). Recently ATR-FTIR has also been used for some quantification studies. ATR-FTIR has been used for determination of nitrate concentration in soils (Shaviv et al., 2003 and Linker et al., 2004). Linker et al. (2005) studied FTIR-ATR to determine nitrate concentration of soil pastes using spectral region around the nitrate band ( $1300\text{-}1550\text{ cm}^{-1}$ ) to build the calibration models. ATR-FTIR technique has also been used to identify

types of agricultural soil based on absorbance bands associated with characteristic soil components including calcium carbonates, clay minerals and organic constituents (Linker et al., 2005, 2006). Linker et al (2005) used wavelength region ( $800\text{-}1200\text{ cm}^{-1}$ ) to classify soils using ATR sampling accessory and reported that each soil type has a distinctive fingerprint in this region and hence the region can be used for soil identification. Previous data has revealed that DR-MIR has better potential to be used for the classification of soils than DR-NIR. However whether the ATR and DR sampling techniques vary in this respect is not known.

Different soil management studies are conducted to address different research questions. Depending on those research objectives, samples collected might differ in diversity. For example if the research question requires collecting samples from different sites and depths, sample set will have more diversity. If the samples from just a single site and depth are needed, the diversity will be much less. Previous results have revealed that it is easier to develop IR calibrations models for less diverse sample sets. For more diverse sample sets it is rather challenging and sometimes necessitates site or depth specific calibrations. So to compare the performance of different IR techniques it is important to understand how the performance is influenced by the diversity of sample sets. For this study we chose to test the performance of ATR and DR IR techniques on samples from a very diverse study and a relatively less diverse study.

The main objective of this study is to compare ATR-MIR with DR-MIR for dry soil OM characterization. The specific objectives are to compare the two techniques with respect to (1) SOC calibration performance (2) spectral basis of SOC calibration (3)

potential for differentiation of SOM types and (4) to evaluate potential advantages of ATR configuration.

## **MATERIALS AND METHODS**

### **Site description and soil sampling**

Samples were collected from two different soil management experimental sites (Table 3.1). The first set was called the single site and depth study (SSSD) because the samples were collected from a single site under a moldboard plowed long-term crop rotation by fertility trial and from a single depth of 0-20 cm. This trial also known as the Hunter Rotation Experiment is located at the Pennsylvania State University R.E. Larson Research Center in Rock Springs, PA (Figure 3.1). The soil type for this on-going experiment started in 1969 is Hagerstown silt loam (fine, mesic, Typic Hapludalfs) which is characterized as well drained and agriculturally productive soil (Braker, 1981). This conventionally tilled experiment consists of four crop rotations and three fertility types replicated 4 times in total of 192 plots of 5.76 m width and 12.8 m length (Mirsky, 2008). The split plot layout of the experiment has year effects as the main plots, fertility treatment as the sub-plot and crop-rotations as the sub-sub-plots. For SSSD study, 192 samples were collected in spring 2004 from 20 cm depth.

Study		n	Sampling depth (cm)	Sampling year	Reference method	OC		
						Mean	SD	Range
SSSD	Cal	144	0-20	2004	LOI	1.55	0.22	0.97-2.48
	Val	48	0-20	2004	LOI	1.55	0.23	1.22-2.39
MSMD		123	0-10,10-20,20-40	1998, 1999,2000	Combustion	1.70	1.07	0.28-6.24

Table 3.1: Description of two sample sets used for the study and descriptive statistics on organic carbon values obtained by the reference method for the two studies (n = number of sample, SD = standard deviation)



Figure 3.1: Aerial photograph of SSSD study trial (Hunter Rotation Experiment)

For the second set, called the multi-site and depth study (MSMD), 123 archived samples were obtained from a cropland biosolid treatment study from 20 different sites in 18 counties of Pennsylvania and three different depths of 0-10, 10-20 and 20-40 cm (Figure 3.2) (Shober et al., 2003). Agricultural soils with a history of biosolids application were identified and also at every site, these soils were paired with fields lacking past biosolids application. Samples were collected in the fall of 1998, 1999 and 2000. Five locations were sampled in each field at a depth of 0-10, 10-20 and 20-40 cm. These five samples were then composited for each depth to produce one sample per field per depth.

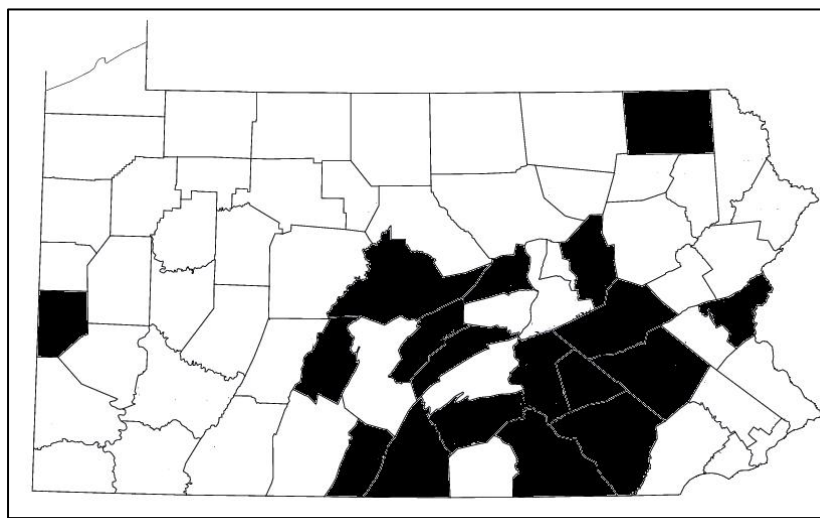


Figure 3.2: Location of MSMD sampling sites in 18 Pennsylvania counties

### **Soil Carbon Analyses**

For the SSSD soils, soil organic matter (SOM) was determined by weight loss on ignition method (Schulte, 1995). Five to ten grams of sieved samples were dried at 105°C followed by ashing at 360°C. For MSMD study, total carbon and inorganic carbon values were obtained using a Shimadzu TOC-5000A Total Organic Carbon Analyzer with the

SSM-5000A Solid Sample Module. Organic carbon was determined by subtracting inorganic carbon from the total carbon.

SSSD soils were analyzed for SOM by weight loss on ignition method while soils from MSMD sets were analyzed for SOC by dry gas combustion method. To make the models comparable in all cases, SOM values were converted to SOC values by using a regression equation developed on a sub-set of SSSD soils ( $n = 10$ ,  $r^2 = 0.96$ ,  $SE = 0.07$ ) (APPENDIX A).

### **FTIR data acquisition**

A Bruker Tensor 27 FTIR equipped with a room temperature La-DTGS detector was used for the DR and ATR measurements (Bruker Optics, Billerica, MA). For DR-FTIR analysis, dry screened samples ( $< 2$  mm) were passed through a 0.25 mm screen and were scanned from  $6998 - 299$   $\text{cm}^{-1}$  at  $6$   $\text{cm}^{-1}$  and 128 scans interval using a Pike technologies XY-autosampler for 96-well plate. Duplicate measurements were taken on separate subsamples.

ATR-FTIR spectra were collected at  $8$   $\text{cm}^{-1}$  and 256 scans with a diamond-ZnSE single reflection ATR accessory (Pike Technologies, MIRacle). For the ATR-FTIR analysis, a small amount of each specimen was screened as above but then further ground into fine powder using mortar and pestle. Samples were then deposited on the ATR crystal one by one. Single beam scan times for DR and ATR measurements were comparable (120 seconds for DR, 180 seconds for ATR). Compared to DR-FTIR analysis of soils, ATR-FTIR is slightly more time consuming and substantially more labor intensive. Milling a small amount of soil with the mortar and pestle adds little time to the process and scan times are slightly longer. ATR crystal cleaning between specimens adds

a little more time and also requires great attention for soils with low SOC content. To ensure better contact with the 1 mm diameter ATR crystal, samples were loaded over the crystal and covered with 1/6" rubber mat pressed using high pressure clamp and then backed off to a lower force. The pressure was released before scanning to minimize small diamond absorbance spectral artifacts due to bending of the diamond layer atop the ZnSe optical element (Figure 3.3).



