

LARS Technical Report 013090

NASA Grant NAGW-925 *EOS*

NASA Grant NAGW-1472 *Applications Miami Co*

**USE OF HIGH-DIMENSIONAL
SPECTRAL DATA TO EVALUATE
ORGANIC MATTER - REFLECTANCE
RELATIONSHIPS IN SOILS**

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HQ. GRANT
IN-43-CR

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1668

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(NASA-CR-186650) USE OF HIGH-DIMENSIONAL
SPECTRAL DATA TO EVALUATE ORGANIC MATTER,
REFLECTANCE RELATIONSHIPS IN SOILS (Purdue
Univ.) 166 p CSCL 05B

N90-24665

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This research was supported by National Aeronautics and Space Administration Grants NAGW-925 and NAGW-1472.

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ABSTRACT

Henderson, Tracey Leslie. M.S., Purdue University, May 1990. Use of High-Dimensional Spectral Data to Evaluate Organic Matter - Reflectance Relationships in Soils. Major Professor: Marion F. Baumgardner.

Recent breakthroughs in remote sensing technology have led to the development of a spaceborne high spectral resolution imaging sensor, HIRIS, to be launched in the mid-1990s for observation of earth surface features. If the high-dimensional HIRIS data are to be applied successfully to soil survey, a thorough understanding of the factors which influence narrow-band soil reflectance, including organic matter, will be essential. This research was conducted to evaluate the effects of organic carbon content on soil reflectance over the spectral range of HIRIS, and to examine the contributions of humic and fulvic acid fractions to soil reflectance.

Organic matter from four Indiana agricultural soils was extracted, fractionated, and purified, and six individual components of each soil were isolated and prepared for spectral analysis. The four soils, ranging in organic carbon content from 0.99% to 1.72%, represented various combinations of genetic parameters such as parent material, age, drainage, and native vegetation. An experimental procedure was developed to measure reflectance of very small soil and organic component samples in the laboratory, simulating the spectral coverage and resolution of the HIRIS sensor. Reflectance in 210 narrow (10 nm) bands was measured using the CARY 17D spectrophotometer over the

400-2500 nm wavelength range. Reflectance data were analyzed statistically to determine the regions of the reflective spectrum which provided useful information about soil organic matter content and composition.

Wavebands providing significant information about soil organic carbon content were located in all three major regions of the reflective spectrum: visible, near infrared, and middle infrared. Although reflectance in the visible bands (425-695 nm) had the highest correlation ($r = -0.991$ or better) with organic carbon content among the soils with the same parent material and age, these bands also responded significantly to iron and manganese oxide content. For soils formed on different parent materials, five long middle infrared bands (1955-1965 nm, 2215 nm, 2265 nm, 2285-2295 nm, and 2315-2495 nm) gave the best correlation ($r = -0.964$ or better) with organic carbon content. Several near and middle infrared bands were identified in which the soils were separable, but the reflectance response was dominated by other soil factors which masked the organic matter effect, indicating that choice of wavebands should not be based on spectral curve separability alone. Although each individual organic fraction had a distinctive curve shape, no diagnostic features were identified in the spectral curves of the unfractionated soils which provided information about organic matter composition. The purified humic acid fractions of the four soils were separable in six bands in the 1600-2400 nm range, suggesting that longwave middle infrared reflectance may be useful as a non-destructive laboratory technique for humic acid characterization. The results of this study indicated the potential of HIRIS data to provide information about soil organic carbon content.

INTRODUCTION

Over the past two decades, high-altitude remote sensing has emerged as important technology for monitoring the earth's agricultural resources. Recent breakthroughs in imaging spectrometry have led to plans for a new high spectral resolution sensor, HIRIS, to be launched on a satellite platform in the mid-1990s for observation of earth surface features. The high-dimensional spectral data generated by HIRIS will provide the first opportunity to characterize detail in soil spectral curves, across contiguous narrow wavebands throughout the reflective portion of the spectrum, from satellite altitudes. If HIRIS data are to be applied successfully to soil survey, a thorough understanding of all factors influencing narrow-band soil spectral reflectance will be essential.

Accurate information about soil organic matter is considered very important for agricultural production, environmental studies, and research on the role of soils in the global carbon cycle. Although soil organic matter is a vital agricultural resource, it has not been adequately mapped over vast areas of the world, particularly in developing countries. The HIRIS sensor, with its capability to detect reflected energy from the earth's surface over large geographic areas, repeatedly over time, promises to facilitate long-term monitoring of the status of organic matter in the world's soils. However, if the enormous quantities of high-dimensional data which HIRIS will generate are to be

put to productive use for organic matter studies, more knowledge about how organic fractions influence soil reflectance in specific wavebands over the spectral range of HIRIS will be necessary.

While it is well known that increasing soil organic matter content causes an overall decrease in soil reflectance, the effects of different levels of organic matter content, and of individual soil organic fractions, on soil reflectance in specific narrow wavebands are not completely understood. Many previous studies of soil organic matter - reflectance relationships considered reflectance only in arbitrarily-chosen regions of the electromagnetic spectrum. Other research identified optimal bands for organic matter - reflectance studies based solely on statistical analysis of soil reflectance data, without consideration of the specific influences of mineral and organic soil materials on reflectance in the chosen bands. This study was designed to identify the important spectral bands in which reflectance data provide useful information about soil organic matter, basing band selection on the physical nature of the reflectance response as well as on statistical analysis.

This research was conducted to evaluate the effects of organic carbon content on soil reflectance over the spectral range of HIRIS, and to examine the contributions of humic and fulvic acid fractions to soil reflectance. The study did not attempt to develop a prediction equation for soil organic carbon content or composition from spectral data, but rather attempted to describe patterns in the relationships between organic matter and high spectral resolution reflectance for selected soils. An understanding of these relationships will provide insight on the potential of HIRIS data to supply information about soil organic

matter.

The objectives of this study were:

1. To develop a method to study organic matter - reflectance relationships in soils, based on narrow-band spectral properties of soil organic matter and organic matter fractions.
2. To identify the regions of the spectrum which provide useful information about soil organic carbon content and organic matter composition, using the spectral range and resolution proposed for the HIRIS sensor.
3. To evaluate the effects of organic carbon content on soil reflectance over the spectral range of HIRIS.
4. To evaluate the effects of organic matter composition on spectral reflectance of soil and organic matter fractions.

It is hoped that the results of this research may contribute a very small step toward the peaceful and productive use of remote sensing technology, leading to wiser stewardship of our agricultural resources and a better understanding of our global environment.

LITERATURE REVIEW

Soil Organic Matter

Agricultural and Environmental Significance

Soil organic matter is a highly complex, heterogeneous soil constituent. Although it comprises only a small percentage of most soils by weight, agriculturally organic matter is an extremely important soil component, influencing soil fertility and structure. It serves as a source and sink for plant nutrients, particularly N, P and S, and as an energy source for soil organisms. Colloidal organic matter is responsible for 20% to 70% of the cation exchange capacity (CEC) of the surface horizons of many soils (Stevenson, 1982). Organic matter is beneficial for maintaining good soil aggregation, aeration, water-holding capacity, and permeability, and so causes soils to be less susceptible to erosion. Also important is the adsorption of certain agricultural chemicals onto organic matter molecules (Stott et al., 1983; Martin and Stott, 1981; Khan, 1978). For this reason, application rates of many herbicides are directly dependent on soil organic matter content.

Soil organic matter is also significant in an environmental sense. Its influences on pesticide transport, soil erosion, and water quality are of particular interest to environmental scientists. Soil organic matter also has an important role in the global carbon cycle. Bohn

(1976) hypothesized that the decay of organic material in the soil is one of the largest CO₂ inputs to the atmosphere, and that soil organic matter is, by far, the greatest reserve of carbon at the earth's surface. Post et al. (1982) estimated the organic carbon content of the world's soils to be 1.395×10^{12} kg. In a more recent study, Bouwman (1989) estimated the amount of carbon in soil organic matter and decaying plant material to be two to three times that in the living biomass. Woodwell et. al (1978) hypothesized that accelerated degradation of soil humus may release as much CO₂ into the atmosphere as harvesting the world's forest reserves. Accelerated loss of soil organic matter is of interest to scientists studying atmospheric CO₂ changes (Franzmeier et al, 1985), and is being investigated as a possible important factor in global warming or the "greenhouse effect" as part of the multidisciplinary research on global change sponsored by the International Geosphere-Biosphere Program of the International Council of Scientific Unions (Bouwman, 1989). A schematic representation of the role of humic substances in the global carbon cycle is shown in Figure 1. Also of environmental significance is the ability of certain organic matter fractions to bind with, transport, or concentrate toxic compounds and heavy metals, possibly contributing to groundwater and surface water contamination (Schnitzer, 1982).

Given its important agricultural and environmental roles, a precise means of determining soil organic matter content is essential. In the laboratory, since organic matter content cannot be measured directly, soil is generally analyzed for organic carbon content, which is multiplied by a constant factor (usually 1.72 to 2.00) for conversion to organic matter content (Nelson and Sommers, 1982). Two laboratory-

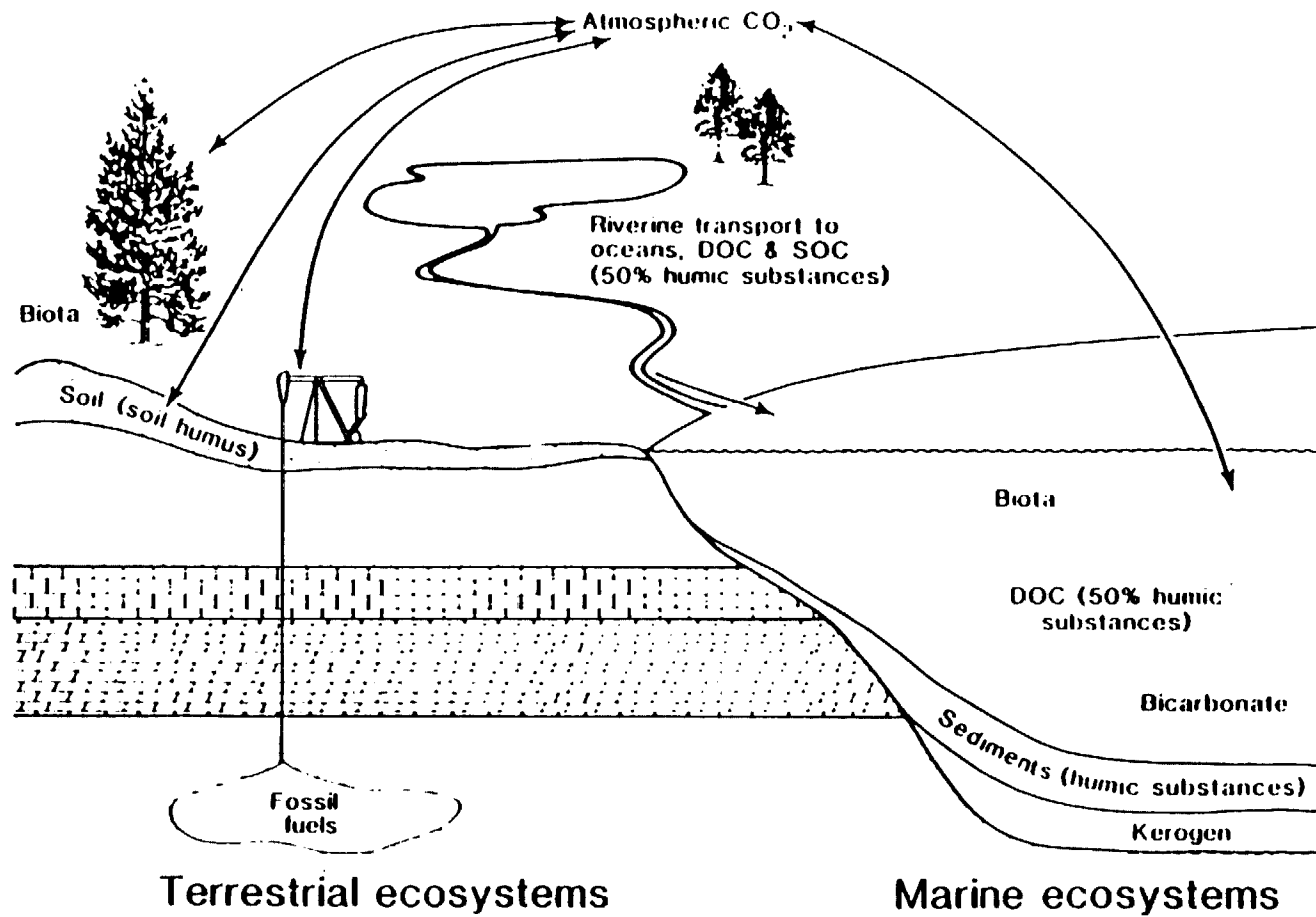


Figure 1. Diagram of the global carbon cycle, indicating the importance of humic substances (Aiken et al., 1985). DOC refers to dissolved organic carbon; SOC to soil organic carbon.

based methods in common use are dry combustion with an automatic carbon analyzer and the Walkley-Black method of wet combustion (Nelson and Sommers, 1982). These methods are relatively time consuming, particularly if one wishes to sample over a large geographic area, or to analyze enough samples to account for spatial variability of organic matter content in the field. Long-term monitoring of soil organic matter content over large geographic areas is important for environmental studies, and real-time determination of large numbers of samples in the field is required if organic matter content is to be used effectively to establish herbicide rates according to soil properties within a field. For these reasons, methods of remote determination of soil organic matter content based on measurement of soil spectral reflectance have been investigated by numerous researchers. Results of these investigations will be described in a later section.

Definitions and Terminology

Considerable confusion exists in the terminology associated with organic matter. Strictly speaking, soil organic matter refers to all of the organic components present in soil, including the living flora and fauna, undecayed plant and animal tissue, partially decomposed organic residues, and fully decomposed material. However, there is no single, universally accepted definition of soil organic matter. Stevenson (1982) rejected the strict definition above, and considered only the fully decomposed organic material in his definition of organic matter, excluding the living biomass and both undecayed and partially decayed tissues. He used the term "humus" synonymously with organic matter, and divided humus into two major components: humic and non-humic substances.

Humic substances are defined by Stevenson as a series of high-molecular-weight, colored substances formed by secondary synthesis reactions, which are distinctive to the soil or sediment environment. Compounds in humus which belong to well-known classes of organic chemistry, including amino acids, carbohydrates, and lipids, are considered to be non-humic substances.

Stevenson's use of the terminology, with the possible exception of his definition of organic matter, is generally accepted by many current researchers in soil chemistry and microbiology. However, others disagree with his definition scheme, so some confusion still exists. For example, Kononova et al. (1966) used the strict definition of organic matter described above, while several others (Tate, 1987; Vaughan and Ord, 1985; Tan, 1982) included undecayed and partially decayed tissues in their definitions, excluding only the living biomass. Many researchers (Sposito, 1989; Kononova et al., 1966; Hayes and Himes, 1986; Schnitzer, 1982; Martin and Haider, 1986; Stott and Martin, 1990) accepted Stevenson's definition of humus as the fully altered biomass, while Vaughan and Ord (1985) included fresh and partially decayed debris in their humus concept. Tan (1982) used an even narrower definition of humus as the decomposed material which does not possess characteristic chemical structure. Many others, including Kononova, Schnitzer, Sposito, and Vaughan and Ord, agreed with Stevenson, and used the term "humic substances" to describe this material, which is resistant to further biological attack. Humic substances exclude what Sposito refers to as the "biomolecules" (proteins, carbohydrates, peptides, amino acids, fats and waxes), which are susceptible to further microbial degradation.

An understanding of the terminology is essential for evaluation of organic matter research. The lack of precise, universally accepted definitions of specific materials included in organic matter, humus, and humic substances makes comparison of research results published by different scientists quite difficult. For the original research presented in this thesis, the terms soil organic matter, humus, and humic substances will be used according to the definitions given in Table 1. Most of these definitions are based on terminology used by Stevenson (1982), with minor modifications.

Extraction, Fractionation and Purification

Many studies of organic matter constituents require extraction of the fully decayed organic matter (humus) from the soil, followed by isolation and purification of humus fractions. A variety of extractants have been used to remove organic material from soils, including NaOH, $\text{Na}_4\text{P}_2\text{O}_7$, and mixtures of the two; chelating resins, and various organic solvents such as DMSO and acetone (Dormaar, 1972; Piccolo, 1988; Stevenson, 1982; Kononova et al., 1966; Schnitzer, 1982). Extractable organic matter generally consists of approximately 65% to 75% humic substances, the amorphous, colored materials which are chemically complex, resistant to microbial attack, and may range in molecular weight from several hundred to several thousand. The remainder is composed of non-humic substances, with still recognizable chemical structures and a short survival span, mainly polysaccharides and proteins (Stott and Martin, 1990; Schnitzer, 1982). The initial crude organic matter extract is often accompanied by a good deal of ash,

Table 1. Organic matter terminology and definitions used for research presented in this thesis. (Modified from Stevenson, 1982)

<u>Term</u>	<u>Definition</u>
Soil Organic Matter	Total of the organic compounds in soil, including undecayed and partially decayed tissues, but excluding the living biomass.
Soil Biomass	Organic material present as living tissue.
Organic Residues	Undecayed plant and animal tissues and their partial decomposition products.
Humus	Total of the organic compounds in soil, excluding undecayed and partially decayed tissues and the soil biomass.
Humic Substances	A series of high-molecular-weight, colored substances, which are distinctive to the soil or sediment environment and are resistant to further microbial degradation.
Non-humic Substances	Compounds belonging to known classes of biochemistry, which are susceptible to further microbial degradation. Includes polysaccharides.
Humic Acid	The dark-colored, alkali extractable humic substance which is insoluble in acid.
Fulvic Acid	The colored, alkali-extractable humic substance which remains in solution after removal of humic acid by acidification. (Does not include polysaccharides.)

depending on the method of extraction (Piccolo, 1988). Extraction efficiency also depends on the method employed.

After organic matter extraction, humic substances may be separated into component fractions based on solubility characteristics. Humic acid (HA), a very dark material which is soluble in alkali but precipitates in acid, has a high molecular weight, averaging between 50,000 and 100,000. Fulvic acid (FA) refers to the pigmented element of the acid-soluble fraction, and is yellow to red-brown in color, soluble in both acid and alkali, and has a much lower molecular weight, typically ranging from 500 to 2000 (Stevenson, 1982). An ethanol extraction of HA will yield hmatomelanic acid, but it is doubtful that this fraction is a distinct chemical entity (Stevenson, 1982). Humic acid may be further fractionated into brown and gray humic acids, based on optical properties and behavior toward electrolytes. A final component of soil humus is humin, the portion of the decayed organic material which is insoluble in alkali and remains with the mineral soil after alkali extraction. Humic acid, fulvic acid, and humin are the principal humic fractions. It has been estimated that in most soils, humic substances are distributed as approximately 50% humin, 40% humic acid, and 10% fulvic acid (Chiou, 1989). Soil polysaccharides, which are colorless and mainly associated with the fulvic acid fraction, comprise the second most abundant component of humus, after humic substances (Foster, 1981). Polysaccharides constitute an estimated 10% to 30% of soil humus (Cheshire, 1977). A diagram of the general extraction and fractionation scheme is shown in Figure 2.