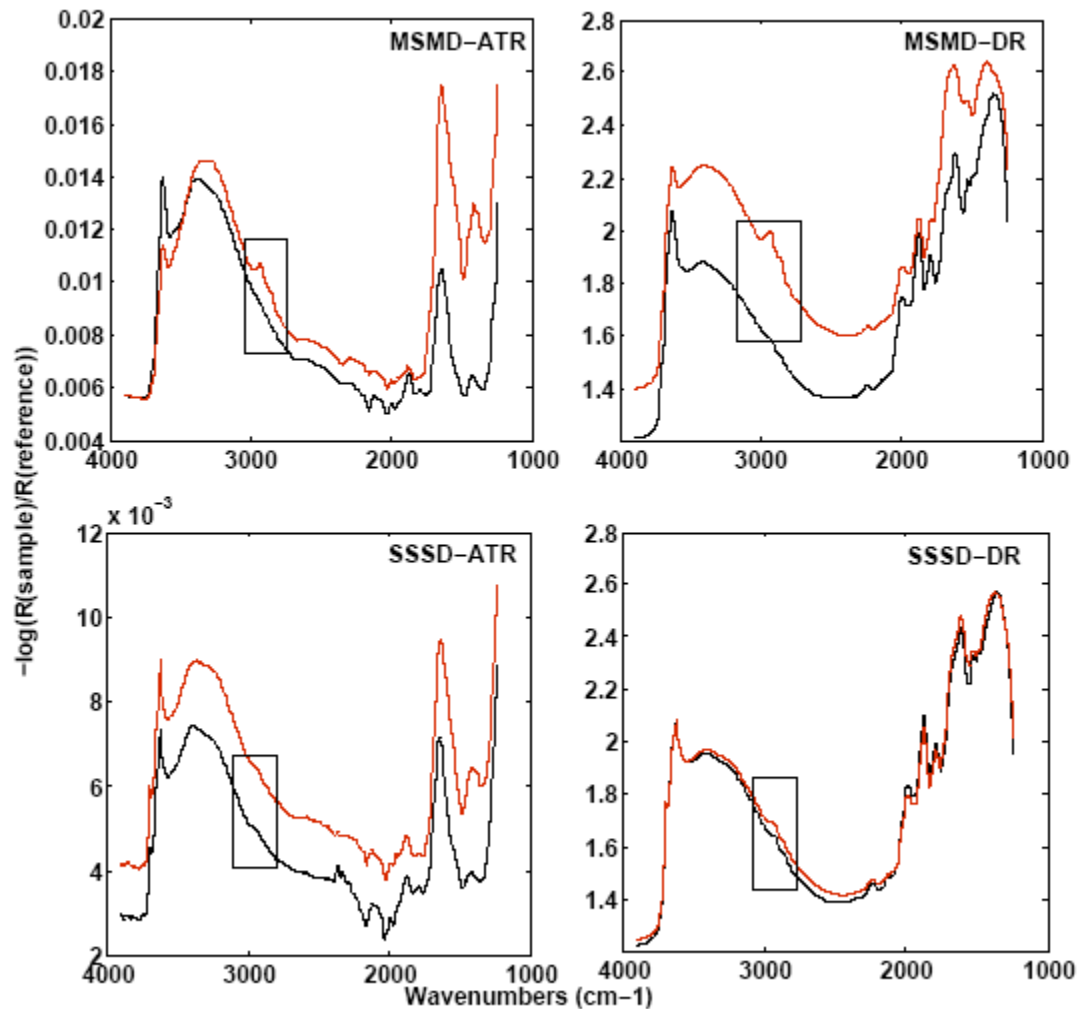


Figure 3.3: Representative FTIR spectra of a high (red) and low (black) carbon soil from both dry screened soil sets obtained with ATR and DR techniques. In the ATR spectra, diamond absorbance artifacts are present in the region. Diamond absorbance artifacts are present in the range  $2500\text{--}1900\text{ cm}^{-1}$  (The blocked area is the C-H stretch region).

### **Multivariate calibration for SOC**

Multivariate calibrations were calculated using partial least squares regression (PLSR) algorithm in MATLAB version 7.5 or Unscrambler software version 7.6 (CAMO, U.S.A). PLS regression is a multivariate calibration method that can decompose spectral data (X) and soil property data (Y) into a new smaller set of latent variables and object scores that best describe all the variance in the data (Haaland and Thomas, 1988 and Wold et al. 2001). Spectral data pretreatment was optimized by testing the performance of various transformation methods like normalization, baseline correction, multiplicative scatter correction (MSC), Savitzky- Golay derivatives (SG), standard normal variate (SNV) correction and various combinations of these transformations to correct undesirable spectral distortion due to scattering and other environmental factors.

For SSSD study (n = 192), a test set approach was used in which a random selection of one third of samples was used for validation and the remaining two thirds for calibration. Cross validation models were developed using calibration set samples which comprised duplicate samples. For deciding on the best transformation and number of factors, we compared root mean square error of cross validation (RMSECV) of different models developed using different transformations and different number of factors. To avoid the overfitting of data by using too many factors, we selected a model with more factors only when the error improvement was more than 0.005% (in units of SOC). Regression vectors obtained using the chosen transformation and number of factors were screened for excessive noise. The best calibration models were evaluated by predicting SOC values of averaged (in cases where duplicates were available) or un-averaged (when

no duplicates were available) validation samples. The root mean square error of prediction (RMSEP),  $r^2$  and slope values for the validation set were reported.

In case of MSMD set, the wide range of soil type and limited number of samples (123) handicapped validation set approach. A leave one out cross validation was used in this case. A number of transformations were tested on the data set and best transformation and number of factors selected based on the calibration model performance. To minimize the chance of overfitting we applied the same precautions noted above for the SSSD set. In both data sets, a soil sample was considered to be an outlier if the difference between its predicted and reference value was 3 times larger than the RMSEP for rest of the samples (Chang et al. 2001, Islam et al., 2003).

## **RESULTS AND DISCUSSION**

### **DR and ATR spectra of soils**

Figure 3.3 shows a typical ATR and DR soil spectra in the mid-infrared range. Previous study for the same sets of soils has revealed that for C-calibration purpose with mid-infrared DR technique, spectral range of 3900-1240  $\text{cm}^{-1}$  was most useful. When high and low C soil spectra with both techniques are compared within the same wavelength regions (3900-1240  $\text{cm}^{-1}$ ), most of the peaks in ATR spectra are found in the DR spectrum albeit with different peak shapes. An exception is the diamonds optical artifacts in ATR spectra over the range (2700-1950  $\text{cm}^{-1}$ ) (Figure 3.3). Comparison of high and low carbon spectrum reveals difference in the weak but important C-H stretch around 2700  $\text{cm}^{-1}$  in both ATR and DR techniques.

ATR spectral peaks in the higher frequency mid-IR range are much weaker than DR. When a broader wavelength region of  $3900 - 540 \text{ cm}^{-1}$  is considered, the peaks in the  $3900-1240$  region are dominated by those in the region  $1240 - 540 \text{ cm}^{-1}$  (Figure 3.4). The ATR-FTIR frequency range  $1240-540 \text{ cm}^{-1}$  does not seem to contain any peaks that are easily assigned to SOC. There is not much difference in the ATR spectra of high and low C soils in this region. This suggests that SOC calibrations built using this region may not work or may have some indirect basis of calibration. Because the relative intensity of this range tends to vary with slight differences in ATR clamp pressure, we removed it from consideration.

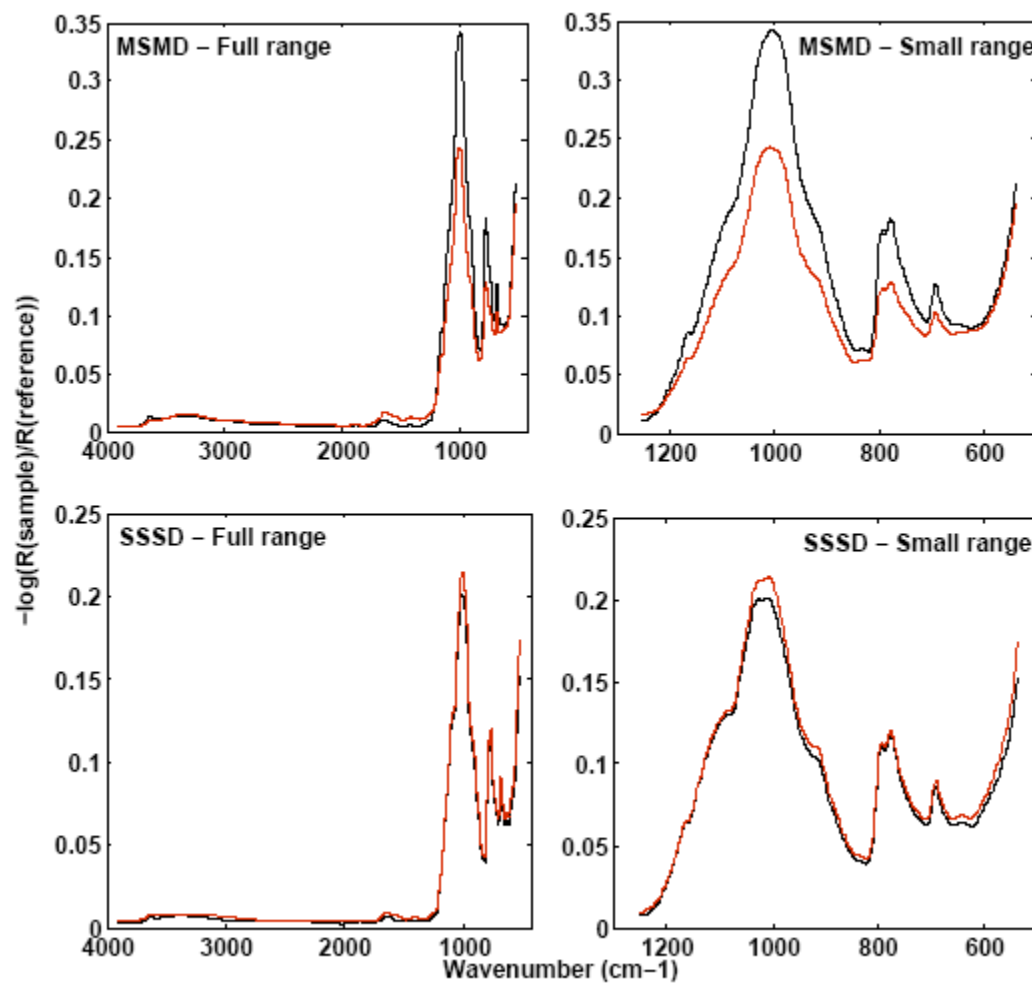


Figure 3.4: ATR-FTIR spectra of high (red) and low (black) carbon soil from both study sets displayed in two frequency ranges: 3900-540  $\text{cm}^{-1}$  and 1250-540  $\text{cm}^{-1}$

For some sample types like soils which absorb too strongly, DR spectra gets distorted in the regions around 1200  $\text{cm}^{-1}$ . Such problem is not encountered with ATR technique and hence ATR spectra are more informative for that region. This suggests that ATR technique maybe better suited for classification purposes than DR.

### **SOC calibrations**

For the SOC-calibration studies, we used a frequency range of 3900- 1240  $\text{cm}^{-1}$  for the DR data. For the ATR data, the wavelength region was not selected a-priori. Various regions between the range 4000-500  $\text{cm}^{-1}$  were tested with respect to their effectiveness in predicting SOC. Two regions gave comparable results 3900-1240  $\text{cm}^{-1}$  and 1750-540  $\text{cm}^{-1}$ .

Table 3.2 summarizes the validation results for both data sets using ATR and DR sampling techniques. For the SSSD set, five outliers were removed from calibration set and two from validation set for the ATR data when wavelength region of 3900-1240  $\text{cm}^{-1}$  was used. A good correlation of 0.80 was obtained with RMSEP of 0.12 and eight factors (Figure 3.5). This was comparable to DR technique where correlation was 0.83 and RMSEP was 0.11. However DR utilized much simpler four factor model. Similar numbers of outliers were detected by DR: four outliers were removed from the calibration set and one from validation (Figure 3.5).

Study		Wavelength region (cm-1)	Samples for validation	Pre-processing	Correlation	RMSEP	Number of factors
SSSD	ATR	1750-540	46	MSC	0.67	0.13	9
	ATR	3900-1240	47	SNV	0.80	0.12	8
	DR	3900-1240	47	SG 11 pt	0.83	0.11	4
MSMD	ATR	1750-540	119	Norm	0.93	0.38	14
	ATR	3900-1240	119	MSC	0.94	0.38	9
	DR	3900-1240	120	SG 9 after norm	0.93	0.37	5

Table 3.2: Carbon calibration model performance for the two studies using ATR and DR-MIR data.

For the MSMD set ATR data and frequency range of 3900-1240  $\text{cm}^{-1}$ , four outliers were removed from the data leaving 119 samples in the model which is comparable to the three found in the DR data set. A good correlation of 0.94 was obtained using 14 factor calibration model but error was much higher (0.38 %) compared to SSSD set (Figure 3.5). The performance was similar to that observed with the DR data for the same set of soil samples (correlation of 0.93 and RMSECV of 0.37% using five factors).

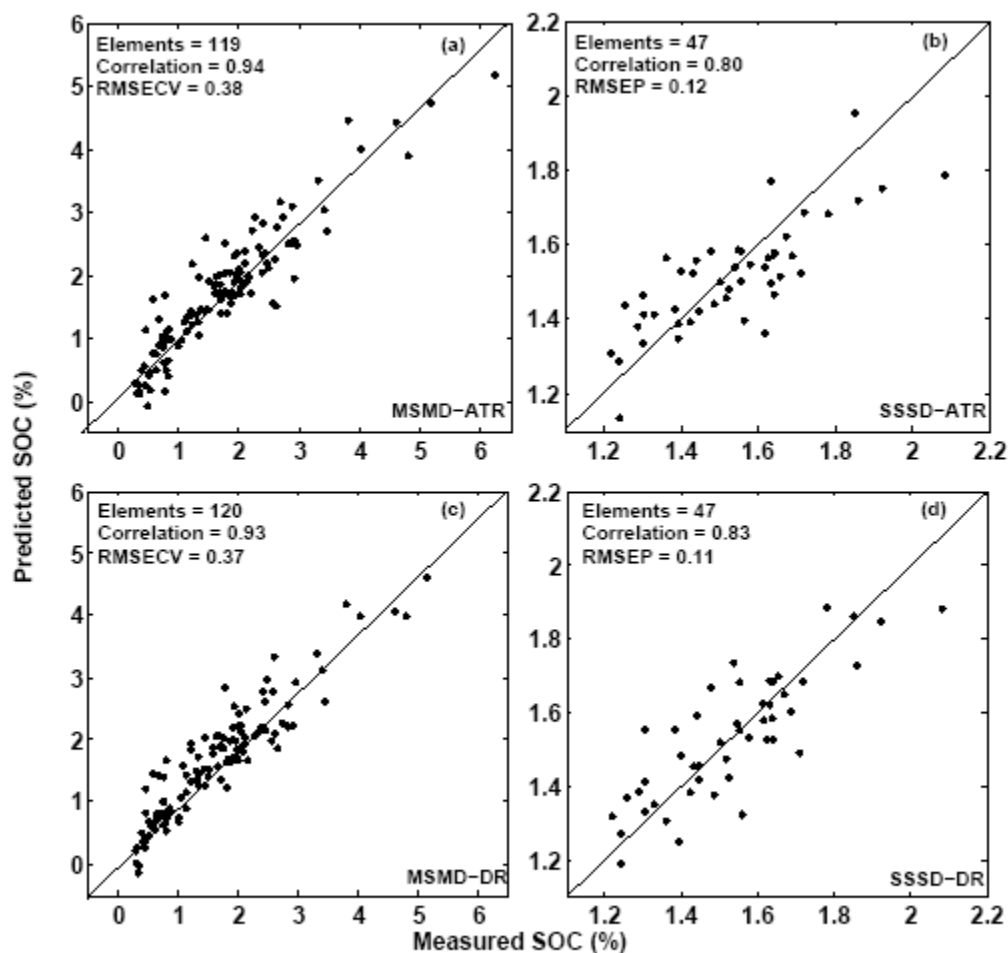


Figure 3.5: Plots of predicted versus measured SOC from the preferred calibration models developed on samples from (a) MSMD study with ATR data (b) SSSD study with ATR data (c) MSMD study with DR data (d) SSSD study with DR data. The frequency range was  $3900\text{-}1240\text{ cm}^{-1}$  in all cases. The lines shown in the graph are ideal.

When comparing frequency range of  $1750\text{-}540\text{ cm}^{-1}$  to the range  $3900\text{-}1240\text{ cm}^{-1}$ , the ATR calibration model performance was poorer in case of SSSD set and similar in case of the MSMD set (Figure 3.6). The narrower range did not reduce the number of



model factors. Previous work suggests that the higher errors of the MSMD set compared to SSSD seem to stem from the structure of the data sets and as such ATR provides no performance advantage over DR.

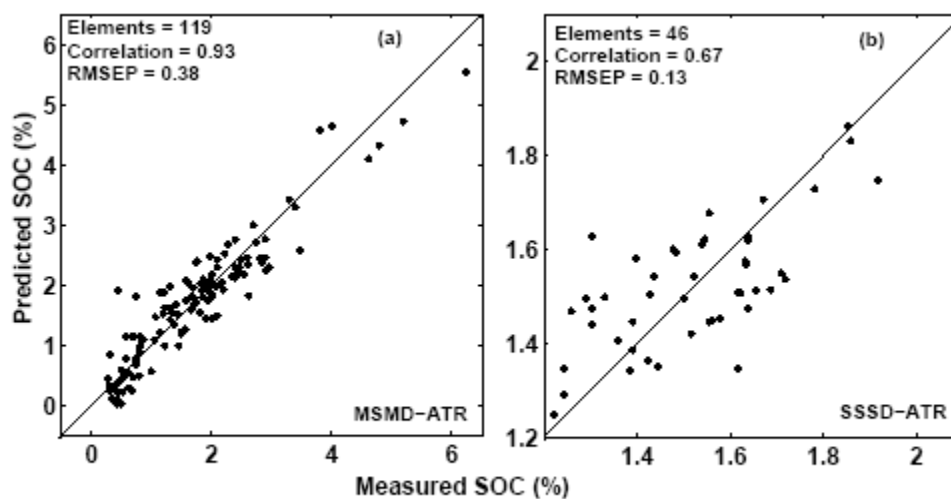


Figure 3.6: Plots of predicted versus measured SOC for preferred ATR calibration model for the 1750-540  $\text{cm}^{-1}$  range developed on samples from (a) MSMD study (b) SSSD. The lines shown in the graph are ideal.

Figure 3.7 and 3.8 compare the regression vectors for the two data-sets obtained with DR and ATR data using two different frequency ranges. In the range 3900-1240  $\text{cm}^{-1}$ , the ATR model and the DR model regression vectors weight bands around 2700  $\text{cm}^{-1}$  (C-H stretch region) and also in the fingerprint range below 1900  $\text{cm}^{-1}$ . However the pattern of weights is quite different between ATR and DR. When the wavenumber range of 1750-540  $\text{cm}^{-1}$  is used, the ATR model weights several bands from 1750-1500  $\text{cm}^{-1}$ . This range may have contributions from lipids, esters, protein, amides and adsorbed water. ATR spectra might be useful to differentiate the types of SOC in specimens.