Early researchers in soil organic matter chemistry believed that the individual humic substances were chemically distinct compounds of

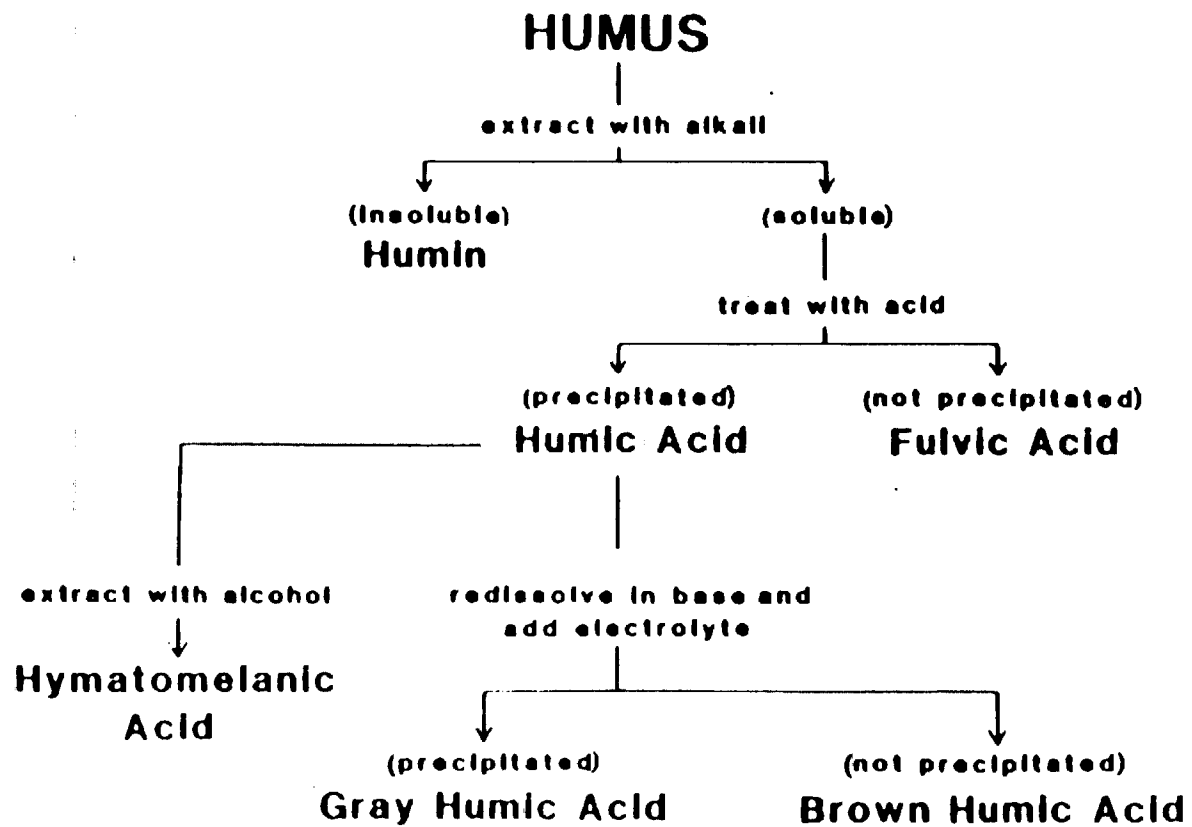


Figure 2. General scheme for extraction and fractionation of humic substances (Stevenson, 1982).

specific composition. A good review of early work in this area is given by Stevenson (1982). However, it is now generally accepted that the humic substances are heterogeneous mixtures of many compounds having similar properties, rather than pure compounds (Aiken et al., 1985). Humic and fulvic acid, separated on the basis of solubility characteristics, are thought to be homogeneous in composition. Differences in their properties are attributed primarily to molecular weight, with less pronounced variation in elemental composition and functional group content (Schnitzer, 1982; Tan, 1982). It has also been theorized that the humin fraction is not chemically distinct, but rather consists of portions of humic and fulvic acid which are so closely bound to mineral particles that they cannot be extracted with alkali (Stevenson, 1985).

Even after alkali extraction and fractionation, humic substances are not easily separated from non-humic materials (Stevenson, 1982). Both inorganic material (ash) and biomolecules are removed with the humic and fulvic acid fractions. Since presence of these contaminants affects molecular weight, functional group and elemental analyses, and absorption spectra of the material (Piccolo, 1988), samples are often purified before any further characterization is attempted.

Purification of humic acid is generally accomplished by repeated treatment with a solution of HF-HCl to remove mineral impurities, mainly hydrated silicate clays. However, this treatment may result in some chemical changes, including an increase in the high molecular weight fraction in purified samples (Piccolo, 1988). Fulvic acid is most commonly concentrated and purified by passing the solution through a column of XAD-8 resin, which is used to separate "generic FA" from the

polysaccharides, proteins, and other biomolecules (Stevenson, 1982; Aiken, 1985; Thurman and Malcolm, 1981). Both purification steps also result in yield loss.

Characterization of Organic Fractions

The most commonly used information about soil organic matter is the percent of organic carbon present in the soil. However, many researchers have attempted to further characterize organic matter fractions according to elementary composition, functional groups, molecular weight, humic to fulvic acid ratio, and optical properties. Methods used include elemental and functional group analysis, infrared absorption spectrometry, pyrolysis-mass spectrometry, electron spin resonance, and nuclear magnetic resonance spectrometry (Stott and Martin, 1990). One way of evaluating organic fractions is the " $E_4:E_6$ ratio", or ratio of the spectral absorbance values at the 465 nm and 665 nm wavelengths (Chen et al., 1977). The $E_4:E_6$ ratio has traditionally been employed to examine differences in molecular sizes of humic extracts (Piccolo, 1988). The value is dependent on pH and sample purity, is independent of concentration, and is inversely related to molecular weight. Attempts to characterize soil organic matter fractions using visible and infrared spectral reflectance have been very limited.

Organic matter characterization studies have significance for several disciplines within soil science, including soil genesis and mineralogy. For example, Goh and Williams (1979) studied changes in the molecular weight distribution of soil organic fractions in three chronosequences, and found significant differences in organic matter

characteristics during soil development as a function of time. Kodama and Schnitzer (1977) found that the concentration of fulvic acid can influence crystallization of ferrihydrite to goethite and hematite, with low FA concentrations favoring hematite and high concentrations completely inhibiting crystallization. In a study of tropical volcanic soils, Griffith and Schnitzer (1975) found that fulvic acid - silica complexes inhibited degradation of organic matter, causing extremely infertile soils.

Chiou (1989) reported that soil humin has been shown to be three times more effective in sorption of certain organic chemicals than humic acid, with fulvic acid sorption much weaker than the other two. Nagel (1989) used humic acid equivalence (Mehlich, 1984) to relate humic acid content to soil color and pesticide adsorption. Dormaar (1983) investigated the role of organic fractions in aggregate stability. These examples illustrate the importance of organic matter characterization for studies involving soil chemistry, groundwater contamination and erosion control. As most existing laboratory methods for organic matter characterization are very time consuming and require expensive equipment, any means of remote evaluation of organic matter composition would be advantageous.

Many difficulties exist in evaluating results of organic matter characterization studies. First is the lack of standardized procedures for extraction, fractionation, purification and characterization of humic substances (Aiken et al., 1985). This problem is further complicated by the lack of universally accepted definitions of soil organic components. Another factor is the extremely heterogeneous nature of soil organic matter, even within a field site (Tate, 1987).

Finally, the lack of statistical information about the variability of humic substances may lead to incorrect generalizations from uncorrelated experiments (Orlov, 1972). These problems need to be resolved before direct comparisons of research results may be made.

Remote Sensing for Soil Characterization

Remote sensing has been defined as "the science of deriving information about an object from measurements made at a distance from the object" (Landgrebe, 1978). This technology has long been considered an important tool for soil mapping, starting with the first use of panchromatic aerial photography for the soil survey of Jennings County, Indiana in 1929. Aerial photographs have been used as base maps for most U.S. soil surveys ever since. Subsequent advances in color and infrared photography extended spectral sensitivity beyond the visible range, but interpretation was based on visual analysis only, and variations in tone were difficult to quantify.

The development of optical-mechanical scanners in the 1960s allowed collection of spectral data in multiple wavebands and extended spectral sensitivity into the thermal infrared region. Availability of multispectral data in digital format facilitated the use of computer pattern recognition techniques to delineate spectrally separable classes of soils, and quantify relationships of observable soil parameters to soil reflectance (Kristof and Zachary, 1974). Digital remotely sensed data from satellite altitudes have been used to map soils according to organic matter content (Kristof et al., 1973), texture (Anuta et al., 1978), parent material (Mathews et al., 1973b), and drainage classes (Kirschner et al., 1978), and to quantify the extent of soil mapping

unit inclusions. Thus, remote sensing techniques have already demonstrated their effectiveness as aids to soil survey, but research must continue if the above parameters are to be predicted accurately over large areas and variable field conditions using remotely sensed data.

Although research has been conducted using other remote techniques such as thermal emission and active microwave attenuation, most of the current remote sensing technology used for soil characterization is based on measurement of electromagnetic energy reflected by a soil surface. Variations in the intensity of reflected energy as a function of wavelength provide the basis for identification and characterization of soil targets.

Measurement of Soil Reflectance

Remote sensing based on reflectance involves detection of energy in the reflective portion of the electromagnetic spectrum (280-3000 nm), or those wavelengths in which the detected energy is primarily solar radiation reflected by objects on the earth's surface if the sun is the energy source (Silva, 1978). For remote sensing purposes, the reflective spectrum is divided into three regions: visible (380-720 nm), near infrared (NIR, 720-1300 nm), and middle infrared (MIR, 1300-3000 nm).

"Reflectance" is a quantitative term, often expressed in percent, meaning the ratio of the energy reflected by a surface to the incident energy (Silva, 1978). Its value is affected by the nature of the reflecting surface and directional characteristics (incidence and view angles). Incident energy is provided either by the sun, in passive

systems, or by an artificial source in active systems. Soil reflectance is illustrated graphically by plotting percent reflectance as the dependent variable against wavelength. This is referred to as the spectral reflectance curve.

The bidirectional reflectance distribution function (BDRF) is the most fundamental property describing surface reflectance characteristics, and is a unique property of the sample (Silva, 1978). However, it is extremely difficult to measure, since the incident irradiance as a function of incidence and view angles must be quantified. Examples of geometric parameters describing light reflectance are shown in Figure 3. The incidence angle is the angle of impinging radiation, and the view angle is the angle of reflected radiation, both with respect to the zenith. A more easily measured quantity is the bidirectional reflectance factor (BRF), which is the ratio of energy reflected by a target to that reflected by a completely reflecting, perfectly diffuse reference surface under given, identical irradiation and viewing conditions. Pressed barium sulfate (BaSO_4) powder is commonly used as the reference surface.

Some laboratory spectrometers are designed to measure directional-hemispherical reflectance, or the fraction of the total incident energy which is reflected back in all directions, rather than BRF. This is possible using an integrating sphere coated with highly reflective material such as BaSO_4 . Hemispherical reflectance as measured in the laboratory has been correlated with numerous soil properties including color, organic carbon content, iron oxide content, degree of erosion, and moisture content (Fernandez and Schulze, 1987; Ruckman et al.,

1981; Pazar, 1983; Christensen and Hummel, 1985; Bowers and Hanks, 1965).

Remote sensing techniques to measure soil reflectance were divided into two categories by Stafford (1988) according to the distance between the soil and the sensor. High-altitude remote sensing, using aircraft- or satellite-mounted instruments, has been used for soil mapping across large geographic areas. The first operational satellite sensor was the Landsat Multispectral Scanner (MSS), launched in 1972, which detected energy in two visible and two NIR bands approximately 100 nm wide, with 80 m ground resolution. This was followed by the Landsat Thematic Mapper (TM), which has six reflective bands (3 visible, 1 NIR, 2 MIR) with approximately the same bandwidth, and a 30 m ground cell. A French sensor, SPOT (Systeme Probatoire pour l'Observation de la Terre) offers an improvement in spatial resolution (20 m for multispectral data), but is limited in spectral resolution, having only three bands (2 visible and 1 NIR). Since the minimum size delineation of a standard Soil Conservation Service Order 2 soil map is approximately 0.57 ha (Soil Survey Staff, 1990), much larger than the 0.09 ha TM pixel size, sensor spectral range and spectral resolution remain the factors most limiting to successful employment of satellite data for soil survey.

Non-contact or close range remote sensing generally involves sensors mounted on field equipment which are capable of repeated, real-time measurement of soil reflectance under field conditions. The main advantages of such sensors are the ability to characterize soil spatial variability across a field, and that ground observations may be made on the same soil sample on which reflectance is measured. Examples of field sensors are the truck-mounted Exotech 20C modified for field use

(Stoner et al., 1980), and the tractor-mounted real-time organic matter sensor developed by Schulze, Van Scoyoc, and others (Shonk and Gaultney, 1988).

Laboratory measurement of soil reflectance can also be considered remote sensing if it does not involve direct contact with the sample. Laboratory studies of soil-reflectance relationships have provided much of the background and justification for the development of high-altitude sensors, and have an advantage in that soil parameters and measurement conditions can be controlled. Examples of laboratory spectrometers are the CARY 17D spectrophotometer, which measures directional-hemispherical reflectance (Fernandez and Schulze, 1987) and the Exotech 20C spectroradiometer, which measures BRF (Stoner, 1979). Both of these instruments are capable of measuring soil reflectance in the range of 380-2500 nm with a spectral resolution of 10 nm or better.

HIRIS Sensor

A new generation of sensors is currently in the design phase as part of the Earth Observing System program sponsored by the National Aeronautics and Space Administration (NASA). Among these sensors is the High Resolution Imaging Spectrometer (HIRIS), which is scheduled to begin operation on the space station polar platform in 1996. Important HIRIS parameters are listed in Table 2. Development of the HIRIS sensor resulted from recent technical advances in imaging spectrometry, a technology which makes possible the simultaneous collection of images in a hundred or more contiguous spectral bands (NASA, 1987). Unlike currently available satellite sensors which detect energy over a small number of non-contiguous broad bands, HIRIS will offer complete

Table 2. HIRIS instrument design parameters (NASA, 1987).

<u>Instrument Parameter</u>	<u>HIRIS Design</u>
Spectral coverage	400-2500 nm
Number of bands	192
Spectral bandwidth	
400-1000 nm range	9.4 nm
1000-2500 nm range	11.7 nm
Ground instantaneous field of view	30 m
Orbit altitude	824 km
Swath width	30 km
Pointing capability	
Down-track	+60 /-30
Cross-track	+24 /-24
Encoding	12 bits/pixel
Maximum data rate	
Internal	512 Mbits/pixel
Output	300 Mbits/pixel
Expected launch date	1996

representation of spectral curves with essentially no information loss (Goetz and Herring, 1989). An illustration of the concept of imaging spectrometry is shown in Figure 4.

The HIRIS sensor will have the capability to generate 192 separate images simultaneously, with each image representing the energy reflected in one narrow (approximately 10 nm) wavelength band. This represents a remarkable improvement over current sensors in spectral resolution. HIRIS will be able to detect energy over the wavelength range of 400-2500 nm, nearly the entire reflective spectrum, while maintaining the 30 m ground instantaneous field of view currently provided by the Landsat Thematic Mapper. Thus, without sacrificing spatial resolution, the problems of poor spectral resolution and limited spectral coverage will be eliminated by HIRIS.

The tradeoff of such improvements, however, is in the enormous quantity of data, approximately 512 megabits per second (Mbs), that will be generated by the sensor. This is well above the current maximum data transmission rate (300 Mbs) from satellite altitudes to Earth (Goetz and Herring, 1989). Such a high data rate makes continuous operation of the sensor impractical. Therefore, HIRIS will operate as a targeting sensor in a request-only mode, allowing selection of certain system parameters to be made by researchers according to their specific needs. Examples of such system parameters are target size and location, pointing angle, and waveband selection.

A great deal of computer time will be required for processing of and information extraction from such high-dimensional data, particularly if data are collected over all 192 spectral bands. Clearly, some form of data reduction will be necessary in order to transmit and process

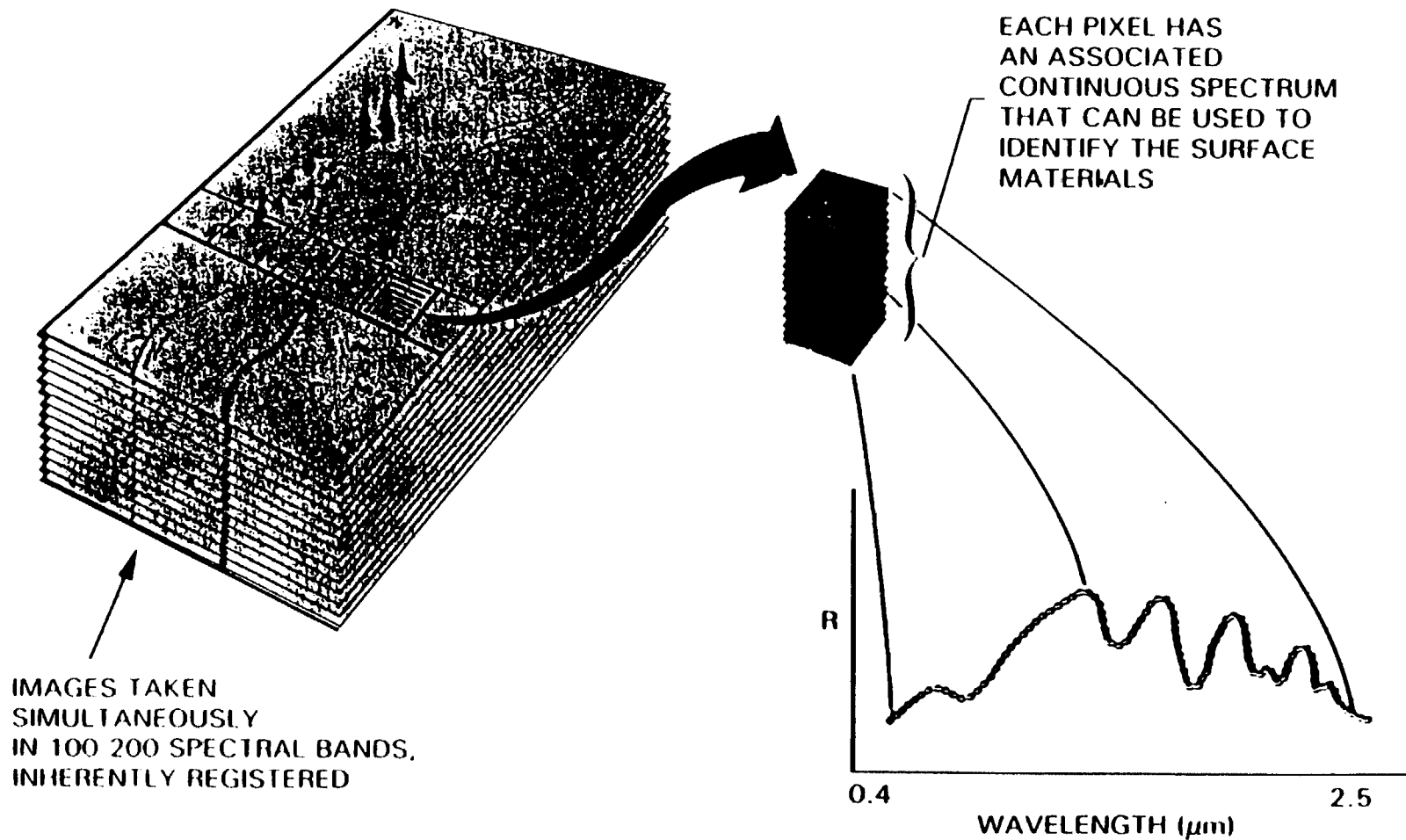


Figure 4. Schematic definition of imaging spectrometry (NASA, 1987).

only those data which are essential for the specific application of any particular mission. Since band selection will be possible, one means of data reduction will be simply to select only those wavelengths which contribute useful data about the soil parameters being investigated, eliminating all bands known to contain little information about the parameters of interest. The capability to tailor wavelength specifications for a given mission will only be useful if intelligent decisions about band selection can be made, based on quantifiable relationships between spectral reflectance and the soil properties under investigation.

Factors Influencing Soil Reflectance

Identification or classification of soils based on their spectral reflectance characteristics will be made possible only by understanding how quantifiable soil properties affect soil reflectance. Reflectance properties depend on the following physical and chemical soil parameters: organic matter content, moisture content, iron oxide content and mineralogy, soil texture or particle size, and possibly salt content (Baumgardner et al., 1985). Under field conditions, soil reflectance is also affected by surface roughness and vegetative cover. In general, reflectance has been found to increase with decreasing organic carbon content, decreasing soil moisture content, and decreasing iron oxide content. At a uniform moisture tension, Stoner and Baumgardner (1981) identified five characteristic reflectance curve shapes based on organic matter and iron oxide content (Figure 5). Soil texture - reflectance relationships are more complicated due to scattering and light traps, but in general reflectance increases with decreasing particle size. By

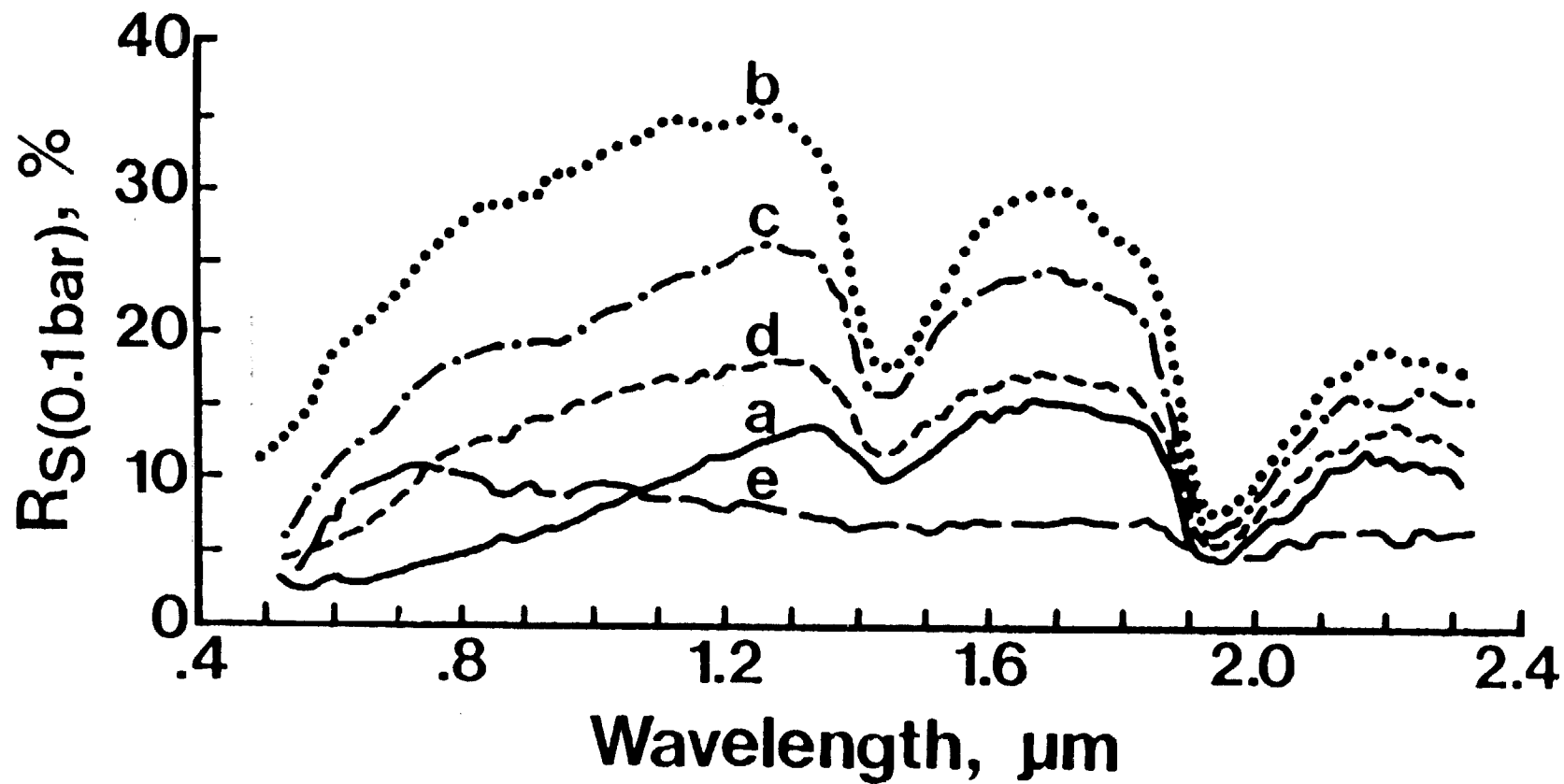


Figure 5. Representative reflectance spectra of surface samples of five mineral soils: (a) organic-dominated; (b) minimally-altered; (c) iron-affected; (d) organic-affected; (e) iron-dominated (Stoner and Baumgardner, 1981).

relating these properties to soil genesis, reflectance has been correlated to genetic factors such as parent material, temperature regime, climatic moisture zone, and drainage class (Stoner, 1979). Soil-reflectance relationships are also wavelength dependent, meaning that soil parameters may affect reflectance differently in different bands and regions of the spectrum.

Use of remote sensing for soil characterization will require a thorough understanding of the interactions between reflectance and all of the soil parameters listed above. The development of HIRIS necessitates a detailed re-examination of soil-reflectance relationships for all of these parameters as functions of narrow wavelength intervals across the entire reflective spectrum.

Soil Reflectance - Organic Matter Relationships

Soil Color and Organic Matter

It has long been known that a strong relationship exists between the amount of organic matter in a soil and soil spectral reflectance. That such a relationship exists is immediately evident, as differences in soil color, which is a function of spectral reflectance over the visible range of wavelengths, are easily observable between soils of differing organic matter content. In general, within a landscape darker soils contain more organic matter than lighter soils. Soil color has been used as a simple method of organic matter content estimation in the field. Several attempts to standardize semi-quantitative estimates of organic matter content based on observed Munsell color have been made for specific agricultural regions. Alexander (1969) developed a chart correlating moist soil color to five levels of organic matter content,

to aid farmers in selecting herbicide rates on Illinois mineral soils. An accuracy of 95% was reported with this technique, but adjustments were necessary for coarse-textured soils, as organic matter content was overestimated for these. To eliminate the subjectivity inherent in visual observations of Munsell color, Page (1974) used a color-difference meter to measure the color of 96 soils on the Atlantic Coastal Plain. A high correlation ($r=0.89$) with organic matter content as determined by the Walkley-Black method was reported, and a prediction equation was proposed. As in Alexander's method, the equation overestimated organic matter content in coarse soils and underestimated it in finer soils, and it was emphasized that regression equations would have to be established based on geographic regions.

A similar method for field estimation of organic matter based on soil color was later developed for Indiana soils (Steinhardt and Franzmeier, 1979). The method was 90% accurate in separating three broad organic matter content classes. The relationship was valid for plowed, bare silt loams, and its geographic limitation was stressed by the authors. In a later statistical study, Franzmeier (1988) attempted to identify the factors causing variability in organic matter - texture - color relationships for 1660 soils throughout the state of Indiana. A high positive correlation existed between organic matter and clay content, while a strong negative correlation existed between organic matter content and moist Munsell value. It was concluded that soil texture was the main factor contributing to variability in the relationship of organic matter content to moist Munsell color. The effects of both geographic location within the state and soil drainage class on the relationship were minimal.

Laboratory-based Studies

Although color relationships are useful for broad field estimations, more quantitative relationships which consider the spectral characteristics of soil reflectance are necessary to apply remote sensing techniques to soil organic matter content determination. Winters (1930) recognized the importance of quantitative reflectance measurements long ago, but in-depth investigations of this nature did not take place until the 1960s, when spectrophotometric instrumentation to measure soil spectral reflectance reliably became available. In 1965, Bowers and Hanks first attempted to identify the influence of organic matter on soil reflectance. They measured spectral reflectance of soil over a wavelength range of 185-3500 nm, before and after oxidation with hydrogen peroxide (H_2O_2) to destroy organic matter. Results showed that removal of organic matter caused increased reflectance at all wavelengths, although the difference between intact and oxidized samples was minimal at wavelengths longer than 1800 nm.

Since then, numerous other laboratory investigations into soil organic matter - reflectance relationships have been conducted, results of which have provided the basis for more applied research with field and high-altitude sensors. In many cases, however, comparison of results between studies is difficult due to differences in wavelengths over which reflectance was measured, spectral bandwidth, band selection criteria, moisture content of soil samples, and reflectance data transformations used in prediction algorithms. Results of several important laboratory studies will be summarized in this section.

Obukhov and Orlov (1964) measured spectral curves of six soils over the visible range only (400-750 nm), with a bandwidth of

approximately 3 nm. For all soils, reflectance was minimal at 400 nm and increased steadily to a maximum at 750 nm. The red region (750 nm) allowed the greatest separation of spectral curves. Relating the curves to measured soil properties, they concluded that humic substances have the most influence on soil visible reflectance, followed by iron oxides which affect soil hue and white substances such as carbonates which affect color saturation. No direct quantitative relationship was found between total organic matter and soil reflectance, but a linear relationship existed between humic acid content and soil reflectance at moderate levels of HA.

Reflectance was later used as a laboratory tool to measure the distribution of humus in soil profiles (Orlov et al., 1966). Again, measurements were made only in the visible range, and humic substances were found to influence reflectance most strongly in the 700-720 nm range. Percent light absorption at 720 nm, expressed as the difference between total incident and percent reflected light ($A=100-R$), was found to be inversely related to humus content. However, the relationship was good only within groups of soils having the same parent material, presumably due to differences in iron oxide content over different parent materials. Later, Mikhaylova et al. (1967) correlated reflectance in four visible bands to humus content and found 430-490 nm to be the best band ($r=-0.93$). They hypothesized that the degree of hydromorphism determined the humus content - reflectance relationship, as the correlation in all bands was much lower for hydromorphic soils. Karmanov and Rozkhov (1972) found that increasing humus content lowered reflectance 1.8 times faster in calcareous clays than in soils formed on shale. They attributed this effect to differences in humus composition

(a lower humic to fulvic acid ratio on the calcareous clays) rather than differences in mineral pigments related to parent material.

Mathews et al. (1973a) extended the spectral range used in previous studies into the middle infrared wavelengths. Reflectance of four soils formed on different parent materials was measured in the 500-2600 nm range, before and after oxidation with H_2O_2 to remove organic matter. Three of the soils exhibited a marked increase in reflectance in the 500-1150 nm range after organic matter removal. One soil, derived from limestone and containing twice the clay content of the other soils, showed very little change, suggesting that a high clay content may interfere with the organic matter - reflectance relationship.

Beck et al. (1976) correlated reflectance of 15 Indiana soils in the 530-2320 nm range to organic carbon content, determined using three different wet combustion methods. Using stepwise multiple regression, organic carbon content was found to be the most important factor affecting reflectance after soil moisture. Bands in the 900-1220 nm range gave the best prediction of organic carbon content from reflectance (r^2 ranged from 0.803 to 0.863 for oven dry soils). The method of organic carbon determination did not affect the relationship.

Montgomery et al. (1976) conducted a similar study, using 56 soils in eight soil orders, from 29 states and five climatic regimes. Reflectance was measured over the 480-2380 nm range, which was divided into eight visible bands of variable width and 59 narrow and 15 broad infrared bands. No clear rationale was given for band selection criteria. For all soils with greater than 2% organic matter, the only visible band in which organic matter made a significant contribution to

explaining reflectance differences was 480-530 nm. Correlations between organic matter and reflectance were much higher for soils within the humid temperate zone than for soils spanning a wide range of climatic regimes. In the infrared bands, organic matter content was less important than silt, iron oxides, and clay content in explaining variations in reflectance. The study concluded that organic matter contents as high as 9% did not mask out effects of other soil parameters on reflectance at any level.

A comprehensive study of contributions of organic matter, texture, mineralogy, drainage class, climatic regime, soil order and parent material was conducted by Stoner and Baumgardner (1981) using 240 soils from 39 U.S. states, Spain, and Brazil. Reflectance was measured in narrow (10 nm) bandwidths over the 520-2320 nm range. Reflectance data were divided into 10 spectral bands over this range for correlation with soil parameters. Band selection was based on existing or proposed Landsat and Skylab bands. Organic matter content was negatively correlated with the log transformation of reflectance in all ten bands, and was the most important contributor to reflectance differences in the 520-1750 nm band. However, the highest correlations occurred in the visible wavelengths.

Leger et al. (1979) correlated organic matter content with area ratios of soil spectral curves, rather than reflectance, over four bands in the 400-650 nm range. The area ratio was defined as the ratio of reflected light (area under the curve) to absorbed light (area over the curve). Two soils, a clay and a sand, were measured before and after oxidation of organic matter. The green band (500-570 nm) showed the highest correlation ($r=-0.78$ and $r=-0.80$ for clay and sand,

respectively). Organic matter removal had a greater effect on reflectance of sand than of clay across the wavelength range measured.

Several researchers have examined combined effects of organic matter and iron oxides on reflectance to facilitate classification of eroded soils using spectral data. Latz et al. (1984) found organic matter content to influence reflectance significantly in the 500-1200 nm range, giving a concave curve shape from 500 nm to 800 nm. Pazar (1983) found reflectance at 660 nm to be the best predictor of organic matter content ($r^2=0.94$), when adjusted for iron oxides using the 485 nm and 560 nm bands. Fernandez (1985) found that eroded soils were detectable due to iron oxide absorption in the 700 nm and 900 nm bands, but that organic matter masked iron oxide effects in non-eroded soils. The shape of the spectral curve in the 700-800 nm band indicated whether organic matter or iron oxides dominated reflectance.

A technique to measure soil organic carbon content in the laboratory using NIR reflectance was recently developed by Dalal and Henry (1986). Three narrow (2 nm) bands at 1744 nm, 1870 nm, and 2052 nm were determined from spectral measurements across the 1000-2500 nm range to be optimal for organic carbon content prediction ($r^2=0.93$). Prediction was successful only when sample particle size was uniform, and samples fell within a narrow range of soil colors.

The variety of bands determined to be optimal for organic matter content prediction in the research described above suggests that organic matter - reflectance relationships are still not completely understood. More laboratory research on the contribution of organic matter to soil reflectance curves will be necessary if remote sensing techniques are to be used successfully for soil organic matter mapping.

High-Altitude Sensors for Organic Matter Mapping

The first organic matter - reflectance study with data from an airborne sensor was conducted by Baumgardner et al. (1970), using an airborne optical-mechanical scanner flown over 25 ha of plowed, bare soil in Tippecanoe County, IN. Reflectance was measured in 12 bands of variable width across a range of 400-2600 nm. Surface soils were sampled and analyzed for organic matter content. The average reflectance values of four ground cells in the 620-660 nm range were plotted against organic matter content with a correlation of $r=-0.74$. Correlations were not presented for the other bands, or for combinations of bands. The authors found that a stronger correlation existed when samples containing less than 2% organic matter were eliminated, and concluded that above this cutoff level, organic matter masked the effects of other soil parameters on reflectance. They hypothesized that high-altitude spectral data could be useful in delineating soil boundaries according to organic matter content. Data from the same airborne scanner were later used to develop models to estimate organic matter content using high-altitude spectral data (Al-Abbas et al., 1972). The best five bands, selected based on linear regression ($r^2=0.631$), were used to delineate five organic matter content levels. There was a close correspondence between boundaries of the computer-generated map and a conventionally-drawn soil map. Organic matter and clay content were found to be significantly correlated.

Kristof et al. (1973) used computer analysis of airborne multispectral data to create a map of soil organic matter content. Spectral data were classified using pattern recognition techniques and each ground cell was assigned to one of five organic matter content

classes. Training data from one field were used "successfully" to classify data from another field in the flightline, and training data obtained in 1969 were used "successfully" to classify data obtained in 1970, indicating the possibility of both spatial and temporal extrapolation of training data. However, no statistical evaluation of the resulting map accuracy was given, and the entire study area still represented a rather small and homogeneous site.

Spectral data were later used to make a soil organic matter map over a very large geographic area covering two regions of the Soviet Union (Kondrat'yev and Fedchenko, 1982). Calibration of visible field reflectance data with sample organic matter content determinations was used to estimate soil organic matter content over the entire area from airborne multispectral scanner data. The resulting map was based on point data rather than organic matter classes, and no evaluation of the accuracy was reported.

Vinogradov (1981) made a spectral map based on soil reflectance, but obtained poor results for soils from different regions and soils formed on different parent materials within a region. Low performance was attributed to interference by iron oxides and varying organic matter composition. Two main difficulties were described in using high-altitude (as opposed to laboratory) spectral measurements: the spatial variation in organic matter content over the relatively large ground target areas, and difficulty in standardizing high-altitude measurements to ground measurements.

No studies were identified using data from satellite sensors to delineate soil boundaries according to organic matter content. However, data collected from field sensors in specific bands have been used to

simulate data from spaceborne sensors. Coleman and Montgomery (1987) measured field reflectance of four Alfisols and Vertisols on 33 sites over seven bands, six of which corresponded to TM reflective bands. The best single band for predicting organic matter content ($r=-0.766$) was 760-900 nm, representing TM band 4. Five other bands (TM bands 3 and 7, the 1150-1300 nm band not present on the TM, and TM bands 2 and 1, in order of importance) were necessary for an unbiased prediction equation. TM band 5 (1550-1750 nm) had the lowest correlation with organic matter content ($r=-0.632$) and was not included in the equation. It was concluded that the set of TM NIR, red visible, and long MIR bands were capable of discriminating between soils of differing organic matter content. These results have yet to be tested using actual satellite data, probably due to the difficulty in obtaining enough ground samples to characterize accurately the variability in soil organic matter content over the area of a TM ground cell (900 m²).

Close-range Organic Matter Sensors

Over the past decade, much of the research on organic matter reflectance relationships has concentrated on development of tractor-mounted sensors for close-range reflectance measurements across a field. Such measurements can be converted directly to organic matter content estimates and used for automatic adjustment of herbicide application rates. Although the 30 m spatial resolution of HIRIS will make it inappropriate for such in-field applications, much of the close-range research involves high spectral-resolution data, and so provides information on the importance of narrow bands for organic carbon content estimation.

Krishnan et al. (1980) measured narrow-band spectral reflectance on 10 Illinois soils in the laboratory, across a range of 400-2400 nm, and developed a prediction equation based on two wavelengths found to be optimal by stepwise multiple regression: 623.6 nm and 564.4 nm. A correlation of $r^2=0.92$ was reported for 10 soils with a range of textures. This prediction model was later tested with 30 soils having a wider distribution of textures and organic matter contents and was found to be unsatisfactory, giving a correlation coefficient of $r^2=0.25$ over the entire range of textures and moisture contents, and $r^2=0.78$ using air-dried soils (Ruckman et al., 1981). Ruckman proposed a simpler prediction equation using a slope-derivative approach with four bands (9 nm bandwidth) on either side of Krishnan's two optimal wavelengths, but results were not significantly improved over Krishnan's equation. Both models improved significantly when sandy-textured soils were eliminated from the analysis, indicating that soil texture is a major contributing factor to variability in the organic matter - reflectance relationship. Pitts et al. (1983) noted that Ruckman did not use soils with the same moisture content for calibration as for testing, which may have contributed to poor performance.