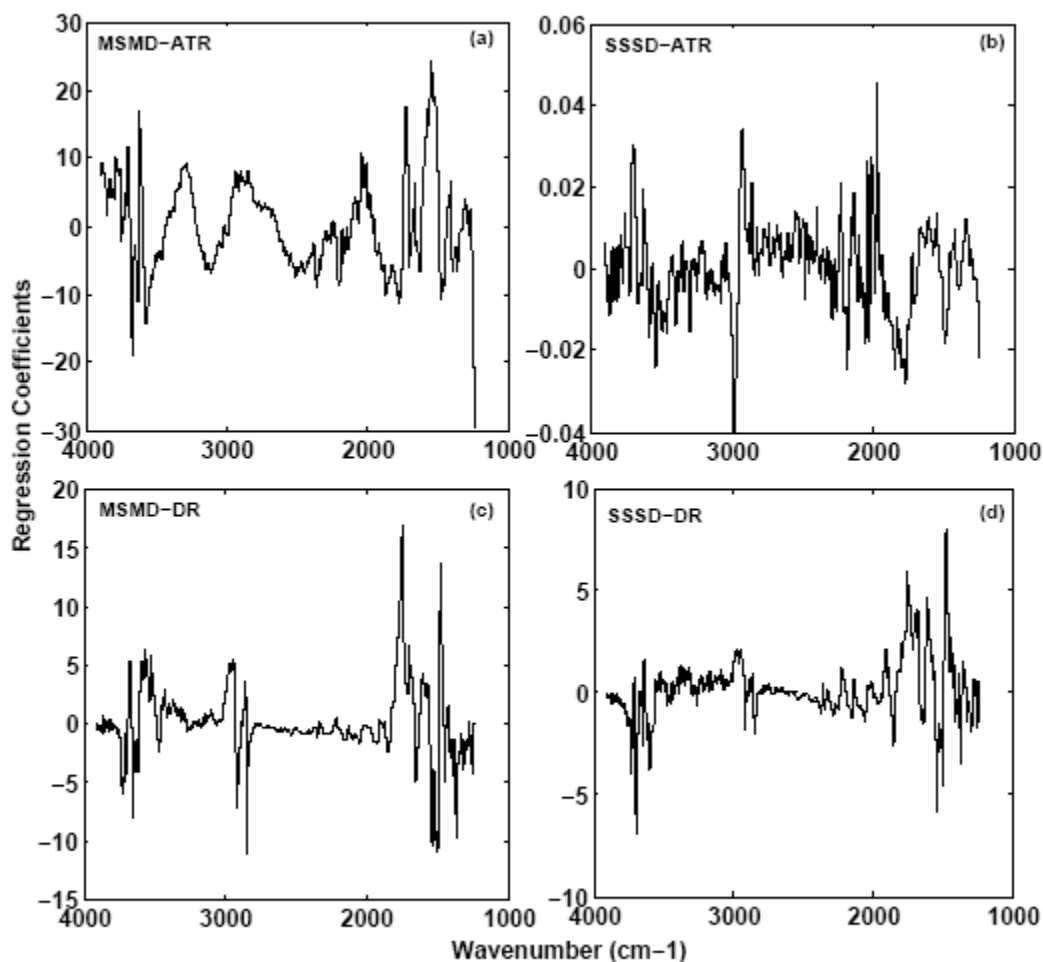


Figure 3.7: Plots of PLS Regression vectors of the preferred calibration models for the two soil collections: (a) MSMD study with MSC pre-processed ATR data (b) SSSD study with SNV preprocessed ATR data (c) MSMD study with first derivation preprocessed DR data (d) SSSD study with first derivation preprocessed DR. All calibrations used the wavenumber range region of 3900-1240 cm<sup>-1</sup>

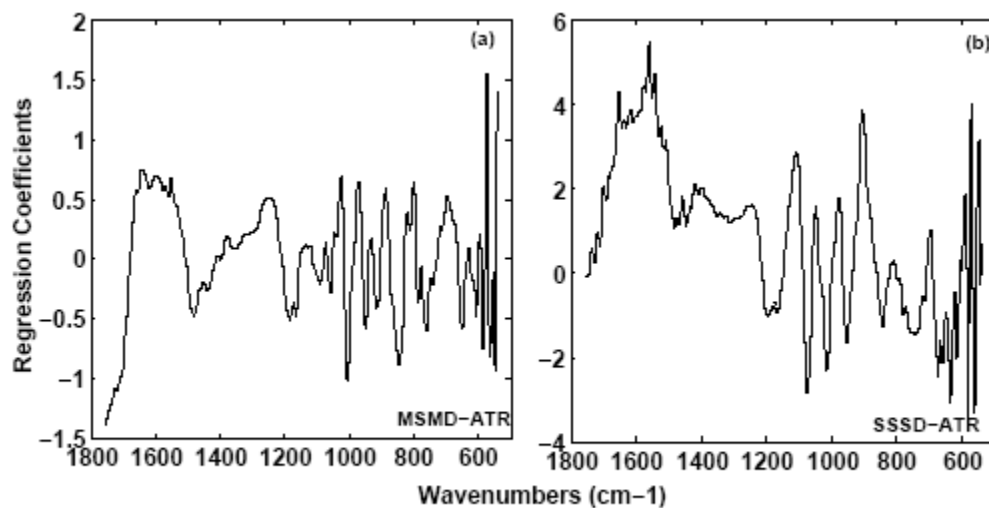


Figure 3.8: Plots of PLS Regression vectors of the preferred calibration models for the two soil collections: (a) MSMD study with MSC pre-processed ATR data (b) SSSD study with SNV preprocessed ATR data. All calibrations used the wavenumber range region of  $1750\text{-}540\text{ cm}^{-1}$

ATR technique has a low error rate and can be easily learned by non-technical personnel. When working through a set of soils we were able to assay about 15 soils per hour by the ATR technique. We needed to collect reference scans more frequently in our DR-FTIR measurements of soils because of fluctuation in water and carbon dioxide gases within the purged sampling accessory. The ATR-FTIR instrument has sealed compact optics and a room temperature detector so we would expect similar performance for portable ATR-FTIR instruments constructed from the same types of components.

### CONCLUSIONS

Diamond ZnSe ATR-FTIR is a promising tool for quick assessment of SOC content with little sample preparation other than to dry and grind a subsample of the soil. Using two different types of soil collections we show that ATR-FTIR calibrations

perform nearly as well as DR-FTIR calibrations. The DR technique is fairly routinely used for infrared determination of SOC and other soil properties. The usable mid-infrared spectral range for soil is different for DR and ATR-FTIR. DR is appropriate for the mid and NIR (above  $1250\text{ cm}^{-1}$ ) while ATR is appropriate for more of the MIR including the fingerprint range ( $3900\text{-}540\text{ cm}^{-1}$ ). There are still unresolved issues about the best spectral range for quantifications and qualitative analysis of SOC. However two ranges were found to perform well for SOC quantitative analysis ( $3900\text{-}1240$ ,  $1750\text{-}540\text{ cm}^{-1}$ ). The better quality MIR spectral signature in the fingerprint region suggests that ATR may perform better than DR for SOC qualitative analyses. Although our diamond-ATR studies utilized a lab based FTIR, the same instrumental components are found in portable instruments. ATR optics is easily sealed so on-site performance should be comparable to what we achieved in the lab.

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**CHAPTER 4**  
**OVERALL CONCLUSIONS**

Soil organic carbon (SOC) is important from both agricultural productivity and environmental quality perspectives. Current methods for SOC characterization are time consuming, laborious and expensive. New efficient approaches for soil characterization are needed for the sample-intensive studies in soil management, ecology and precision agriculture, especially when there is a need to map soil properties. In the present study we compared the ability of mid-infrared (MIR) and near-infrared (NIR) spectroscopy in combination with partial least squares regression (PLSR) to determine SOC across three distinct types of soil management studies which varied in sample diversity. For the least diverse set, samples were collected from a single site and depth (SSSD), for a slightly more diverse set, samples were collected from a single site at multiple depths (SSMD) and for the most diverse set (MSMD) samples were collected from different sites and depths.

In general MIR could provide more accurate estimation of SOC compared to NIR for all the three studies. The prediction errors were smaller for MIR than NIR and also the MIR technique used fewer PLS factors. MIR also seems to have better quality information with some very distinguishable bands associated with SOC. We however could not get good calibration performance based on just those bands. This might be because of the complex nature of soils and interaction between organic and mineral matrices. Nevertheless we could get better performing models by eliminating uninformative regions of the spectra.

Both spectral regions gave good predictions of SOC for less diverse sample sets (SSSD and SSMD). When the sample set was very diverse (MSMD), the model performance deteriorated. This could have been anticipated as the MSMD samples were

derived from more than 20 different soil series which were formed from 13 different parent materials and composed for very different minerals. The mineralogical differences in MSMD samples were also evident from the spectra of those soils. We believe that this huge mineralogical diversity made it challenging to achieve good overall accuracy in MSMD study.

We hypothesized that when sample sets are too diverse (because of multiple depths or sites) it can be beneficial to develop separate calibration models on subsets generated by splitting or by removing unusual samples. Samples can be effectively divided into different calibration groups based on known information (site or depth) or by unsupervised clustering. If the cluster analysis reveals the presence of two or more very different groups of samples, separate calibrations can be developed for those groups. For SSMD samples we found that samples were clearly divided into two major clusters. Separate calibration for those two clusters improved the model accuracy compared to using a single model for all samples. This approach did not work well for MSMD because of insufficient number of samples in some clusters. And as such poorly represented spectral types that are too small for a stand-alone calibration were predicted better by the full diverse set.