Pitts et al. (1983) tested a redesigned version of Krishnan and Ruckman's derivative sensor and found that it did not respond to changes in organic matter content. He designed a color sensor based on four channels using band ratios of reflectance in narrow red (619.5 nm) and green (560.0 nm) bands with overall visible reflectance. While organic matter content classes predicted using individual channels overlapped, an exclusion algorithm using all four channels allowed prediction of organic matter content within a range of 1.4% organic matter, using the

same 30 soils as in Ruckman's study. However, no statistics were given on the number of samples correctly classified.

Smith et al. (1987) developed another organic matter sensor and tested it on the same set of 30 Illinois soils. Using eight narrow bands and a multiple regression algorithm, organic matter content was predicted with $r^2=0.86$. Narrow-band reflectance data performed better than broadband data.

More recently, Sudduth and Hummel (1988) used the same data set in a study to determine the best bands, algorithm, and transformation for use with reflectance data to predict total carbon content. They found a partial least squares regression analysis of data from 15 narrow bands, spaced 100 nm apart in the infrared region (800-2580 nm) and transformed to absorbance as the log of inverse reflectance ($A=\log R^{-1}$) to give the best carbon content prediction ($r^2=0.91$). The improved performance of infrared as compared to visible bands was attributed to greater effects of soil moisture on reflectance in the visible range.

The research described above attempted to establish universal relationships between reflectance and organic matter content for soils representative of the entire state of Illinois, a relatively large geographic area. Fernandez et al. (1988) hypothesized that soil reflectance and organic matter content are more highly correlated within a landscape than across large geographic distances. They tested this hypothesis by measuring reflectance over 380-830 nm of six soils in two toposequences within a landscape. Munsell color values were calculated from reflectance data using the method described by Fernandez and Schulze (1987). Correlations between calculated Munsell value and organic matter content for the six soils ($r^2=0.94$) were considerably

better than previous results over large geographic areas, confirming the hypothesis. The complicating effects of larger geographic areas were attributed to soil texture differences.

These results led to the development of a tractor-mounted organic matter sensor which can be calibrated on a field-by-field basis (Shonk and Gaultney, 1988). Reflectance is measured in one band only (visible red, 660 nm) with a 40 nm bandwidth. Laboratory tests showed high correlations ($r^2=0.871$ to 0.980), but the sensor had difficulty differentiating between soils with high (>6%) organic matter contents. Field tests gave a correlation of $r^2=0.83$. The sensor has also been used to produce organic matter maps on a field scale (Gaultney et al., 1988).

Narrow-band Reflectance and Band Selection

One trend common to laboratory, high-altitude and close-range studies of organic matter - reflectance relationships is that no conclusion has been reached on the optimal number and location of wavebands for organic carbon content prediction. Bands have often been selected arbitrarily or based on selection criteria including convenience and availability on existing sensors, rather than spectral characteristics of soil organic matter. Even studies basing band selection on quantitative procedures such as correlation and regression analyses fail to prove the contribution of organic matter to soil reflectance in those bands, if reflectance due to the organic fraction itself is not quantified.

HIRIS, with its high spectral resolution and complete coverage of the visible and reflective infrared regions, will offer the first

opportunity using a spaceborne sensor to base spectral band selection on application-specific predictive capability rather than sensor limitations. To maximize the usefulness of HIRIS, optimal bands for specific applications must be identified in advance. Chen and Landgrebe (1988) proposed a band selection algorithm for use with HIRIS data. The algorithm was later tested using high spectral resolution soil reflectance data measured in the laboratory to classify 514 soils from across the U.S. according to organic matter content (Henderson et al., 1989). The substantially higher probability of correct classification (PC) obtained in this study using the selected HIRIS bands (PC=0.883) compared to MSS and TM bands (PC=0.600 and 0.640, respectively) indicates the great potential of HIRIS data for characterization of soil properties, even across very large geographic areas. The optimal bands selected by this algorithm are listed in Table 3. The algorithm did not rank the bands in order of importance relative to information content about organic matter. The relationship of reflectance in these selected bands to the reflectance properties of organic matter itself remains to be proved.

Reflectance of Organic Matter Fractions

Although a great deal of research has been presented on soil reflectance - organic matter content relationships, and the necessity of more basic studies of the theory of organic matter reflectance has been stressed to give credence to empirical data (Orlov, 1972), very little work has been done to study the spectral reflectance of specific organic matter fractions. Numerous studies characterizing organic matter fractions using absorption spectrometry have shown differences in

Table 3. Optimal simulated HIRIS bands for classification of soils according to organic matter content, selected using the band selection algorithm (Henderson et al., 1989). The algorithm did not rank bands in order of importance.

<u>Band Number</u>	<u>Wavelength Range (nm)</u>
1	520 - 590
2	590 - 850
3	850 - 870
4	870 - 900
5	900 - 1200
6	1200 - 1360
7	1360 - 1380
8	1380 - 1390
9	1390 - 1620
10	1620 - 1640
11	1640 - 1660
12	1660 - 1690
13	1690 - 1700
14	1700 - 1750
15	1750 - 2280
16	2280 - 2320

absorption spectra of humic and fulvic acids of different soils (MacCarthy and Rice, 1985; Ghosh and Schnitzer, 1979). Mehlich (1984) proposed visible light absorbance at 650 nm to estimate extractable organic matter content, expressed as humic acid equivalence. These demonstrated relationships between light absorption and organic matter composition have led to the question of whether such relationships exist with light reflectance.

Shields et al. (1968) hypothesized that organic matter composition affects soil color. They compared color, calculated from reflectance data, of 16 Chernozemic (Mollisols) and Gray Wooded (Alfisols) soils formed on similar parent material and grouped according to similar organic carbon content. Within groups, color value differences of approximately one Munsell unit were found between the Chernozemic and the Gray Wooded soils. The differences were attributed to organic matter composition, but no information was provided on iron oxide content, texture, or drainage class. $E_4:E_6$ ratios were measured for humic acid extracted from these soils and plotted against Munsell value, and distinct curves were found for Chernozems and Gray Wooded soils. These results suggested that direct relationships existed between HA $E_4:E_6$ ratios and soil reflectance, but the nature of the relationship differed according to soil type. The only other organic matter composition factor found to differ between the two soil types was the HA:FA ratio, but FA content was never measured directly, and the validity of information provided by this ratio is questionable in light of recent advances in fractionation and purification methodology.

Grandmaison (1986) used reflectance at 650 nm to estimate the degree of decomposition of peat samples by correlating reflectance to

concentration of soluble humic substances, but did not investigate the individual contributions of humic and fulvic acids to reflectance.

The only direct investigation of organic fraction reflectance was conducted by Obukhov and Orlov (1964), in which they isolated soil humic and fulvic acid and measured spectral reflectance of the dry powders in narrow (3 nm) bands over the visible range (400-750 nm). However, no identification, description, or reflectance data of the soil from which these fractions were extracted was given, so comparison of the overall soil reflectance curve with those of the organic matter fractions was impossible. Results showed humic acid to have an extremely low reflectance (<2%) throughout the entire visible range, while fulvic acid had a higher reflectance, particularly in the green and red regions, reaching a maximum of nearly 20% around 750 nm. No data were provided on infrared reflectance, sample purity, or repeatability of spectral measurements.

In spite of many unanswered questions about this study, numerous other researchers have since attributed reflectance differences among soils of similar organic matter content to organic matter composition differences. Variation in relative contents of humic and fulvic acids in the organic fraction, expressed as HA:FA ratio, was given as evidence for differences in organic matter quality (Orlov et al., 1966; Karmanov and Rozhkov, 1972; Michaylova et al., 1967; Vinogradov, 1981). However, relative contributions of humic and fulvic acid to surface soil reflectance were not investigated. Montgomery (1976) found correlations between percent humic acid carbon and reflectance in the 600-1100 nm range, but this effect was weaker than the organic carbon content effect, and did not mask out other soil parameters. Reflectance of

specific organic fractions was not measured. It was concluded that better techniques for isolation of specific organic constituents would be necessary before their contributions to soil reflectance could be quantified.

Recent advances in organic matter fractionation and purification methods are cause for reevaluation of previous conclusions about organic fractions and reflectance. The validity of the HA:FA ratio as a means of organic matter characterization is now questionable due to the presence of impurities, particularly in the fulvic acid fraction (Stevenson, 1985). The nature of relationships between organic matter composition and soil reflectance throughout the reflective spectrum, using samples prepared with currently accepted fractionation and purification techniques, is not well understood at this time. One goal of the research presented in this thesis is to provide insight into these relationships.

MATERIALS AND METHODS

Soil Sample Selection, Collection and Preparation

Surface horizons of four agricultural soils in Indiana were chosen for this study based on their organic carbon contents, and various combinations of certain genetic parameters such as parent material, internal drainage characteristics, and native vegetation (Tables 4 and 5). Three soils, Chalmers, Raub and Fincastle, were collected from Tippecanoe County in Northwestern Indiana, in the Tipton Till Plain physiographic region. This is a nearly flat to gently rolling glacial plain formed during the Wisconsin glacial period. The three soils are located on a relatively flat landform with a characteristic swell and swale topography (Schneider, 1966). The glacial till ranges from loam to clay loam in texture, and the Peorian (late Wisconsin age) loess cover is moderately thick (Franzmeier and Sinclair, 1982). The fourth soil, Clermont, was obtained from the southeastern part of the state in Jennings County, which is located in the Muscatatuck Regional Slope physiographic region. The soil was found on a broad, flat upland area formed during the Illinoian period (Schneider, 1966). Parent material was moderately thick Peorian loess over weathered loamy glacial till (Franzmeier and Sinclair, 1982). All four soils had a similarly flat slope (<2%) and minimal evidence of erosion. For convenience, the three soils from the northern location (Chalmers, Raub and Fincastle) will be

Table 4. Series name, geographic location in Indiana and taxonomic classification for each of the soils used in this study.

<u>Series</u>	<u>Location</u>	<u>Classification</u>
Chalmers	Agronomy Farm, Tippecanoe Co.	Fine-silty, mixed, mesic Typic Haplaquoll
Raub	Agronomy Farm, Tippecanoe Co.	Fine-silty, mixed, mesic Aquic Argiudoll
Fincastle	Wildlife Farm, Tippecanoe Co.	Fine-silty, mixed, mesic Aeric Ochraqualf
Clermont	SEPAC Farm, Jennings Co.	Fine-silty, mixed, mesic Typic Ochraqualf

Table 5. Native vegetation, parent material, source of loess, and internal drainage classification of each of the soils used in this study.

<u>Soil</u>	<u>Native Vegetation</u>	<u>Parent Material</u>	<u>Source of Loess</u>	<u>Internal Drainage</u>
Chalmers	Prairie	Loess/Glacial Till	Northern (Wabash River)	Poor
Raub	Prairie	Loess/Glacial Till	Northern (Wabash River)	Somewhat Poor
Fincastle	Forest	Loess/Glacial Till	Northern (Wabash River)	Somewhat Poor
Clermont	Forest	Loess/Glacial Till	Southern (White River, East Fork)	Poor

referred to as "northern soils", and Clermont as the "southern soil", when discussing geographic and parent material differences in the remainder of this thesis.

The four soils, which represent two soil orders (Alfisol and Mollisol), have various combinations of similarities and differences in their genetic factors and soil properties. Although the surface horizons of the four soils were formed on similar kinds and ages of parent material (Peorian loess deposited in the Wisconsinan period), the source of the loess was different in the northern and southern locations. The northern loess was derived from the Wabash River, while the southern loess originated from the eastern fork of the White River. A mineralogy difference exists between these two sources. Granitic rocks, which contain dark iron-bearing minerals such as augite and magnetite, predominate in the northern location, while rocks in the southern location contain more quartz and feldspars. This difference in the loess parent material from the two locations can be expected to influence certain soil physical and chemical properties.

The age of the underlying glacial till differs significantly for the two locations. The till underlying the northern soils is of Wisconsinan age (approximately 20,000 years), while that under the Clermont soil is considerably older, deposited about 100,000 years ago in the Illinoian period (Wayne, 1966). The age of the glacial till is not expected to affect properties of the soil surface horizon, which developed from the loess cover. However, the depth from the surface to the level of calcareous till is related to surface properties. For example, in the Clermont soil the depth to calcareous till is about three to four meters, compared to about one meter for the northern

soils. Thus the weathering environment is less acidic in the northern soils due to the influence of the shallower calcareous till. Although both Chalmers and Clermont are wet, poorly drained soils, the more acidic environment of the Clermont provides better reducing conditions for weathering and subsequent leaching of iron-bearing minerals.

The four soils were formed under two types of native vegetation. Chalmers and Raub, both Mollisols, were formed under prairie vegetation. They have the two highest organic carbon (O.C.) contents, 1.72% and 1.46% O.C., respectively, and are darker in color than the other soils. The two Alfisols, Fincastle and Clermont, were formed under hardwood forest and have similarly low organic carbon contents (0.99% and 1.11% O.C., respectively). Chalmers and Clermont soils are poorly to very poorly drained, while Fincastle and Raub are classified as somewhat poorly drained. Chalmers soil has a silty clay loam texture classification, while the other three soils are silt loams. Results of soil physical and chemical analyses are presented in a later section.

Surface samples were collected from the top 5 cm of the soil, after removal of surface debris by scraping with a shovel. At each site, samples were obtained from six locations within the field, a few meters apart. In most cases, soil cores were also collected using a truck-mounted hydraulic probe. Soil descriptions were made in the field for each of the sampling sites, and a more detailed examination of the soil cores was conducted in the laboratory. The soil samples were placed in labelled plastic bags and transported to the laboratory, where the six samples for each site were mixed, air-dried and ground to pass a 2 mm sieve. The <2 mm fraction was stored at room temperature in large metal cans and used for all subsequent procedures and analyses.

Organic Matter Extraction, Fractionation and Purification

To quantify the contributions of specific organic components to soil reflectance, isolation of individual soil fractions was necessary. Each soil was fractionated into seven components. Crude organic matter (extractable humic substances plus impurities) was first extracted and then fractionated into crude humic acid and crude fulvic acid. Purification methods were then used to produce pure humic and fulvic acids. Once isolated, samples of all organic fractions were freeze-dried and retained for spectral analysis with the exception of crude fulvic acid, which was present in a very dilute solution. A diagram of the fractionation scheme is shown in Figure 6. The mineral soil plus humin fraction, which remained after crude organic matter extraction, was retained. A portion of this fraction was then subjected to oxidation with hydrogen peroxide (H_2O_2) to remove all remaining organic material according to the method outlined by Franzmeier et al. (1977), leaving only the mineral soil. Later, a seventh component was produced by treating the mineral portion with a citrate-bicarbonate-dithionite solution to destroy iron and manganese oxides. Reflectance measurements of the iron-extracted minerals were not included in the original spectral analysis, but were performed at a later time. The procedure developed by D.G. Schulze (personal communication, 1989) was used for iron and manganese oxide extraction, and is described in Appendix I.

The extraction, fractionation and purification techniques used for the humic substances were those recommended by the International Humic Substances Society (IHSS). To date, no single procedure for all these techniques, specifically designed for isolation of purified humic

SOIL FRACTIONATION SCHEME

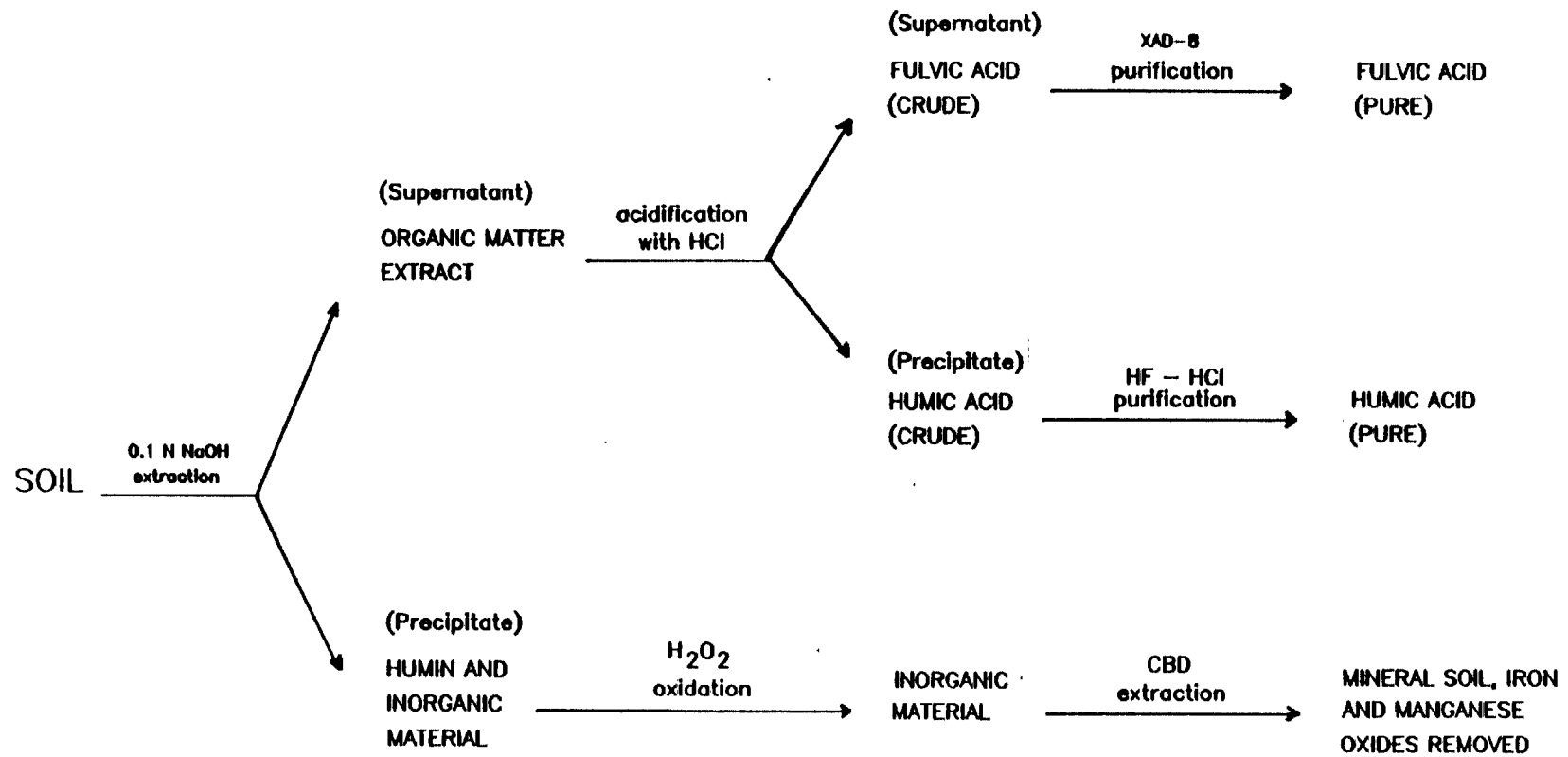


Figure 6. Diagram of the soil fractionation scheme used to isolate soil components for spectral reflectance measurements.

substances from soils, has been published. However, methods for each phase of the isolation process have been published in separate chapters of a book sponsored by the IHSS and edited by Aiken et al. (1985). A variety of organic matter extraction techniques from soil are described by Hayes (1985), and fractionation procedures for soil humic substances are discussed by Swift (1985). The extraction and fractionation procedure used in this study, based on solubility and precipitation at various pH values, is described by Schnitzer (1982). Aiken (1985) outlines a procedure for concentration and purification of aquatic humic substances. For this study, Aiken's procedure was applied to soil humic substances with minor changes suggested by F.J. Stevenson (personal communication, 1987). Purification methodology for fulvic acid, using XAD-8 resin, is described in detail by Thurman and Malcolm (1981). A copy of the unpublished IHSS recommended procedure (Outline of Extraction Procedures) was obtained by personal communication (P. MacCarthy, 1987, to D.E. Stott). Minor modifications to these procedures were made to accommodate large soil sample sizes, according to recommendations by F.J. Stevenson (personal communication, 1987, 1988). The complete extraction, fractionation and purification procedures used in this study are outlined in Appendix II. They were based mainly on the IHSS outline with inputs from the other references listed in this section.