In case of MSMD study, our clustering analysis revealed that mineralogy is not the only variable responsible for grouping of samples into various clusters which means that other factors like quantity and quality of SOC might be playing a part too. Soils with different mineralogy were sometimes clustered together and likewise samples from same series were sometimes put in different clusters. So just with the information on soil series

it can not be said that the spectra of soils will be different and that the samples belonging to different soil series would require more than one calibration models.

In IR calibration studies most researchers have focused on the accuracy of the technique. However to take the economic advantage of this technique, we also need to evaluate its efficiency. That will determine how many samples have to be analyzed by standard methods and put in the calibration set to get good predictions of the remaining samples. We defined efficiency as the percentage of samples that are not needed in the calibration. We found that for all sets MIR calibrations proved to be slightly more efficient than NIR which means that for MIR calibrations fewer samples have to be analyzed by the standard techniques. Also the efficiency was much better for sets that are less diverse ( $SSSD > SSMD > MSMD$ ).

The study also revealed that the calibration models developed for one soil management study can be used for a new study by adding some samples from the new study to the calibration. If the study for which the model was originally developed is diverse or is similar in mineralogy to the new site, fewer samples from the new study are needed in the updated calibration. If the new study has more sample diversity than the old one, more samples from the new study are needed. However in all three studies we found that it is more efficient to build a calibration model using samples from that site instead of trying to extend an existing model.

In precision agriculture and other soil management studies, in-situ (on-site) measurements are sometimes very desirable. Potential of MIR to make in-situ measurements in soil studies has not been assessed. Therefore in the second part of this study we explored the ability of a sampling technique called ATR (Attenuated Total

Reflectance), which has a potential to be used for direct measurements in the field, to determine SOC. ATR-FTIR is a promising tool for quick assessment of SOC content with little sample preparation other than to dry and grind a subsample of the soil. Using SSSD and MSMD samples, we provided evidence that ATR-FTIR calibrations perform nearly as well as DR-FTIR calibrations. There are still unresolved issues about the best spectral range for quantifications and qualitative analysis of SOC. However two ranges (3900-1240, 1750-540  $\text{cm}^{-1}$ ) were found to perform well for SOC quantitative analysis. The better quality MIR spectral signature in the fingerprint region suggests that ATR may perform better than DR for SOC qualitative analyses. Although our ATR studies utilized a lab based FTIR, the same instrumental components are found in portable instruments. ATR optics is easily sealed, so on-site performance should be comparable to what we achieved in the lab.

## APPENDIX A:

**Conversion of organic matter values to organic carbon values**

10 SSSD samples that were analyzed for both SOC and SOM were used to develop a regression equation between the two parameters. For SOC determination, the soil samples were sent to Agriculture Analytical Laboratory at the Pennsylvania State University. The carbon values were determined using Combustion-Fisons NA 1500 Elemental Analyzer. The SOM was determined in the lab using loss on ignition method. The procedure used is given below:

**Procedure:**

- Weigh crucibles
- Weigh out 5-6 gm of air dried soil in crucibles
- Dry the samples in the crucibles for 2 hours at 105°C
- Record weight of the crucible plus soil
- Preheat the furnace to 360°C and ash samples for 2 hours
- Let the sample cool in a desiccator and record weight

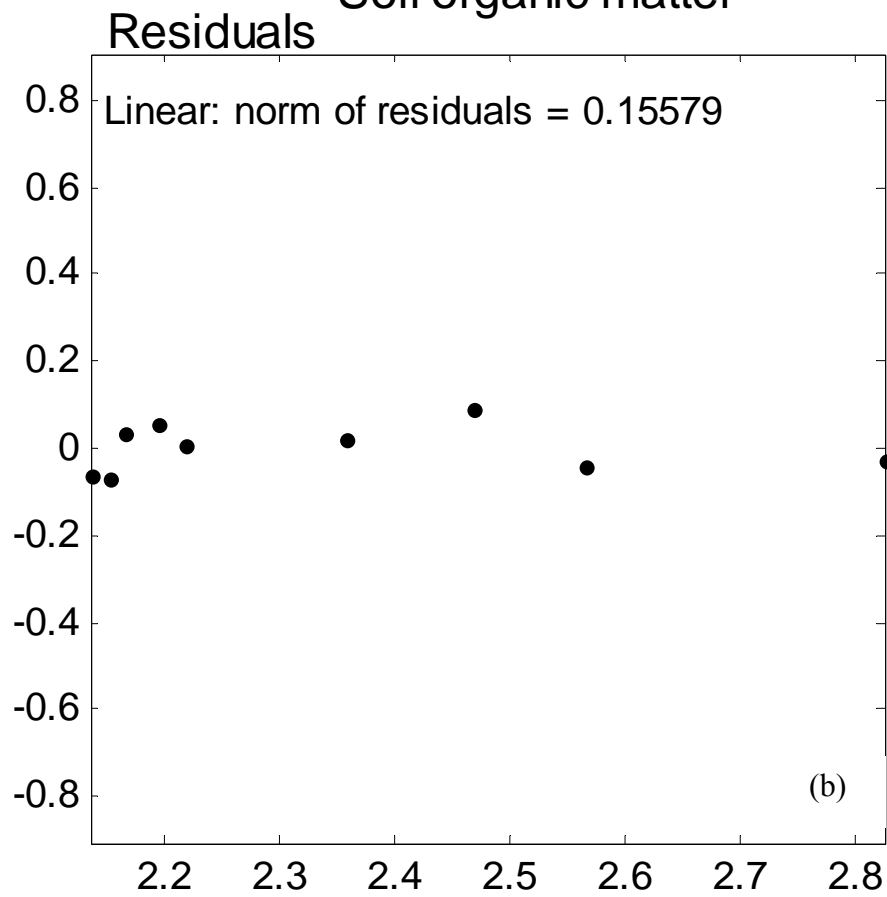
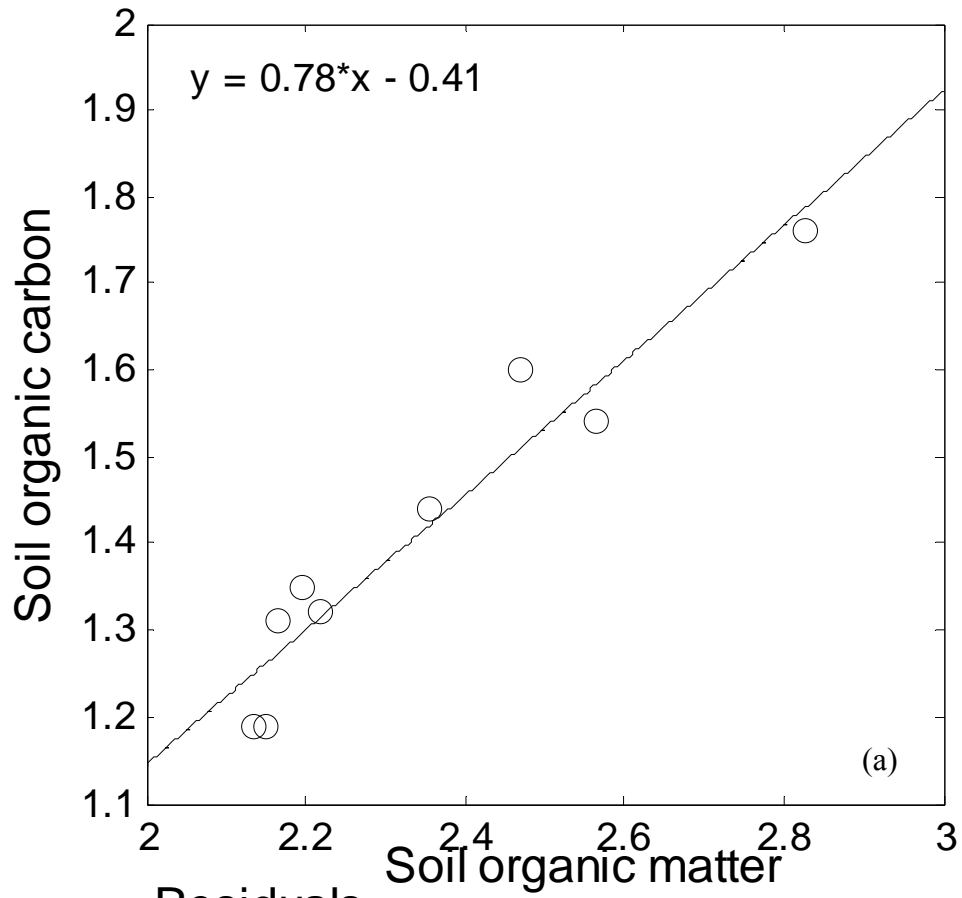
$$\% \text{ Loss on ignition (LOI)} = (\text{wt at } 105^{\circ}\text{C} - \text{wt at } 360^{\circ}\text{C}) \cdot 100 / (\text{wt at } 105^{\circ}\text{C} - \text{crucible wt})$$

$$* \% \text{ Organic matter} = -0.23 + (0.8 \times \% \text{LOI})$$

\*This is a generalized equation used for Pennsylvania soils to convert LOI to organic matter, developed by relating the LOI and Walkely Black procedure results.

After getting both SOC and SOM values for the samples, regression analysis was used to determine the regression equation and correlation between the two methods.