Measurement of Spectral Reflectance

Sample Preparation

The CARY 17D spectrophotometer (Varian Instrument Division, Palo Alto, CA) was chosen to measure reflectance because of its minimum

required sample size. Although an Exotech 20C spectroradiometer (Exotech, Inc., Gaithersburg, MD), which was also available, had been used successfully to measure soil reflectance over the desired spectral range and resolution (Stoner, 1979), its smallest possible sample holder was a cylinder with an inside diameter of approximately 4 cm. The amount of material required to fill the sample cavity to the approximately 7 mm depth necessary to assure an opaque sample (Fernandez and Schulze, 1987) was far greater than the quantity of purified humic and fulvic acids that could be produced in a reasonable amount of time. The design of the CARY 17D allowed much smaller samples to be measured. Twelve sample holders were made following the design of Fernandez and Schulze (1987), each consisting of a plexiglass block (44 mm x 44 mm x 12 mm) with a rectangular sample cavity (25 mm x 10 mm x 12 mm) and rounded ends to facilitate cleaning. A plexiglass plunger, which fit snugly into each hole, was used to hold the sample in place and was secured with a metal screw. The sample volume required to fill the cavity to 7 mm depth was sufficiently small to allow preparation of enough purified humic and fulvic acids to make three samples of each component for each soil.

One drawback of this instrument was that sample and reference ports were oriented such that both samples and standards had to be mounted vertically (Figure 7). It was therefore necessary to secure a glass slide to the front of the sample holder to prevent the dry powder sample from falling into the integrating sphere. A 44 mm x 44 mm x 1.6 mm transparent glass plate was fixed to the sample holder by taping the edges as described by Fernandez and Schulze (1987). SI-UVA fused silica glass (ESCO Products, Inc., Oak Ridge, NJ) was used because of its

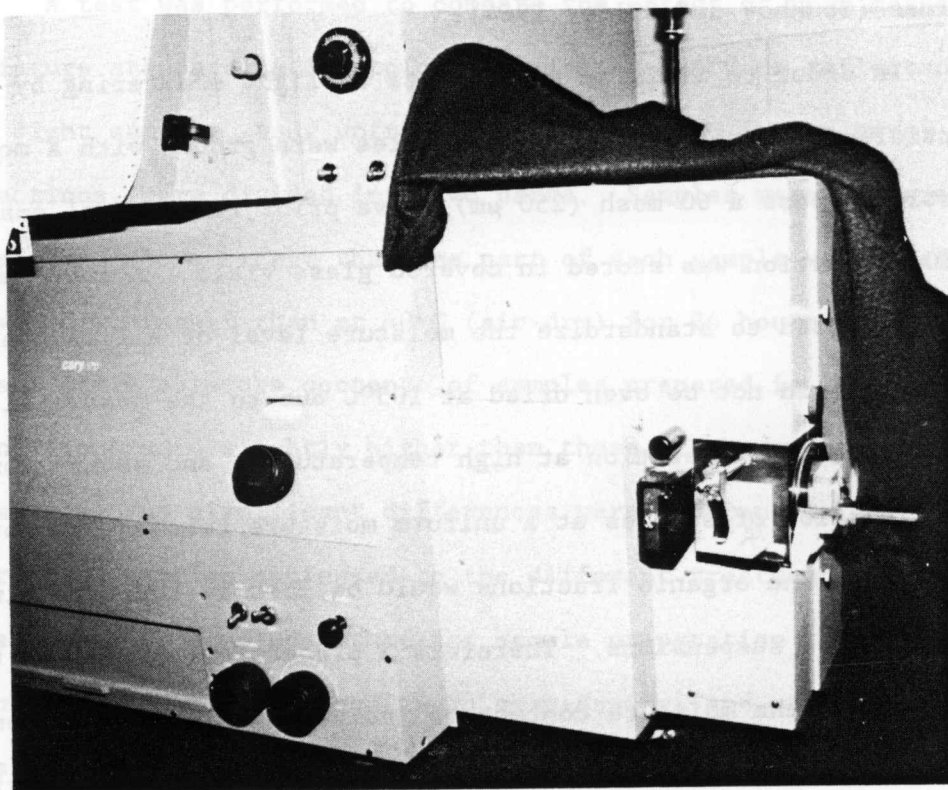


Figure 7. The CARY 17D reflectance accessory with vertically-mounted reflectance standard (front) and sample (right side).

extremely high transmittance (99.9% for 400-2000 nm) over most of the desired wavelength range. Although the transmittance decreased for wavelengths longer than 2000 nm, the effect was not critical since the same glass was used on all samples and reference standards. The use of identical glass plates for samples and standards has been shown to eliminate the influence of the glass on the resulting sample reflectance values (Obukhov and Orlov, 1964).

In order to minimize the effects of light scattering by individual particles of variable size, all samples were ground with a mortar and pestle to pass a 60-mesh (250 μm) sieve prior to spectral analysis. The <250 μm portion was stored in covered glass vials. A uniform treatment was then used to standardize the moisture level of all samples. The samples could not be oven dried at 105°C due to the possibility of organic matter alteration at high temperatures, and saturation and equilibration of samples at a uniform moisture tension was not possible, as some of the organic fractions would be lost in the resulting solutions or suspensions. Therefore a procedure was devised to standardize the moisture content of individual samples between measurement runs to closely resemble the oven-dry condition without significantly altering sample properties. The glass vials containing the sieved samples were covered with paper wipes, secured with rubber bands, to allow airflow to the samples while preventing sample loss. Sample loss was a potential problem for the fulvic acid samples which were very light and fluffy. The vials were then placed in an oven at 40°C for 24 hours. Clean, dry sample holders were placed on clean paper with the glass side down. Immediately upon removal from the oven, a quantity of sample sufficient to fill the sample cavity to 7 mm depth

(approximately 400 mg) was placed in each sample holder. The plexiglass plunger was set into the cavity and light, even pressure applied to hold the sample in place, and the plunger was secured with the metal screw. The sample holders were then placed in a desiccator over CaSO_4 for at least 24 hours, and stored there until spectral measurements were made (usually on the day following sample preparation).

A test was performed to compare the effect of this method of moisture standardization to oven-drying at 105°C on reflectance. Each of eight samples, four unfractionated soils and four mineral soil fractions, were divided into two parts. Samples were prepared as described above except that one part of each sample was dried at 105°C (oven-dry) rather than at 40°C (air-dry) for 24 hours. Although the gravimetric moisture contents of samples prepared in the air-dry conditions were slightly higher than those of the oven-dry samples (Table 6), no significant differences were apparent in the reflectance curves of samples subjected to the different treatments (Figure 8). It was therefore concluded that the sample preparation technique described above gave comparable results to oven-drying, and was a valid method of standardizing moisture content between measurement runs without changing sample composition.

Instrument Operation

The CARY 17D spectrophotometer was equipped with an integrating sphere accessory (model 1711) allowing measurement of diffuse directional-hemispherical reflectance. Monochromatic illumination was provided by a tungsten-halogen lamp. A continuous reflectance spectrum was measured in 1 nm wavelength increments with a single scan starting

Table 6. Average gravimetric moisture content, expressed on an oven-dry (105°C) basis, of four unfractionated soil samples and four mineral component samples prepared for spectral analysis by air-drying at 40°C for 24 hours.

<u>Component</u>	<u>Soil</u>	<u>Moisture content (%)</u>
Unfractionated soil	Chalmers	2.87
	Raub	0.93
	Fincastle	0.81
	Clermont	0.63
Mineral component	Chalmers	0.53
	Raub	0.12
	Fincastle	0.36
	Clermont	0.26

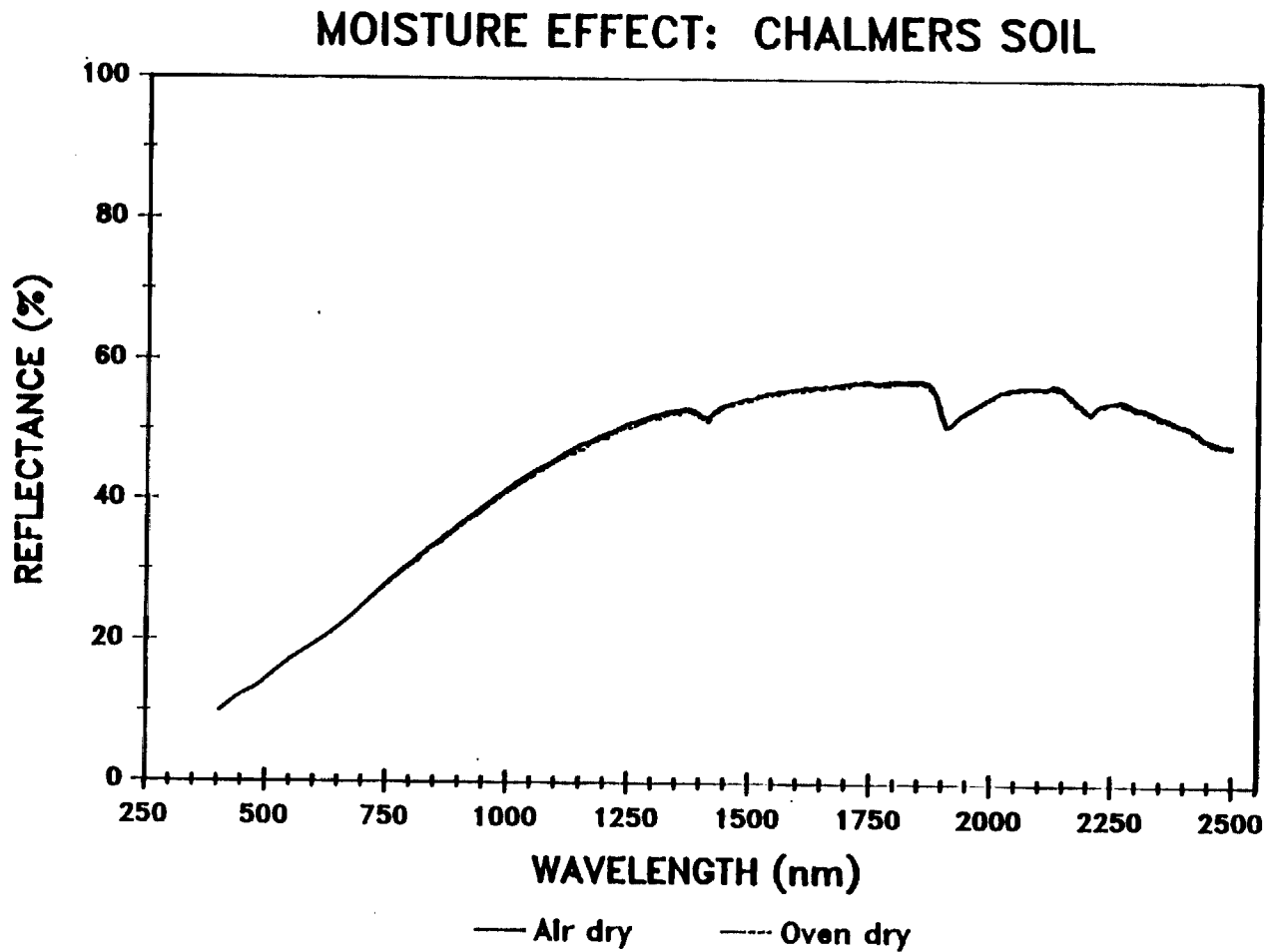


Figure 8. Spectral curves for Chalmers unfractionated soil, comparing air-dry (40°C) and oven-dry (105°C) sample preparation.

at 2500 nm and ending at 400 nm. Two detectors were used: a photomultiplier tube for the 400-799 nm range and a lead sulfide detector for the 800-2500 nm range. The scan was halted for approximately two minutes after the 800 nm reading to allow the instrument readings to re-equilibrate after the detector change. There was no significant difference between reflectance values at 799 nm and 800 nm as a result of the detector change, but readings obtained with the lead sulfide detector had slightly more noise than those detected using the photomultiplier tube.

Previous attempts to measure soil infrared reflectance using the CARY 17D were successful only in wavelengths shorter than 1220 nm (Pazar, 1983). Since data were required out to 2500 nm for this study, many trials were performed using a Wingate soil sample to investigate instrument settings which would allow accurate reflectance measurements over longer wavelengths. Wingate soil was chosen because its visible reflectance had been previously measured on the CARY 17D (Fernandez, 1985) and its reflectance over the 520-2320 nm range had been recorded using the Exotech 20C spectroradiometer (Stoner, 1979), thus providing a basis for comparison of spectral curves. A combination of instrument settings were identified which provided an acceptable signal-to-noise ratio over the entire 400-2500 nm range, while maintaining the 1 nm spectral resolution and a reasonably fast scan speed. The optimal instrument parameters were as follows: slit control, 1.4; scan speed, 2 nm/s; slit height, 10 nm; and pen period, 5. Wavelength calibration was tested by measuring the reflectance of two wavelength calibration standards: an erbium oxide standard (Spectralon no. WCS-EO-020-3282E) and a dysprosium oxide standard (Spectralon no. WCS-DO-020-3282D)

(Labsphere, Inc., North Sutton, NH). Reflectance of the calibration standards was measured with and without a glass slide over the standard plates. The presence of the glass slide did not affect the location of peaks. Measured and certified peak locations were compared and found to agree within 3 nm. Repeatability of spectral measurements was analyzed by comparing results of twelve measurement scans of a single sample taken over two days. Reflectance data were measured on three separate samples of each component for each soil to facilitate assessment of sample-to-sample variability.

Reflectance Standards

The CARY 17D was set up to measure relative reflectance, or the reflectance of a sample relative to the reflectance of a known reference standard. Pressed BaSO₄ powder (Eastman White Reflectance Standard, Eastman Organic Chemicals, Eastman Kodak Co., Rochester, NY) was used as the reference standard due to its stability and its extremely high reflectance: >99.9% in the visible range and >98% in the 700-1400 nm range (Eastman Kodak Co., 1990; Billmeyer, 1969; Wyszecski and Stiles, 1982). Reflectance of BaSO₄ is greater than 90% throughout most of the 1400-2500 nm range and never falls below 88% in this range (Figure 9).

To provide a consistent means of referencing sample reflectance to standard reflectance from day to day, two identical BaSO₄ standards were used, prepared in plexiglass holders according to the method described by Fernandez and Schulze (1987). The first, designated as the working standard, remained mounted over the reference port throughout the measurement period. The other, a reference standard, was treated as a sample and placed over the sample port to be scanned at the start of the

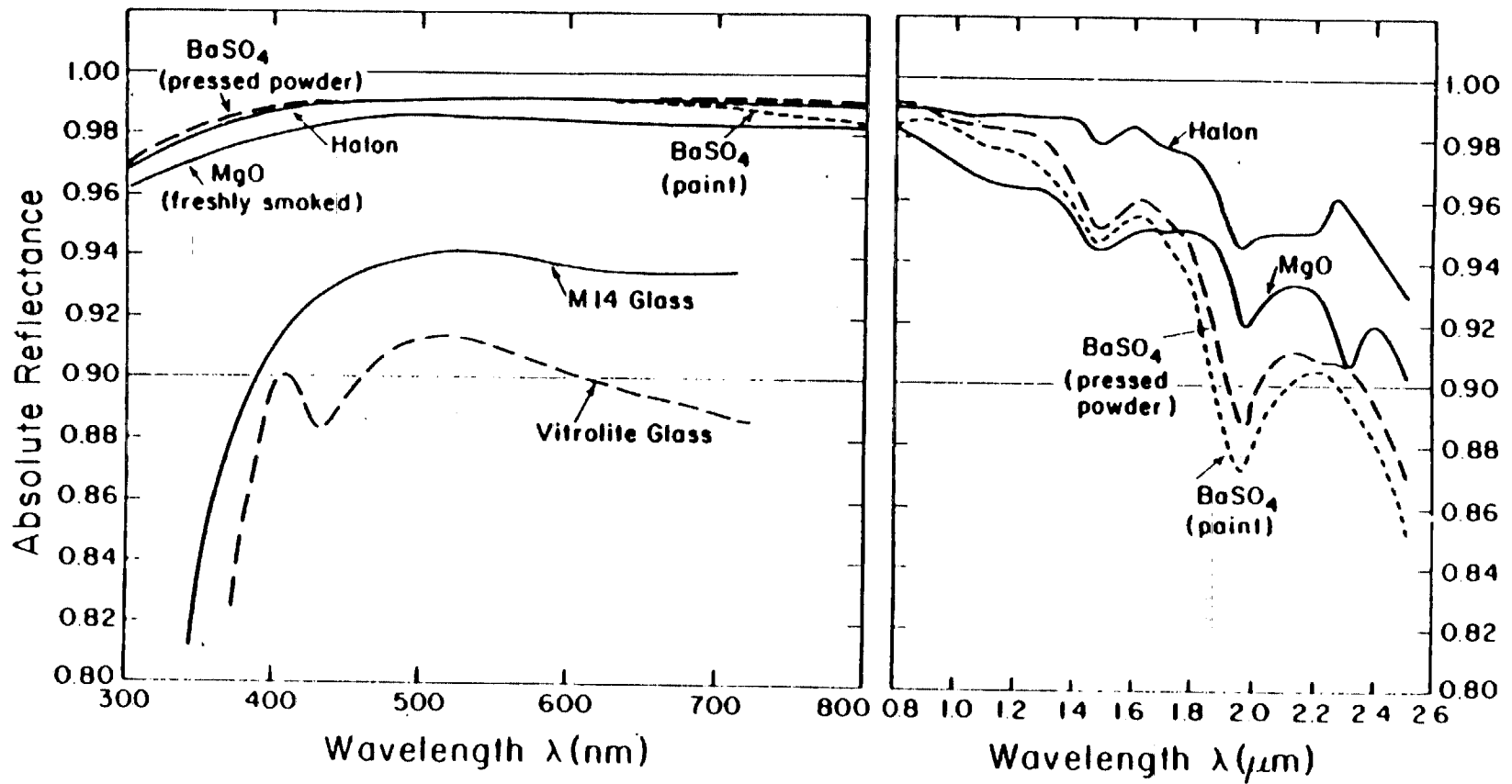


Figure 9. Reflectance spectrum of a pressed BaSO₄ standard (Wyszecki and Stiles, 1982).

day and at periodic intervals over the course of the measurement period. The first reference scan was run only after the machine had been allowed to warm up (about one hour) and equilibrate for 15 minutes with the reference standard on the sample port and the slits open. The balance control dial was adjusted so that the standard reflectance in the visible region read 1.00, and then locked. Machine parameters were kept constant for all subsequent sample scans. The reference scan was followed by a block of six sample scans. A block consisted of the three samples with two scans each for one component of one soil, in random order. Two to four sample blocks could be scanned in a day. Following each sample block, a new reference scan was made to minimize the effects of machine drift. The balance control was readjusted if necessary immediately prior to each new reference scan.

After completion of all reference and sample scans for a day, sample relative reflectance was calculated using a computer program (D.G. Schulze, Purdue University, personal communication) to adjust (reference) the sample reflectance values relative to the reference spectrum. The computer program was based on the assumption that the reference standard had 100% reflectance at all wavelengths. While this assumption is adequate for BaSO_4 in the visible range, it is less valid in the near and middle infrared range because BaSO_4 reflectance decreases in the longer wavelengths. Therefore, a method was developed to correct sample reflectance based on the absolute reflectance of BaSO_4 . Since no published values existed for the absolute reflectance of the BaSO_4 pressed powder standards prepared using the method described above, relative BaSO_4 reflectance was measured and converted to absolute reflectance using a known transfer reflectance standard

(Wyszecki and Stiles, 1982). A Spectralon diffuse reflectance target (no. SRT-99-100-3282-A; Labsphere, Inc., North Sutton, NH) with reflectance greater than 93% throughout the 400-2500 nm range was used as the transfer standard and was scanned as a reference standard on the CARY 17D. A glass plate was placed over the standard during the scan to maintain consistent measurement conditions for samples and standards. The BaSO₄ reference standard was then scanned as a sample, and its relative reflectance based on the Spectralon standard was calculated using the computer program.

Absolute reflectance values of the Spectralon target at every 50 nm throughout the desired range were certified and published by the manufacturer. As the target reflectance curve was flat throughout this range with no sharp peaks at 1 nm intervals, linear interpolation was used to assign reflectance values between the certified points. Since the computer program assumed 100% reflectance for the Spectralon reference standard, multiplication of the BaSO₄ relative reflectance by the absolute reflectance of the Spectralon standard resulted in an absolute reflectance spectrum for the BaSO₄ standard. For correctly referencing sample reflectance relative to the BaSO₄ standard, the computer program was modified to multiply each reference spectrum by the absolute reflectance of BaSO₄. All six samples in each block were referenced to the previous reference spectrum. The use of absolute BaSO₄ reflectance as a reference standard for the soil and component samples eliminated the erroneous assumption of 100% BaSO₄ reflectance in the infrared wavelengths.

It is recommended that determination of the absolute reflectance of the BaSO₄ standards be repeated whenever new standards are prepared.

Data Handling

Raw reflectance data were collected in digital format and stored on a floppy disk at the completion of each measurement scan. Each raw data file was copied to a "working" version of the file at the time of storage. This file was later converted to a referenced data file based on the absolute reflectance of the BaSO₄ reference standard, using a computer program as described in the previous section. Due to the large volume of data generated in this study, all raw and referenced data files were transferred from floppy disks to two 20 Mb Iomega data cartridges (Iomega Corp., Roy, UT) for permanent storage. Spectral curves were plotted using Sigmaplot version 3.1 software (Jandel Scientific, Sausalito, CA).

Soil Chemical and Physical Analyses

Each of the four unfractionated soils was analyzed for organic carbon content, iron and manganese oxide content, pH, cation exchange capacity (CEC) and particle size distribution. Soils were ground to pass a 2 mm sieve prior to all analyses. Soil color was observed and calculated for all samples. In addition, E₄:E₆ ratios were measured on the purified humic and fulvic acid samples.

Organic carbon content was measured on samples of the humin plus mineral components of the four soils, as well as the four unfractionated soils, using the modified Walkley-Black wet combustion method (Franzmeier et al., 1977). It was assumed that total carbon and organic carbon contents of the samples were equivalent, as all soils were sampled from the surface and contained no carbonates. The analyses were performed in triplicate. Organic matter content estimates were obtained

by multiplying the percent organic carbon by 2.00 (Nelson and Sommers, 1982). Average organic carbon contents and organic matter content estimates for the eight samples are listed in Table 7. Measured organic carbon content values, rather than organic matter content estimates, were used in all subsequent statistical analyses. Each soil was analyzed for pH and CEC (by sum of cations) at the Soil Testing Laboratory of the Purdue University Agronomy Department. Analyses were done in duplicate, and average results are shown in Table 8.

Iron and manganese oxide contents were determined in duplicate for each unfractionated soil (Table 9), at the National Soil Survey Laboratory of the U.S. Department of Agriculture - Soil Conservation Service in Lincoln, Nebraska. The citrate-dithionite extraction method was used for both analyses (Olsen and Ellis, 1982). At the Purdue University Soil Characterization Laboratory, particle size distribution was measured in duplicate for each of the unfractionated soil samples. Eight size fractions were measured using the pipette method (Franzmeier et al., 1977). The texture classification and average percent sand, silt and clay for each soil are reported in Table 10.

Observed soil Munsell colors were recorded for the unfractionated soils and for all soil components. For a more quantitative evaluation of color differences that may not have been detectable visually, Munsell color was also calculated for the same samples using reflectance measurements in 1 nm intervals over the 400-830 nm range, according to the procedure developed by Fernandez and Schulze (1987). Calculated and observed colors agreed reasonably well (Table 11), in spite of the lack of reflectance data in the 380-399 nm range. Differences between the observed and calculated hues can be attributed to the greater precision

Table 7. Organic carbon (O.C.) content and estimated organic matter (O.M.) content of four unfractionated surface soils and the humin plus mineral components of the four soils.

<u>Sample</u>	<u>Soil</u>	<u>% O.C.</u>	<u>% O.M.</u>
Unfractionated Soil	Chalmers	1.72	3.44
	Raub	1.46	2.92
	Fincastle	0.99	1.98
	Clermont	1.11	2.22
Humin + Mineral Component	Chalmers	0.66	1.32
	Raub	0.56	1.12
	Fincastle	0.44	0.88
	Clermont	0.44	0.88

Table 8. Cation exchange capacity (CEC) and pH of the four unfractionated soils used in this study.

<u>Soil</u>	<u>CEC (cmol Kg⁻¹)</u>	<u>pH</u>
Chalmers	21	5.8
Raub	16	5.1
Fincastle	8	5.9
Clermont	6	6.0

Table 9. Free iron (Fe) oxide and manganese (Mn) oxide contents of four unfractionated soils.

<u>Soil</u>	<u>Fe (%)</u>	<u>Mn (%)</u>
Chalmers	1.25	trace
Raub	1.3	0.1
Fincastle	1.3	0.2
Clermont	0.4	0.0

Table 10. Particle size distribution (percent sand, silt, and clay) and texture class of four unfractionated soils.

<u>Soil</u>	<u>% Sand</u>	<u>% Silt</u>	<u>% Clay</u>	<u>Texture Class</u>
Chalmers	10.2	59.6	30.2	Silty Clay Loam
Raub	11.0	68.5	20.5	Silt Loam
Fincastle	10.3	76.0	13.7	Silt Loam
Clermont	21.1	66.8	12.1	Silt Loam

Table 11. Dry colors of surface horizons of four unfractionated soils and soil components, determined visually by matching with Munsell Soil Color Charts and by calculation from reflectance spectra. (CH = Chalmers; R = Raub; F = Fincastle; CL = Clermont).

<u>Sample</u>	<u>Soil</u>	<u>Observed Color</u>	<u>Calculated Color</u>		
			<u>Hue</u>	<u>Value</u>	<u>Chroma</u>
Unfractionated Soil	CH	10YR/4/1	0.94Y	4.74	1.81
	R	10YR/5/2	0.23Y	5.31	2.08
	F	10YR/6/3	1.18Y	6.06	2.49
	CL	10YR/6/2	1.38Y	6.60	2.14
Humins + Mineral	CH	10YR/7/2	1.53Y	6.83	2.05
	R	10YR/7/2	1.00Y	6.92	1.89
	F	10YR/7/3	0.57Y	6.46	2.28
	CL	10YR/7/2	1.13Y	7.34	1.62
Mineral Only	CH	10YR/7/2	1.52Y	7.36	2.02
	R	10YR/7/2	0.98Y	7.30	1.95
	F	10YR/7/2	0.49Y	6.98	2.14
	CL	10YR/8/2	0.67Y	7.72	1.69
Crude Organic Matter	CH	10YR/2/2	9.49YR	3.63	1.25
	R	10YR/3/2	9.09YR	3.95	1.55
	F	10YR/3/2	8.82YR	3.61	1.89
	CL	10YR/3/3	9.25YR	4.32	2.24
Crude Humic Acid	CH	7.5YR/2/0	8.59YR	2.10	0.46
	R	7.5YR/2/0	8.18YR	2.18	0.56
	F	7.5YR/2/0	9.32YR	2.67	1.07
	CL	10 YR/2/1	8.75YR	2.70	1.04
Pure Humic Acid	CH	7.5YR/2/0	9.56YR	1.98	0.21
	R	7.5YR/2/0	9.37YR	2.13	0.54
	F	7.5YR/2/0	8.56YR	2.12	0.34
	CL	7.5YR/2/0	8.99YR	2.13	0.69
Pure Fulvic Acid	CH	10YR/5/6	8.60YR	5.26	5.26
	R	10YR/5/6	8.42YR	5.20	5.27
	F	10YR/5/8	8.86YR	5.60	5.52
	CL	10YR/5/8	9.13YR	5.71	5.56

of the measured reflectance, as hue is reported in units of 2.5 hue in the Munsell Soil Color Charts. Since hue is a continuum in the Munsell system, a hue calculated to be slightly yellower than 10YR would be expressed as a low "Y" hue. The calculated and observed values agreed well, although calculated values were slightly higher than observed values for low value (2 or 3) colors. Measured chroma were slightly lower than observed chroma in most cases. These trends agree with the findings of Fernandez and Schulze (1987). Use of reflectance measurements to calculate color allowed smaller differences in soil and component colors to be quantified than could have been accomplished by visual observation alone.

$E_4:E_6$ ratios were determined for the purified humic and fulvic acid samples of each soil, using the method described by Chen et al. (1977). A small amount (4 mg) of each sample was diluted in 10 ml of 0.05 N NaHCO_3 solution to standardize the pH to 8.0. Percent spectral absorbance was measured at 465 nm and 665 nm using a Bausch and Lomb Spectronic model 1001 spectrophotometer. Analyses were performed on six replicates. The $E_4:E_6$ ratio was calculated for each replicate as the ratio of the absorbance values at the two wavelengths, and then averaged for the six replicates.

To test the hypothesis that spectral absorbance measurements are related to spectral reflectance measurements, and therefore that $E_4:E_6$ ratios may be predicted from reflectance data, two standard reflectance-to-absorbance transformations were used with reflectance data. The first transformation ($A=1-R$, where A is percent absorbance and R is percent reflectance) was based on the concept, described by Silva (1978), that the sum of reflectance plus absorbance plus transmittance

is equal to one. Transmittance in this case was assumed to be zero, as the soil samples were opaque. This transformation was previously used to relate absorbance based on reflectance data to soil humus content (Orlov et al., 1966). The second transformation was based on the logarithm of the inverse of reflectance ($A = \log(R^{-1})$). This transformation was used by Krishnan et al. (1980), who found that the curve of this function more closely resembled spectral absorbance curves than direct reflectance measurements. Average $E_4:E_6$ ratios for the eight samples, based on directly measured absorbance values and calculated absorbance values using the two transformations, are presented in Table 12.

Statistical Analyses

Each of the original raw and referenced reflectance data files contained 2101 data points (reflectance values) corresponding to the 2101 wavelength bands in the 400-2500 nm range with 1 nm spectral resolution. Since an objective of this research was to simulate HIRIS data (10 nm spectral resolution) using laboratory-measured reflectance, data in each set of ten contiguous 1 nm bands were averaged to provide a single data value representing one 10 nm band. Each resulting absolute reflectance data file then contained 210 data points corresponding to 210 HIRIS (10 nm) bands over the same wavelength range. Delineation of the 1 nm bandsets to be averaged started at 2500 nm and continued to the shorter wavelengths, and each new 10 nm band was identified by the location of its center wavelength. For example, the first converted HIRIS band represented 10 data points from 2500 nm to 2491 nm, and was designated as the 2495 nm band. For convenience, the center wavelength

Table 12. $E_4:E_6$ ratios of purified humic acids (HA) and fulvic acids (FA) from four soils, computed using direct absorbance (A) measurements and transformed reflectance (R) data.

Organic Fraction	Soil	$E_4:E_6$ Ratio		
		<u>Direct Measurement</u> A	<u>Reflectance Transformations</u> A-1-R A- $\log(R^{-1})$	
Pure HA	Chalmers	3.55	1.00	1.037
	Raub	4.11	1.01	1.095
	Fincastle	4.47	1.01	1.063
	Clermont	4.51	1.02	1.127
Pure FA	Chalmers	13.77	1.55	2.680
	Raub	14.65	1.55	2.711
	Fincastle	16.63	1.68	2.530
	Clermont	15.04	1.94	3.000

used for band identification was rounded to the nearest whole number, and therefore was shifted down by 0.5 nm (for example, 2495 nm rather than 2495.5 nm). This approximation is considered reasonable since the wavelength calibration of the CARY 17D was shown to be accurate within 3 nm, and no sharp peaks were apparent in any of the original 1 nm resolution curves.

After conversion of the 1 nm referenced reflectance files to 10 nm bands, the six spectral reflectance curves (three samples in duplicate) for each of the original six components of each soil and the four unfractionated soils, were plotted for a total of 168 spectral curves. Analysis of variance (ANOVA) was computed for data in each of the 210 wavelength bands in each set of six spectral curves, in order to evaluate measurement error and sample variability (Neter et al., 1985).

The six spectral curves for each soil and component were then averaged together to produce one average spectral curve representing the set, to allow for comparison of soil and component effects on reflectance. The separability of the resulting average spectral curves for each of the four soils within each component was computed at the 99% significance level in each of the 10 nm bands, using the Tukey method of multiple means comparison (Neter et al., 1985). Machine error and sample variation were accounted for in this analysis. The relative importance of spectral regions for specific applications was based on analysis of curve separability for various combinations of soil components, and will be explained in the following section. Discriminant analysis was then used to confirm the separability results (Johnson and Wichern, 1982). Lastly, correlation analysis was performed to evaluate statistically the relationships between reflectance and

organic carbon content in the important spectral regions (Neter et al., 1985). The averaged reflectance files representing the sets of six curves were used for correlation analysis, and the individual curves (six in each set) were used for discriminant analysis.

RESULTS AND DISCUSSION

Evaluation of Reflectance Measurement Technique

The instrument chosen to measure spectral reflectance had to meet the following criteria: the capability to simulate the spectral range and resolution of HIRIS, the capability to measure reflectance of very small (<1 g) dry powder samples, accuracy of wavelength calibration throughout the desired spectral range, and high repeatability of spectral measurements. The first three criteria were met by the CARY 17D spectrophotometer, as described in the previous section. After some adjustment of the instrument, an adequate signal-to-noise ratio was achieved which allowed spectral measurements in 1 nm bands, ten times finer than the spectral resolution of HIRIS, over the entire 400-2500 nm range. The minimum required sample size was approximately 400 mg for unfractionated soil. Wavelength calibration was accurate within 2 nm for the shorter wavelengths (400-1500 nm), and within 3 nm for longer (>1500 nm) wavelengths (Table 13). Calibration accuracy within 3 nm was considered acceptable, as none of the soil and component spectral curves showed sharp peaks, and measurements were averaged over 10 nm increments to simulate HIRIS bands. The results of this wavelength calibration test also proved the capability of the CARY 17D to resolve sharp, narrow peaks. This indicated that any lack of sharp peaks in the spectral curves of the soil and component samples was due to the nature of the

Table 13. Comparison of peak locations, certified and measured with the CARY 17D spectrophotometer, on spectral reflectance curves for an erbium oxide wavelength calibration standard plate (Spectralon no. WCS-EO-020-3282E; Labsphere, Inc., N. Sutton, NH).

Certified Peak Location (wavelength in nm)	Measured Peak Location (wavelength in nm)	Difference (nm)
408.4	407	1.4
454.2	453	0.8
489.1	488	1.1
522.1	521	0.9
538.9	538	0.9
654.3	653	1.3
660.8	659	1.8
799.3	799	0.3
976.7	978	1.3
1471.8	1470	1.8
1536.7	1534	2.7

spectral properties of the samples themselves, rather than an inability of the instrument to detect distinctive curve features. For comparison, the reflectance of the same calibration plate was measured on the Exotech 20C spectroradiometer. Results showed that the CARY 17D had a greater capability to resolve spectral features than the Exotech 20C.

Repeatability of Spectral Measurements

The excellent repeatability of spectral measurements with the CARY 17D has been demonstrated in the visible range by Fernandez and Schulze (1987), indicating a very low measurement (machine) error. To verify the repeatability of measurements using the current instrument parameters, twelve scans of a test sample of Clermont unfractionated soil were performed on two occasions, several days apart, and plotted on the same set of axes. The 12 spectral curves lay directly on top of each other with no visually apparent differences. The consistency of these results indicated a very low machine error throughout the range of wavelengths measured. Further testing with more samples of the same material indicated that although sample-to-sample variation was low, machine error was negligible in relation to sampling error. It was concluded that three samples of each material, with two scans of each sample, would be sufficient to characterize the measurement and sampling error in the data. Therefore, the reflectance of each of the 28 materials (four unfractionated soils and the six components for each soil) was measured in a block of six scans.

An analysis of variance (ANOVA) was performed on the reflectance data in each wavelength for the six scans within each block. Although the numerical results differed according to wavelength and the material

measured, in all cases the machine error was negligible in relation to the sampling error. Although the ANOVA tables for each of the 210 wavebands in the 28 blocks are too lengthy to present here, the results showed that machine error was not significant until the fourth digit on a scale of 10,000, or one tenth of one percent reflectance point.

The degree of sample-to-sample variation differed according to the material measured. In most cases, six of the seven materials measured initially (unfractionated soil, mineral soil plus humin, mineral soil portion only, crude organic matter extract, crude humic acid, and purified humic acid) had relatively low sampling error. However, in all cases sampling error was larger than machine error, by about one half to one order of magnitude (in the order of one percent reflectance) for components having the least amount of sampling error. An example of a material having low sampling and measurement error was the Clermont crude organic matter extract. The six spectral curves for this block of scans were indistinguishable (Figure 10). As with all the other samples, Fincastle purified fulvic acid had extremely low machine error, but it also had a very high degree of sample-to-sample variation (Figure 11). Three distinct curves can be seen for the six scans, corresponding to the three physically different samples of Fincastle fulvic acid used in the block. The two duplicate scans for each sample are indistinguishable, showing evidence of the minimal machine error.