Except for the one outlier, regression line between SOM and SOC values suggested that the two values had a good linear relationship with slope equal to 0.78 and intercept equal to -0.41 (Figure 1 ). The Pearson correlation coefficient between the SOM and SOC values was found to be 0.96. For most HRE samples the SOM values range from 2.2-2.8% (Figure 1 (c)). Thus the fitted line equation developed on a sub-set of HRE soils can be used for to calculate SOC for all HRE soil samples.





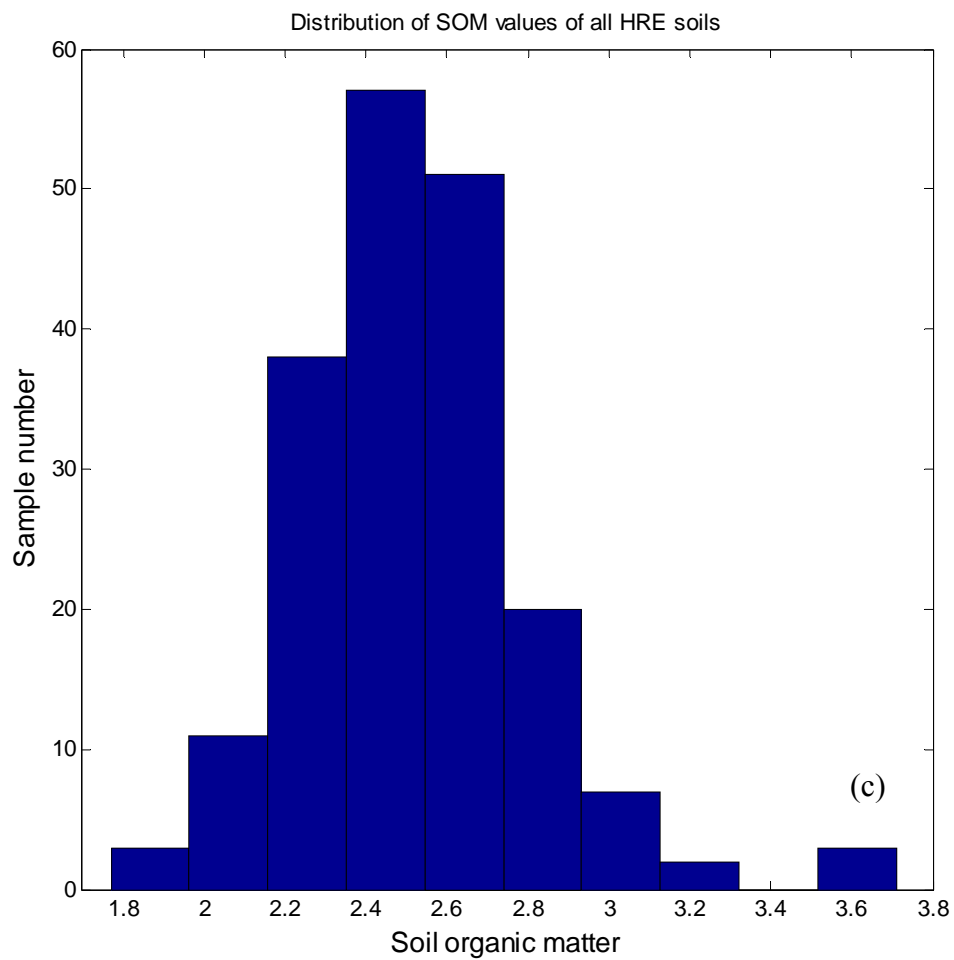


Figure 1 (a) Fitted line between SOM and SOC values for a sub-set of HRE soils (b) Difference between SOC values and corresponding fitted values (c) SOM distribution of all the SSSD soils

## APPENDIX B

### **KCl dilution experiment to get true absorption spectrum of soil**

**Objective:** To obtain a true DRFTIR spectra of soil and assess the spectral distortions of the strongly absorbing bands (especially at  $1100\text{ cm}^{-1}$ ) that result from scanning neat soils

Three soil specimens, high, low and medium in carbon were selected from the SSSD set. Samples were ground very fine with mortar and pestle and dried at  $105^{\circ}\text{C}$  for 2 hours. Each sample was run in triplicate. Samples were diluted to get 1, 2, 4 and 8% soil in potassium chloride (KCl). Besides this, 6 replicates were run of pure salt and the three neat soils. Samples were loaded in 96 well diffuse reflectance plate and analyzed by DR-FTIR.

DR-FTIR spectra revealed that diluting samples with KCl eliminated the appearance of inverted peaks in the region of  $1100\text{ cm}^{-1}$ . 1% and 2% soil spectra looked more similar to salt spectrum than 4 and 8%. C-H stretch region near  $2800\text{ cm}^{-1}$  and other small peaks around  $2400\text{ cm}^{-1}$  were more evident at soil level of 4 and 8% than the lower levels.

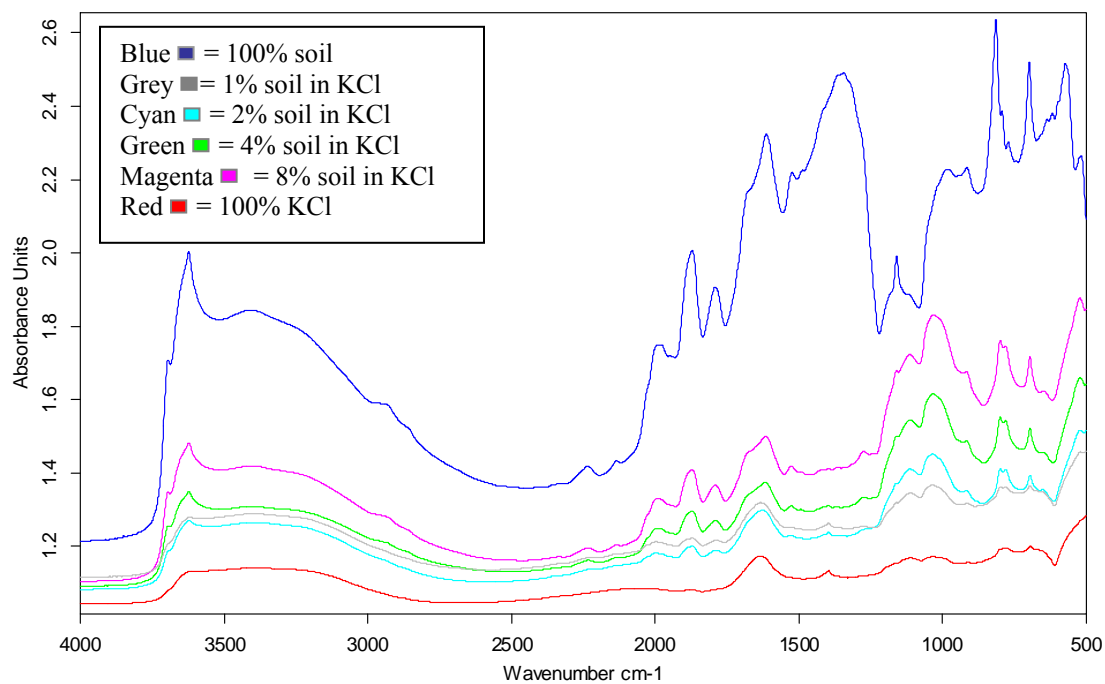


Figure 1: Spectra of salt, 100% soil and soil at different dilution level with KCl

For the spectral subtraction of salt spectrum from salt +soil spectrum, 4% soil dilution treatment was selected.