Both machine error and sampling error were considered in all subsequent statistical analyses. However, the fulvic acid fraction was the only component which showed a high degree of sample-to-sample variability, and this variability existed for the fulvic acid isolated from all four soils. In each case, the variation between the three

MACHINE ERROR AND SAMPLE VARIATION (CLERMONT CRUDE ORGANIC MATTER EXTRACT)

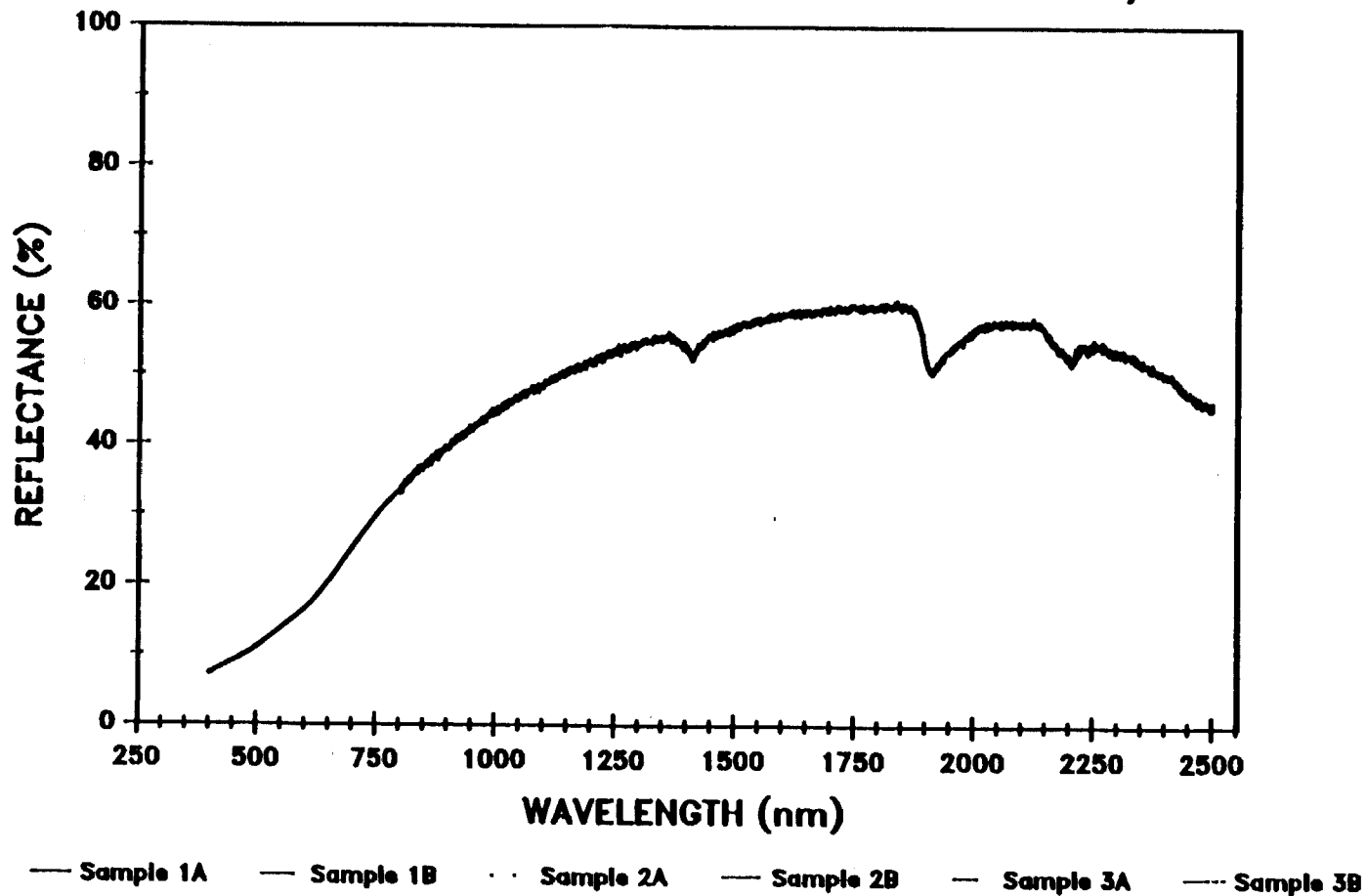


Figure 10. Spectral reflectance curves for the six scans in the Clermont crude organic matter sample block, illustrating the very low measurement error and sample-to-sample variation.

MACHINE ERROR AND SAMPLE VARIATION (FINCASTLE FULVIC ACID)

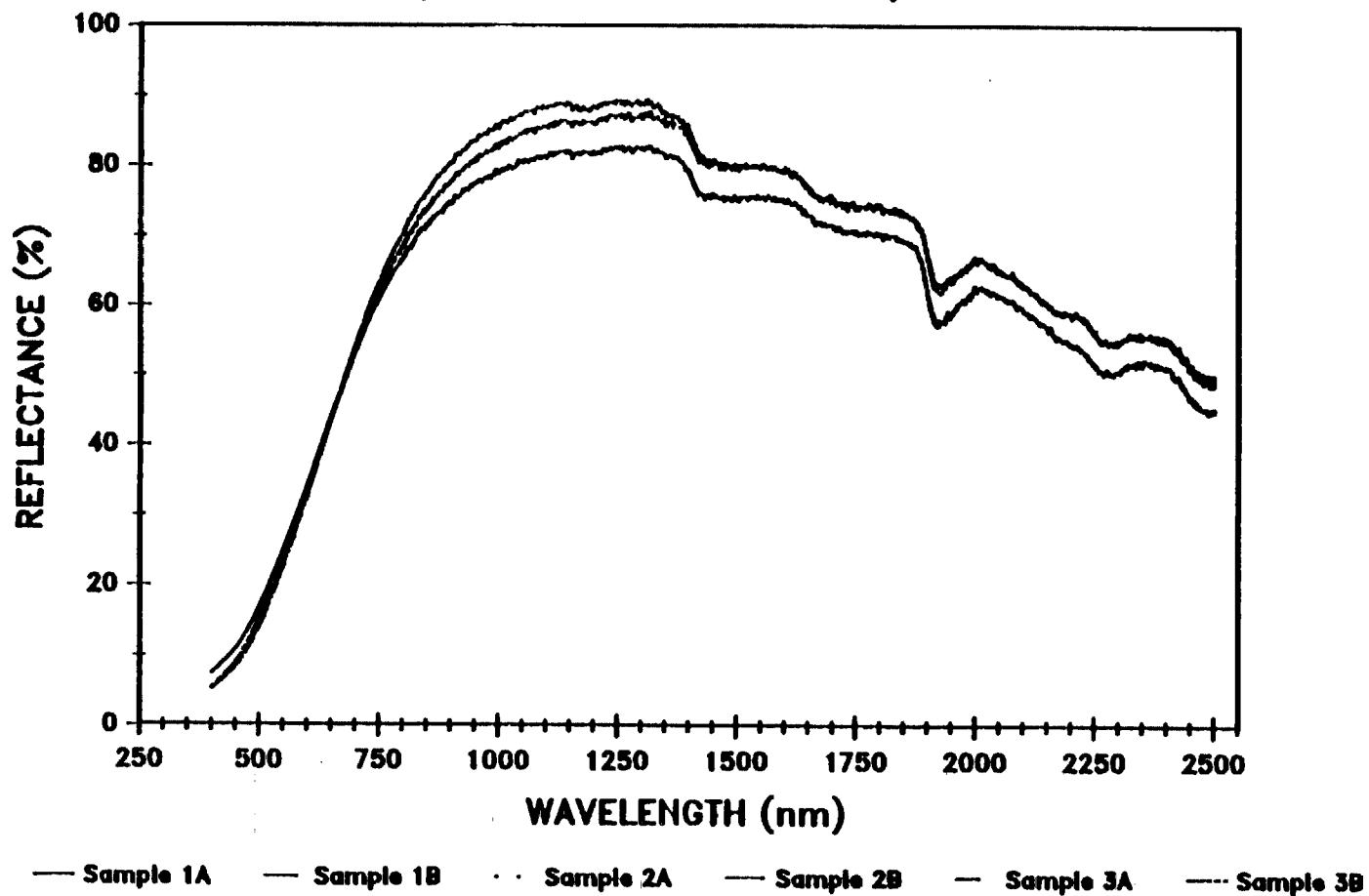


Figure 11. Spectral reflectance curves for the six scans in the Fincastle purified fulvic acid sample block, illustrating the very low measurement error and the high degree of sample-to-sample variation.

fulvic acid samples for a soil was greater than or equal to the variability between the average fulvic acid samples of the different soils. For the unfractionated soils and all other soil components, the variation in average reflectance between different soils was far more important than the variation of the three samples within a single soil.

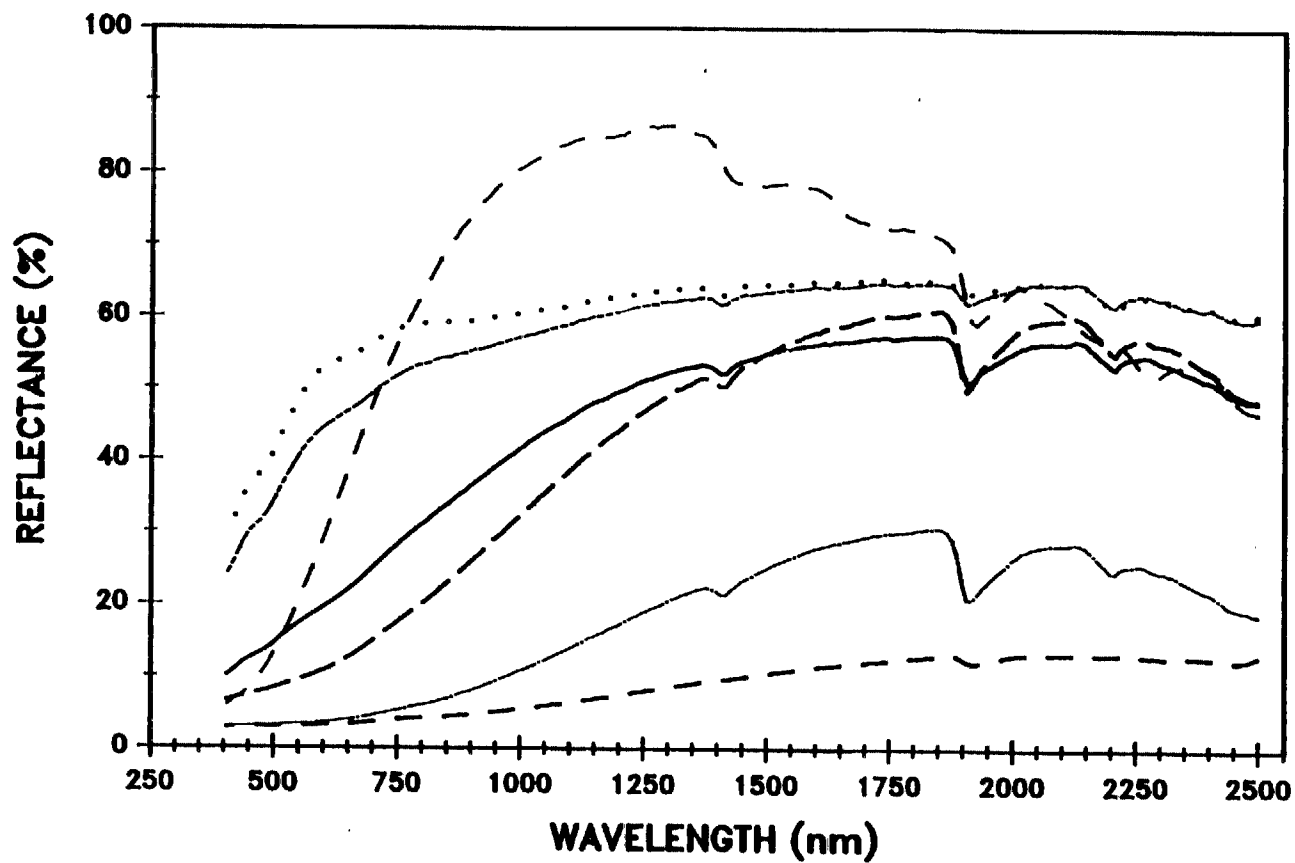
The high variability of the fulvic acid samples may have been due to slight differences in the pH of the purified FA solutions, resulting from inconsistencies in the extraction or purification processes. The visible reflectance of FA responds to pH, and the color of a fulvic acid solution can change from wine-red to orange to yellow as the pH decreases from 5 to 2. The final pH of all the FA samples was approximately 2 for all samples.

Effect of Organic Carbon Content on Soil Reflectance

Visual Analysis of Spectral Curves

After the 168 individual reflectance data files had been collected, the six curves (three samples with two replications each) in each sample block were averaged to produce one representative spectral curve for the material, resulting in 28 composite reflectance curves. Prior to any further statistical analysis, these composite curves were plotted in various combinations and analyzed visually. Two sets of plots were made. In the first set of four plots (Figures 12-15), the spectral curves of the seven materials (unfractionated soil plus six components) for each soil were plotted on the same set of axes. This allowed examination and comparison of the curve shapes for each fraction, within a single soil. Comparing the four plots, it can be noted that each individual component had a distinctive curve shape, easily identified by

CHALMERS SOIL COMPONENTS



— Unfractionated soil - - - Humin + Inorganic material ··· Inorganic material
 — Organic matter extract — Humic acid (crude) — Humic acid (pure) — Fulvic acid (pure)

Figure 12. Average spectral reflectance curves of the Chalmers unfractionated soil and six Chalmers soil components.

RAUB SOIL COMPONENTS

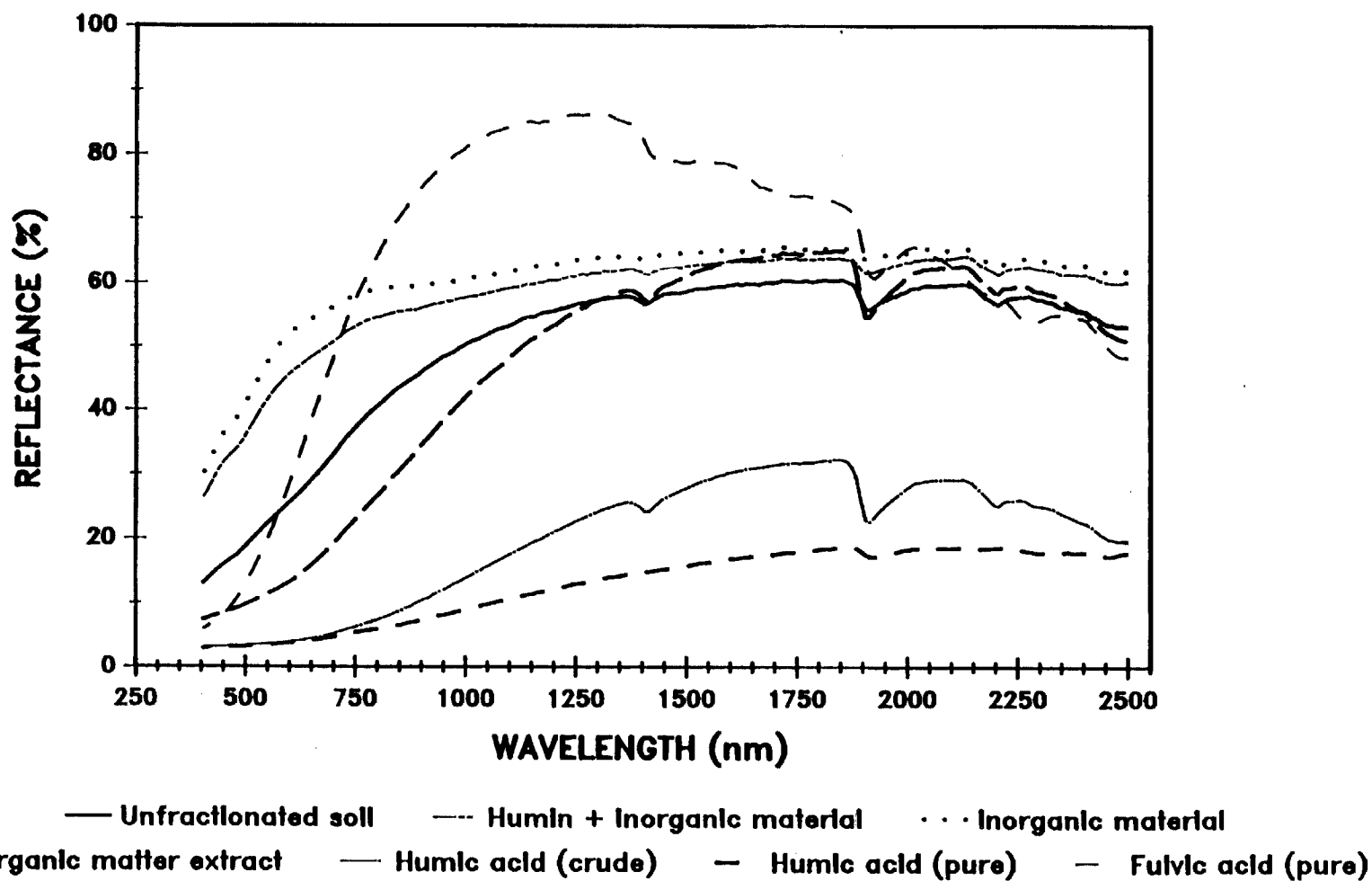


Figure 13. Average spectral reflectance curves of the Raub unfractionated soil and six Raub soil components.

FINCASTLE SOIL COMPONENTS

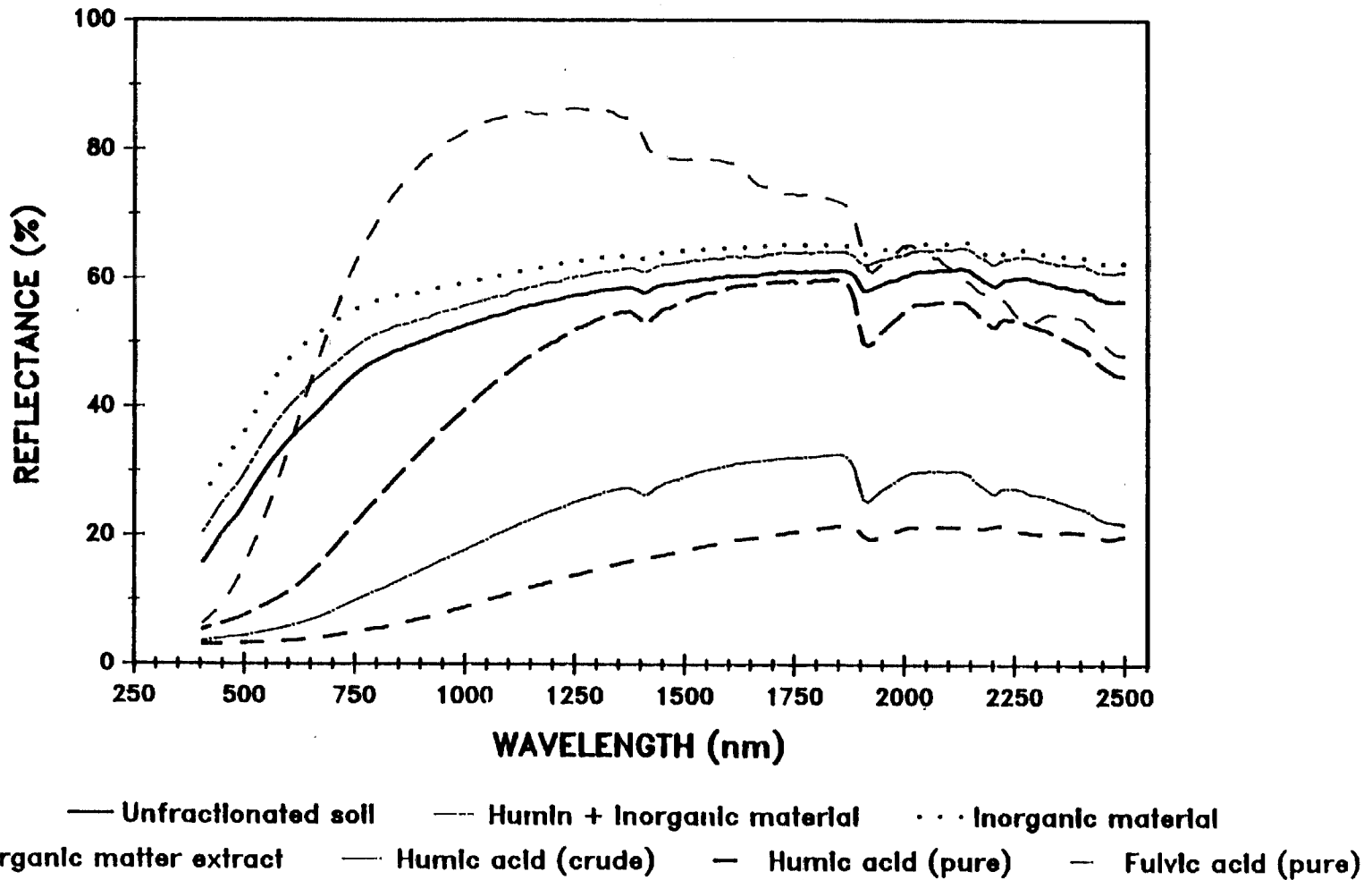
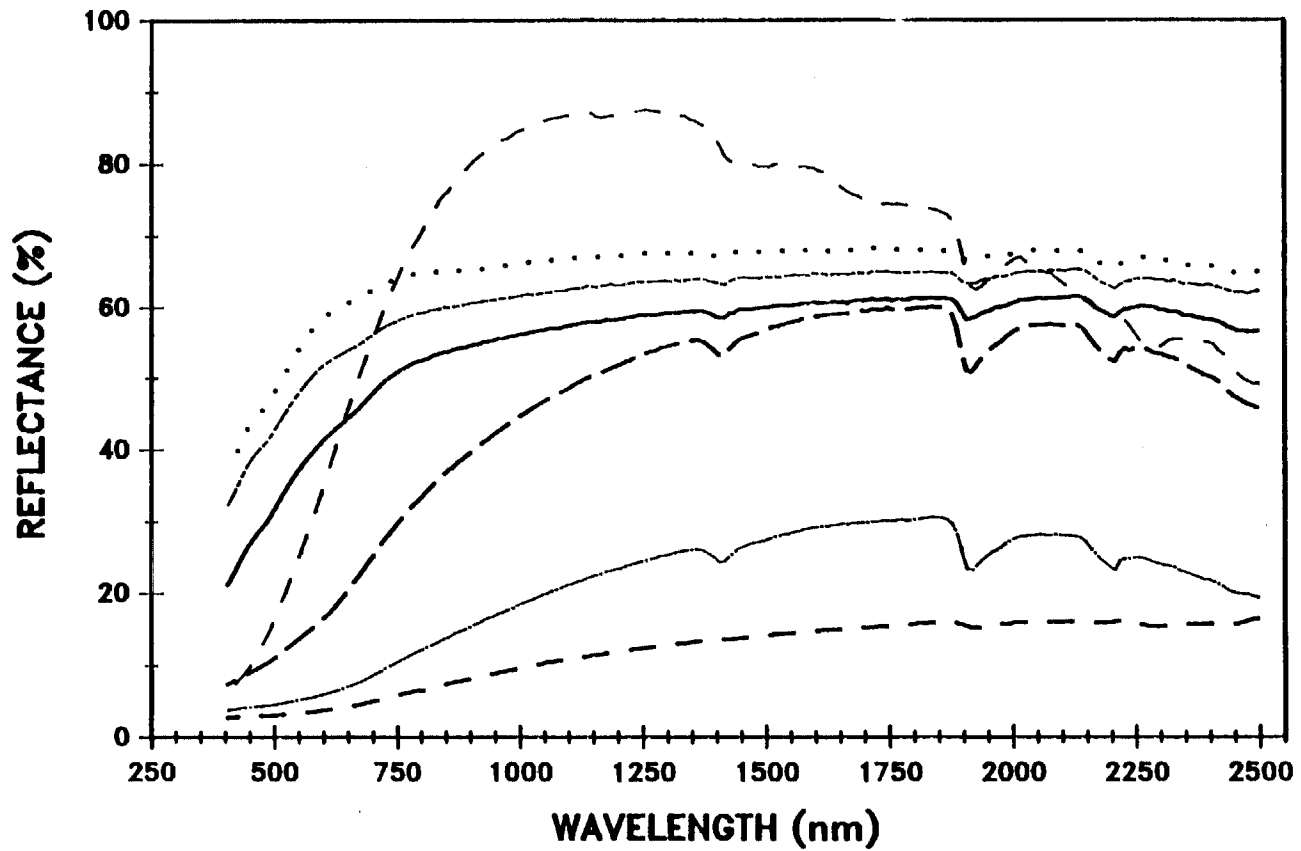