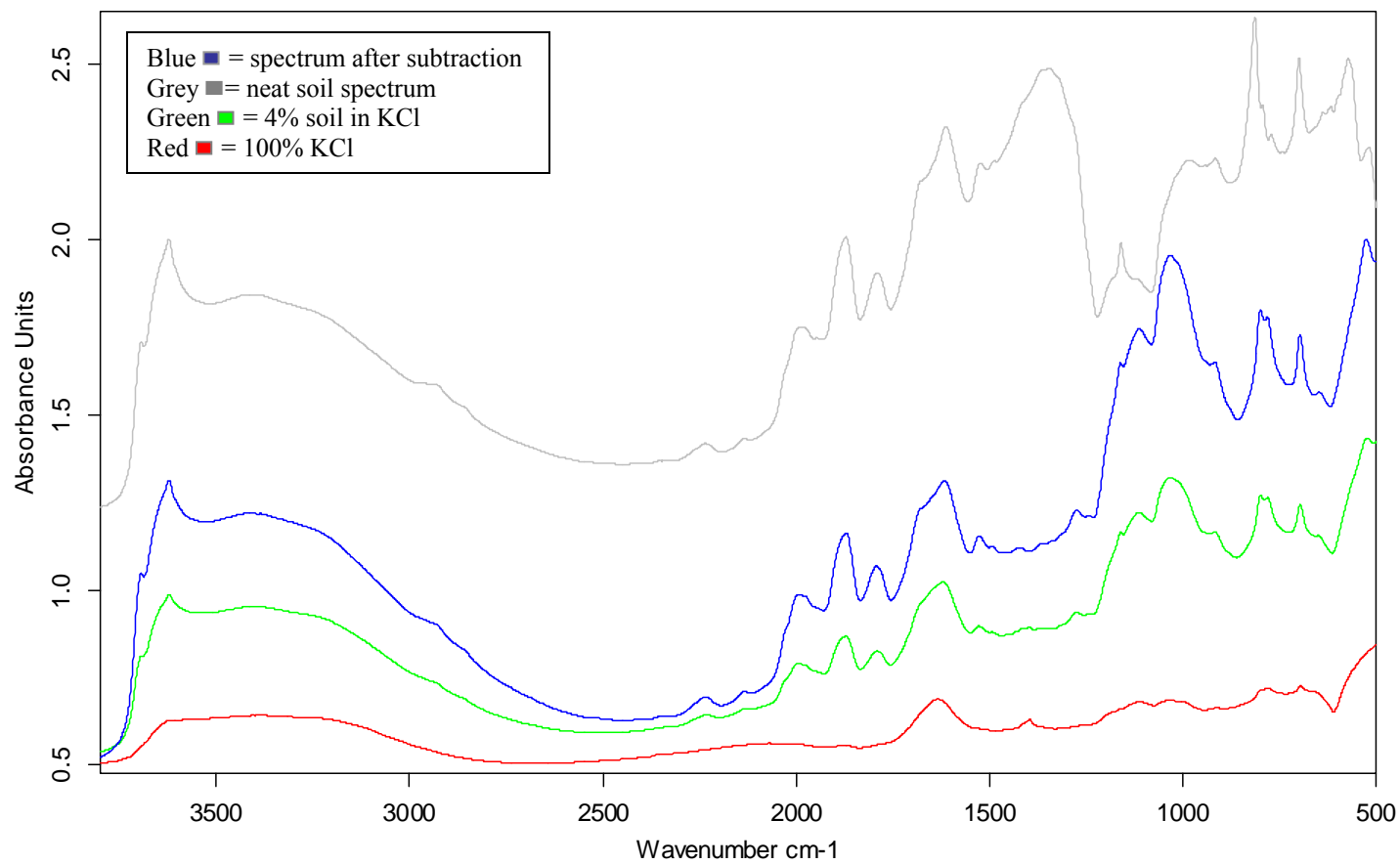


Figure 2: Spectral subtraction of salt from 4% soil mixture

## APPENDIX C

### Identifying the soil spectrum peaks by addition of different soil components

The experiment was conducted in order to identify peaks found in the MIR spectrum of soil. Some organic and inorganic compounds that are known to be found in soils were mixed with a soil sample and the spectra thus obtained were compared with the spectrum of neat soil. Enhancement of any neat soil peak when a particular compound is added would just the association between that peak and the compound.

For this purpose, a soil sample was selected from SSSD set. The OM content of this soil was 2.5%. Assuming that humic acid (HA) forms about 70% of OM content in soils, this soil sample should have around 1.75% HA. Polysaccharides and other proteins form 30% of OM, so the soil should have about 0.75% of these compounds.

#### Procedure

- HA, agar and pepsin were weighed out and mixed with known weight of soil to form mixtures of 1.75% HA and soil, 0.75% agar and pepsin in soil.
- Soil was also mixed with silica and gibbsite in the 80:20 ratio
- A blank was prepared which contained only soil
- Small amount of water was added to the vials containing these samples and the vials were put in the oven at 50°C overnight. Since pepsin is known to destroy some soil proteins, no water was added to soil-pepsin mixture to prevent any reaction.
- When the samples were dried, they were loaded in the DR plate for scanning
- Additionally all these samples were diluted with KCl salt to 4% and scanned

- Pure samples of soil alone, salt, silica and gibbsite were also run

#### Results:

With the addition of organic compounds including HA, agar and pepsin, there was enhancement of peaks in the regions 2938, 2853 (C-H stretch region) and also in the region  $1620\text{ cm}^{-1}$  (Figure 1) which has been reported to be associated with C=C stretching. Spectra obtained by subtracting soil spectrum from soil + organic compound spectra (Figure 4) confirmed the presence of organic peaks in this region. However the organic signature is very small compared to some other regions of the spectrum which are mostly due to inorganic components (Figure 2).

Addition of silica resulted in a new peak at 3550 and enhancement of peak at  $3623\text{ cm}^{-1}$  (Figure 3). In the literature these peaks have been reported to be associated with silicate vibrations. Enhancement of peaks near  $3610\text{ cm}^{-1}$  by the addition of gibbsite has been reported to be associated with the hydroxyl stretching caused by minerals including gibbsite (Madari et al., 2006).

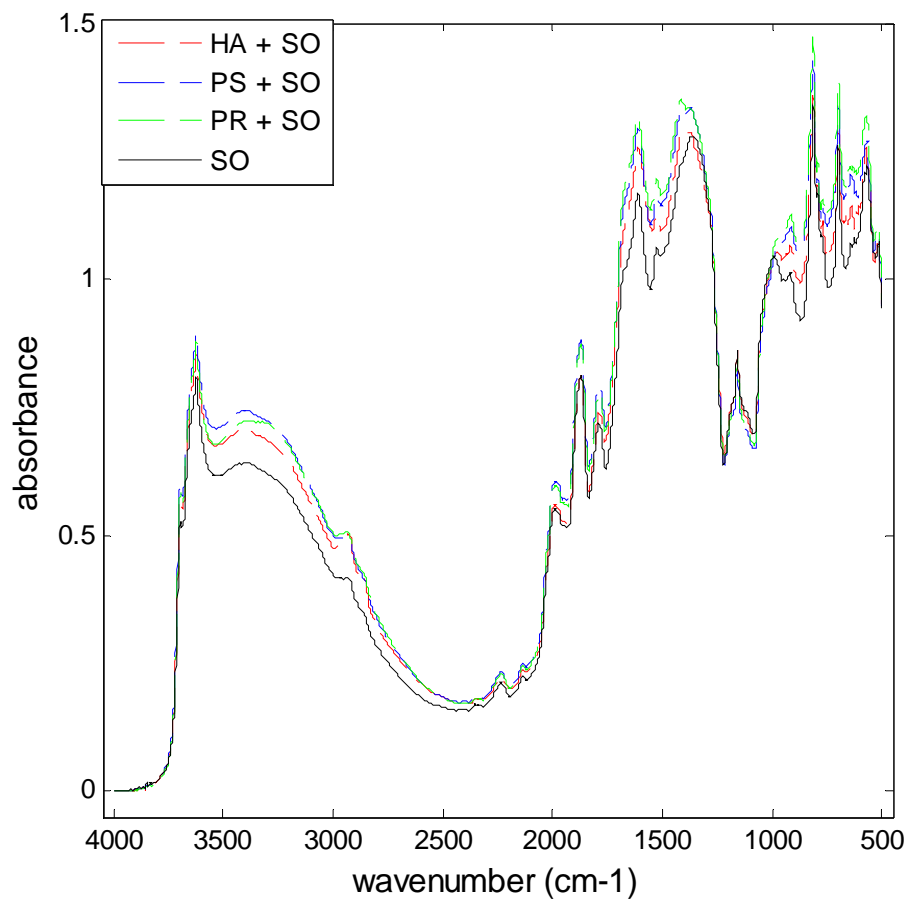


Figure 1: Changes in the soil spectral features after addition of organic compounds including humic acid (HA), a polysaccharide, agar (PS) and a polypeptide, pepsin (PR)

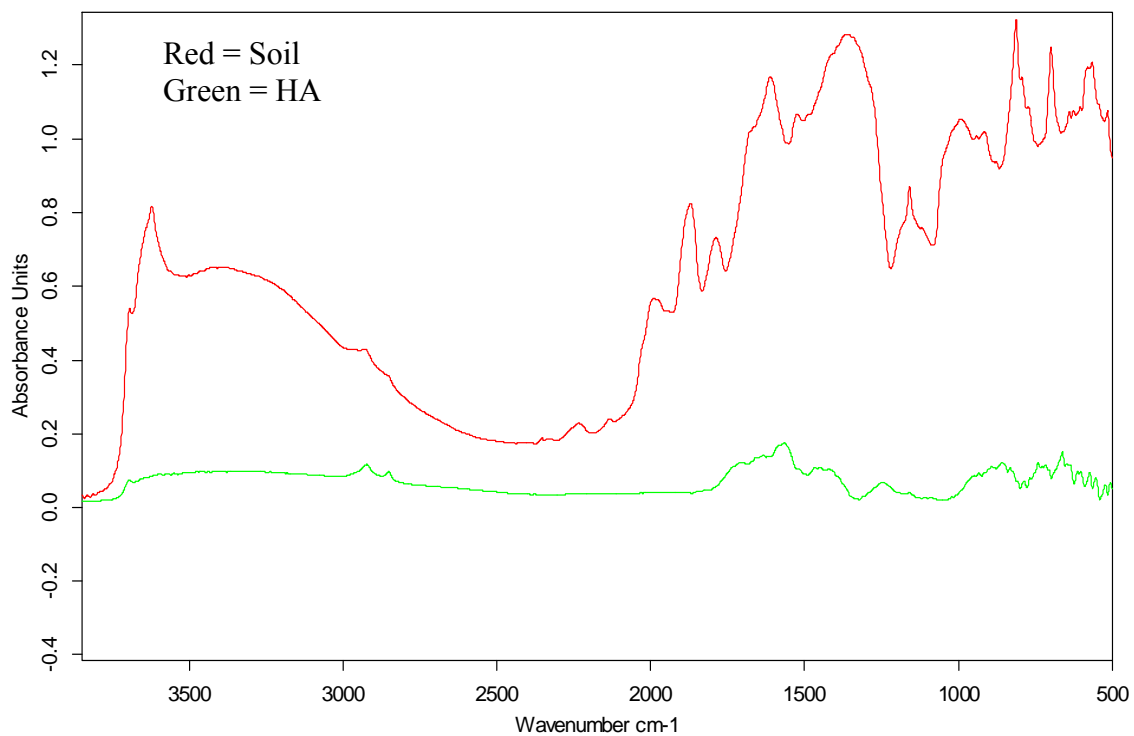


Figure 2: Spectrum of humic acid illustrates the small magnitude of the organic components as compared to the large mineral peaks found in the soil spectrum



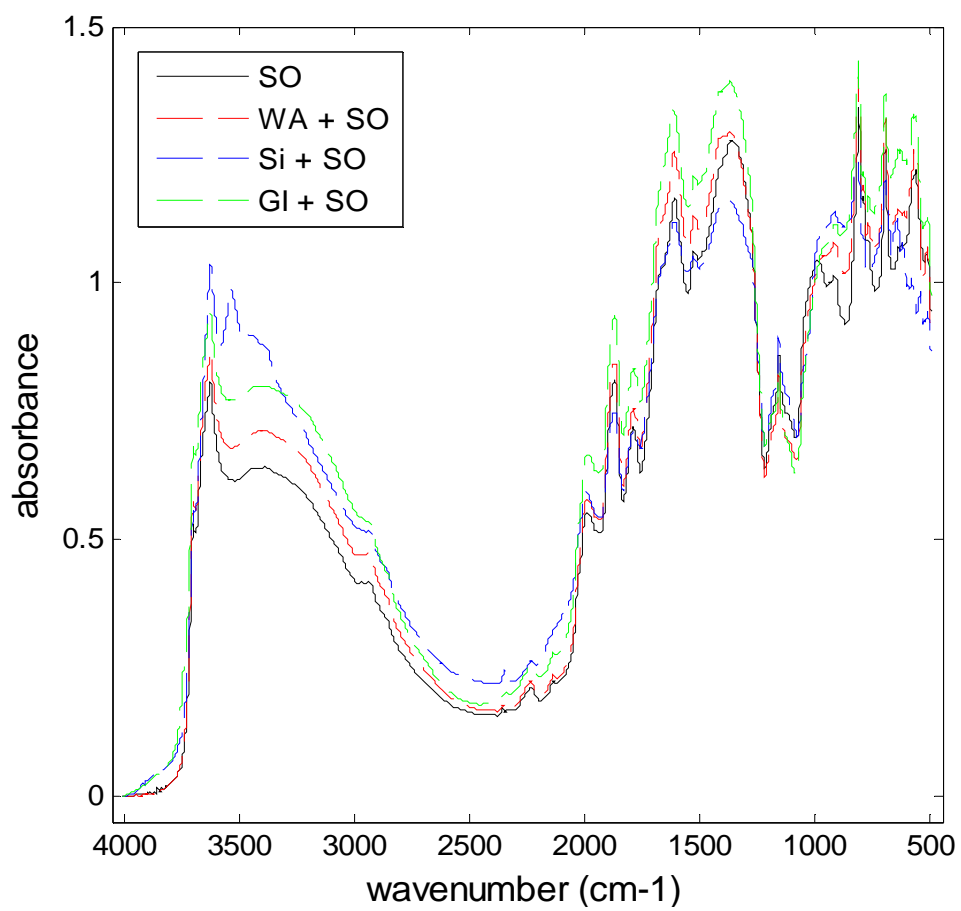


Figure 3: Changes in the soil spectral features after addition of organic compounds including water (WA), silica gel (Si) and gibbsite (GI)

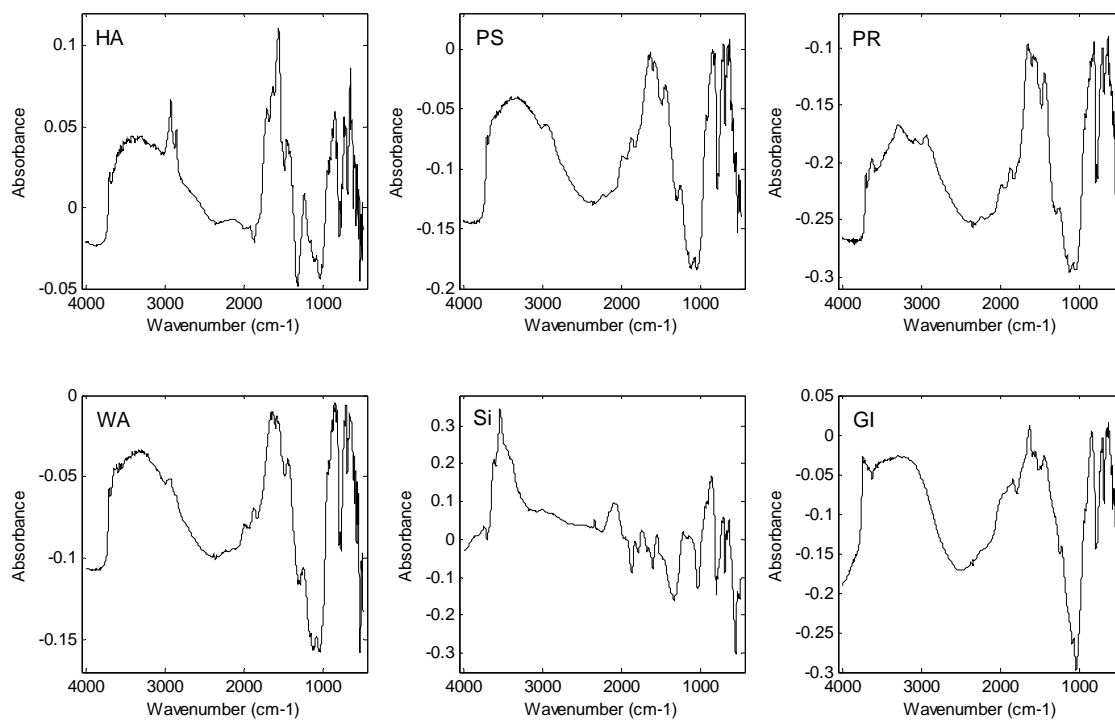


Figure 4. Spectral signature of different organic and inorganic components added to the soil obtained by subtracting soil spectrum from the spectrum of soil + added component.

## APPENDIX D

### Prominent soil minerals found in different soil series used for MSMD and SSMD study

Data shown in Tables 1 and 2 has been taken from Penn State Soil Characterization laboratory website (The Penn State Soil Characterization Lab, 2008). This data was collected on soils sampled from various locations across Pennsylvania (Ciolkosz et al., 1998). Distribution of different minerals in various soil series that were used for MSMD sampling is provided in the Table 1. The values were averaged across different soil series pedon depths (equally weighted) of sampling and are a good illustration of the fact that mineralogy of various soil series could be very different from each other.

Series Name	Kaolinite	Illite	Vermiculite	Montmorillonite	Chlorite	Quartz
Allenwood	16.97	45.15	17.12	1.06	3.48	0.00
Athol	25.74	47.04	12.22	2.78	1.67	1.30
Atkins	23.67	31.17	18.83	0.00	0.08	21.75
Bedington	13.70	51.37	30.04	0.21	0.12	2.78
Berks	48.57	40.00	7.14	0.00	0.00	2.14
Duffield	15.56	48.89	22.78	3.89	2.22	0.00
Edom	47.14	50.66	13.55	2.03	0.64	1.40
Elliber	8.42	43.16	31.05	0.53	2.63	0.26
Gilpin	4.29	66.79	16.43	1.07	6.07	0.36
Glenelg	0.56	53.89	15.83	0.28	7.22	0.00
Hagerstown	12.92	63.33	15.00	1.67	1.25	1.25
Hublersburg	5.83	31.67	1.67	0.00	5.00	0.00
Manor	1.62	66.42	22.36	1.28	4.59	0.41
Opequon	32.19	34.38	24.69	0.63	0.63	0.63
Shelmadine	13.97	36.18	19.85	9.85	11.47	2.35
Volusia	22.86	40.00	17.14	1.43	9.29	0.00
Watson	22.12	46.86	20.34	2.46	1.69	2.63
Weikert	11.09	56.74	23.70	0.00	5.65	0.33

Table 1: Percentage of different minerals found in some soil series from which the samples were obtained for MSMD study.

Samples for SSMD study were collected from 3 different soil series and from 3 different depths. Table 2 shows the mineralogy difference in the A and B horizons of these soil series. The values are percentage of minerals averaged over various depths of horizon A and B and reveal that even within the same soil series, mineralogy can vary with the depth of sampling.

Soil Series	Horizon	Kaolinite	Illite	Vermiculite	Montmorillonite	Chlorite	Quartz
Hagerstown	A	20	33	27	1	3	10
	B	24	49	19	1	2	3
Hublersburg	A	28	33	21	1	3	13
	B	24	44	15	3	3	10
Opequon	A	5	63	20	0	4	8
	B	4	67	16	2	7	4

Table 2: Percentage of different minerals found in the A and B horizons of three soil series from which the samples were obtained for SSMD study.

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State University, [Online WWW]. Available URL: <http://soilislife.psu.edu/>

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