Figure 14. Average spectral reflectance curves of the Fincastle unfractionated soil and six Fincastle soil components.

CLERMONT SOIL COMPONENTS



— Unfractionated soil - - - Humin + Inorganic material · · · Inorganic material

— Organic matter extract - - - Humic acid (crude) - · - Humic acid (pure) - Fulvic acid (pure)

Figure 15. Average spectral reflectance curves of the Clermont unfractionated soil and six Clermont soil components.

visual inspection, which remained relatively constant for the four soils. The relationship of the extractable organic component curve shapes to overall soil reflectance will be discussed in a later section.

In the second set of seven composite plots, the four spectral curves for each component, one for each soil, were plotted on one set of axes. This allowed examination of reflectance differences between soils, and was more useful for relating individual soil properties to soil reflectance. The unfractionated soil and the mineral soil component were the most important materials for examining the effects of organic carbon content, in relation to other soil properties, on reflectance. The spectral curves of these two materials are shown in Figures 16 and 17. Visual inspection of the plots showed that not all the soils, or their mineral components, were separable across all wavelengths. For example, Figure 16 shows that all four unfractionated soils were spectrally separable in the visible (400-700 nm) portion of the spectrum, and Chalmers (a northern soil with 1.72% O.C.) had the lowest reflectance in this spectral region, while Clermont (a southern soil with 1.11% O.C.) had the highest reflectance. However, the reflectance curves for the Clermont and Fincastle soils, each having a similarly low organic matter content, were inseparable in wavelengths longer than 1400 nm. Examination of the spectral separability of soil and component curves, and interpretation of relative reflectance in the separable regions in terms of soil physical characteristics and genetic factors, provided the basis for studying relationships between observable soil properties and soil reflectance.

The spectral curves of the other soil components (humin plus mineral fraction, mineral fraction with iron oxides removed, crude

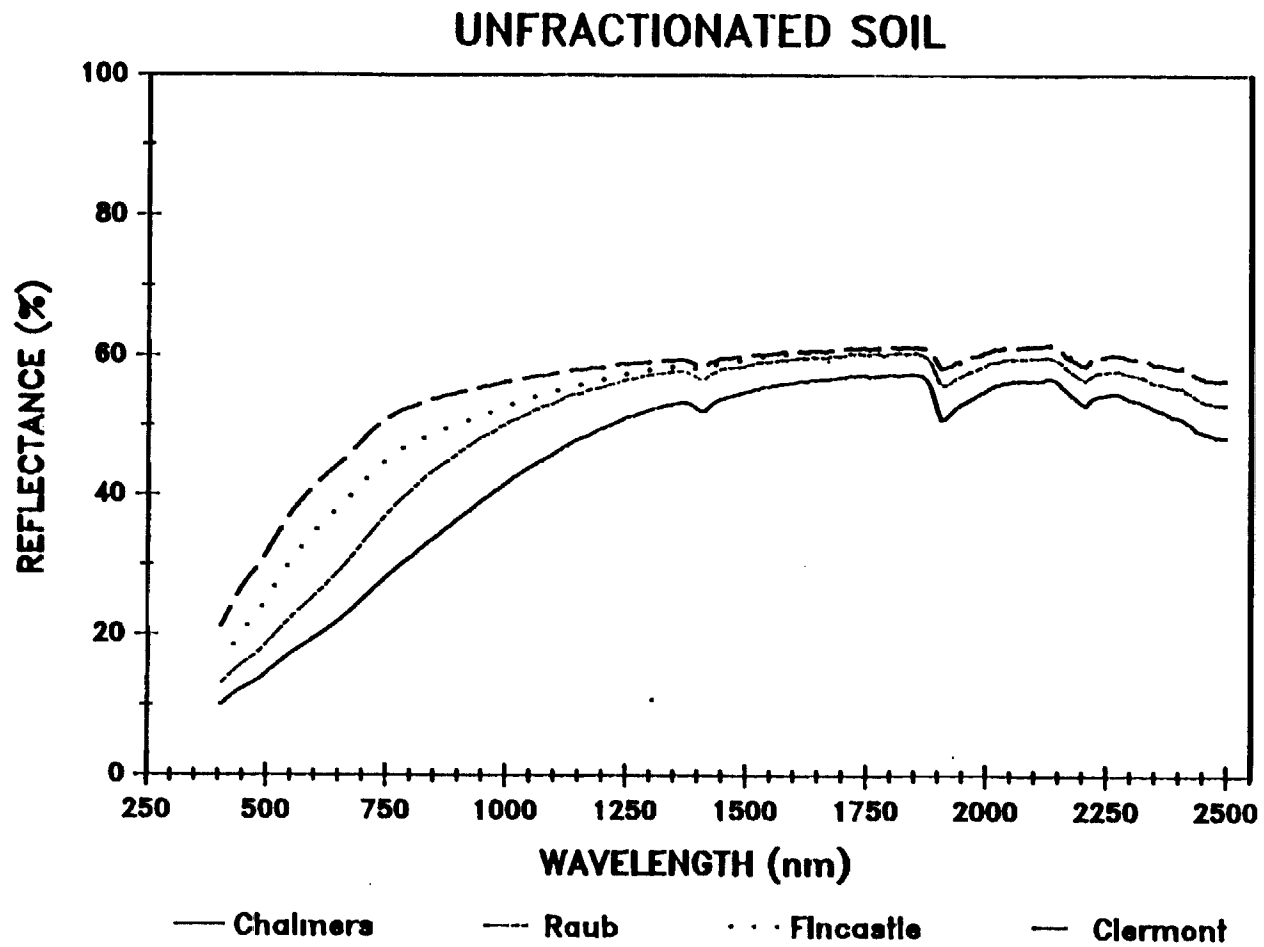


Figure 16. Average spectral reflectance curves of the un-fractionated soil samples of the four soils.

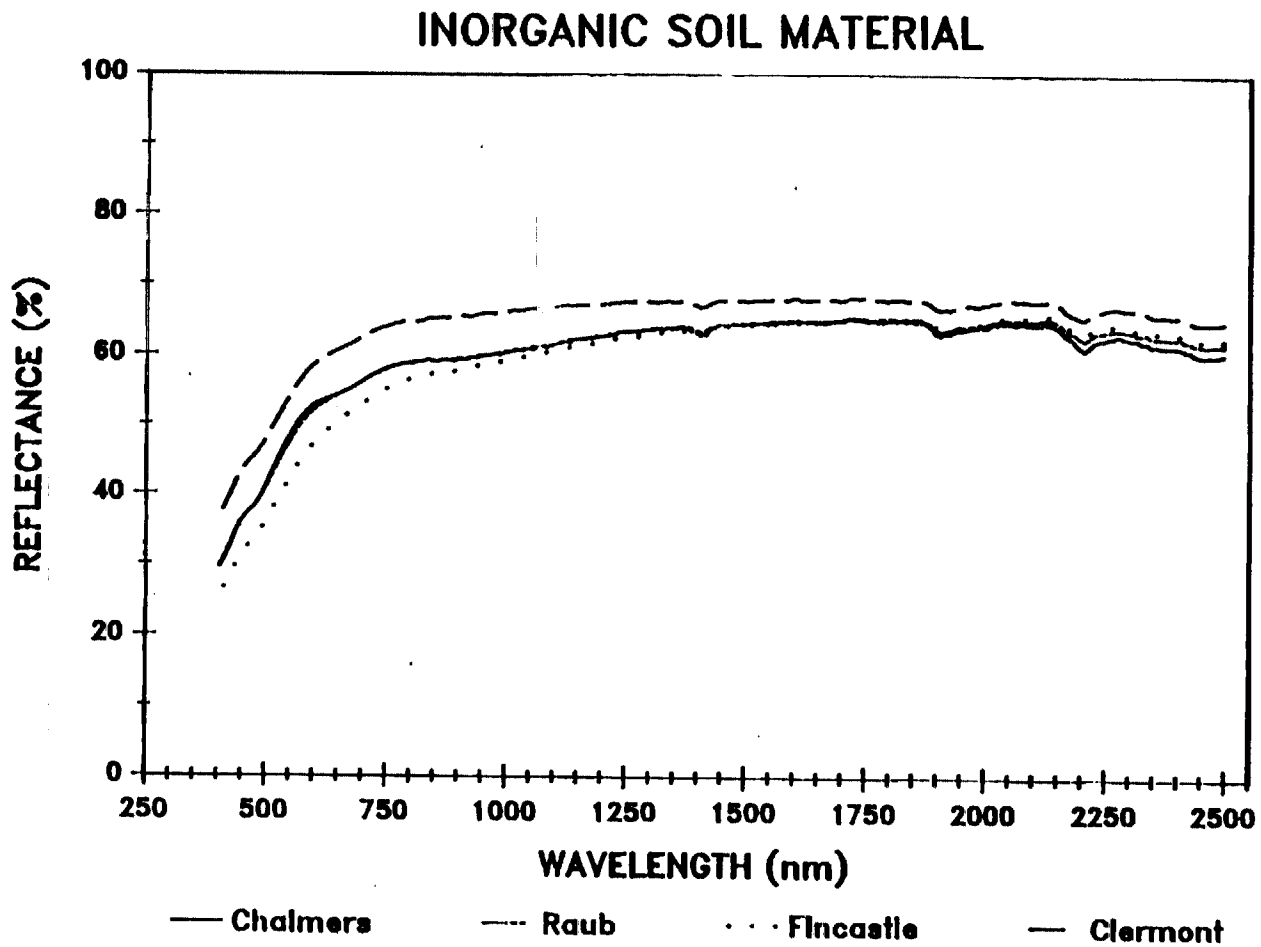


Figure 17. Average spectral reflectance curves of the inorganic (mineral only) components of the four soils.

extractable organic matter, crude humic acid, pure humic acid, and pure fulvic acid) will be presented and discussed in a later section.

Determination of Optimal Bands

The term optimal bands refers to the broad or narrow wavelength bands, or specific regions of the spectrum, in which reflectance measurements provide the most information for a particular application, in this case soil organic carbon content. The first step in the determination of optimal bands was to establish precisely where in the reflective spectrum the four soils, and their components, were separable. Although visual inspection of spectral curves was useful to identify gross differences in reflectance, statistical analysis was necessary for objective determination of separability.

Spectral separability of the composite curves was determined using the Tukey analysis for multiple means comparison to compare the reflectance value of each material over the four soils, in each of the 210 wavelength bands. A 1% Type I error criterion (99% significance level) was used to establish a very conservative test of separability. Separability analysis was performed to compare the differences in reflectance between the four soils for each of the seven materials (unfractionated soil and six components) individually, using the same procedure. The procedure used for unfractionated soil was as follows: The separability of each pair of unfractionated soils was analyzed separately using the Tukey method in each band, for a total of six pair combinations for the four soils. The bands in which the reflectance curves were statistically separable were noted for each pair. The intersection of the six sets of separable bands was taken as the final

set of separable bands for the four unfractionated soils. In other words, each of the four soils had to be statistically distinct from every other soil in a band, for that band to be considered a "separable" band. In this way, a strict separability criterion was maintained. The final set of separable bands was noted for the four unfractionated soils, and for each set of components as the analyses were performed.

The separability analysis of the unfractionated soils alone did not provide the desired information about how soil organic matter affects reflectance. It simply indicated that for some reason, the four soils had different spectral responses in the separable bands. To examine the specific effects of soil organic carbon content on soil reflectance, the causes of the different spectral responses had to be investigated in terms of observable soil properties, including organic carbon content. This was accomplished by comparing the separable bands of the inorganic fractions of the soils (in which the extractable organic matter had been removed, and the humin remaining with the mineral soil had been destroyed with H_2O_2) with the separable bands for the unfractionated soils. In bands where the mineral fraction curves were not separable, the spectral characteristics of the mineral soil components were considered the same. If, in the same bands, the unfractionated soil curves (organic matter intact) were separable, the soil factor causing the differences in spectral response was concluded to be organic matter.

Interpretation of the results of the separability analysis was a long process, involving comparison of separable and inseparable bands for different soils and components, followed by study of the magnitude of spectral response in the separable bands, and attempts to relate the magnitudes of reflectance to measured soil physical and chemical

properties. The logic involved in the interpretation of the separability analyses will now be explained, using a simple example that can be observed visually as well as statistically.

The reflectance curves of the mineral fractions of the Chalmers and Raub soils were not separable in the 750-1000 nm range (Figure 17). However, the unfractionated soil spectral curves of the same two soils were highly separable in the same 750-1000 nm range (Figure 16). Since the two mineral fractions had the same spectral response, the difference in the unfractionated soil reflectance in these separable bands can be attributed to the organic matter in the two soils.

Examination of the magnitude of unfractionated soil reflectance in bands where the mineral soils were inseparable but the unfractionated soils were separable showed the intensity of the reflectance response to be related to organic carbon content. Using the same example in the 750-1000 nm range, Raub unfractionated soil had a higher reflectance than Chalmers unfractionated soil (Figure 16). The difference in spectral response was previously attributed to differences related to the soil organic matter of the two soils. Such differences could be due to organic matter content, organic matter quality, or some combination of the two. In general, an increase in soil organic matter content causes decreased soil reflectance, so it was hypothesized that the Chalmers soil, which showed the lower spectral response, contained more organic matter than the Raub soil. A check of the measured organic carbon content of each of the two soils proved this to be true. Chalmers had 1.72% O.C., while Raub had 1.46% O.C. It can therefore be concluded that soil organic carbon content is a soil property which influences soil reflectance in the 750-1000 nm range.

It cannot be concluded from the above example that soil organic carbon content is the only soil factor which influences soil reflectance in this wavelength range. For the two soils considered, organic matter composition may also be a factor. This possibility will be investigated in a later section. Although the mineral reflectance curves for the Raub and Chalmers soils were inseparable in this range, it is possible that mineral factors which did not differ between these two soils may also have influenced reflectance. An examination of the Fincastle and Clermont spectral curves proved this to be the case. Figure 17 shows that the Fincastle and Clermont mineral fraction curves were distinctly separable from the Raub and Chalmers curves in the same 750-1000 nm range. Since none of the mineral fraction samples contained organic matter, some soil property inherent in the mineral fraction of these two soils affected the reflectance response. Another examination of the unfractionated soil curves (Figure 16) shows that in the 750-1000 nm range, the spectral curves of all four of the intact soils were separable. Figures 12-15 show that for each soil series, the reflectance of the unfractionated soil was lower than that of the mineral component. From these observations, and the organic carbon contents of the four soils, it can be inferred that both organic carbon content and some (yet to be identified) mineral characteristic influenced the reflectance of the unfractionated soils in the 750-1000 nm bands.

Analysis and interpretation using the logic described in the above example, based on statistical rather than visually-observed curve separability, was performed on the unfractionated soils and mineral fractions over the set of four soils for all 210 bands. It should be

noted that in no region of the spectrum were the mineral fractions of all four soils inseparable. Over all bands, the Clermont mineral fraction had a considerably higher reflectance than the other three soils, while the spectral curves of the Chalmers, Raub, and Fincastle mineral soils were very close to each other, and were indistinguishable throughout a large portion of the spectrum (Figure 17). The only major soil genetic factor which differed between Clermont and all of the other three soils was parent material, or the source of the loess cover. This parent material difference is related to geographic location and the presumed mineralogy differences in the loess originating from the northern and southern sources.

Stoner (1979) found that parent material had an indirect effect on soil reflectance by influencing soil texture and mineralogy. This finding gives credence to the hypothesis that the difference in Clermont mineral reflectance from the other soils is related to the complicating effects of parent material. Fernandez et al. (1988) found that linear relationships between visible reflectance and organic matter content of unfractionated soils were valid within toposequences, but the relationships broke down when comparing soils from different geographic regions. They hypothesized that reflectance would be useful as a tool to estimate soil organic matter content only within relatively small, homogeneous geographic areas. The limitation of the organic matter - reflectance relationship over larger areas was not attributed to any quantifiable soil property.

One of the major advantages of high-altitude remote sensing systems such as HIRIS is the ability to collect data simultaneously over very large geographic areas. If HIRIS data are to be employed to estimate

and monitor levels of soil organic matter, the feasibility of extending organic carbon content - reflectance relationships over large areas must be investigated. It is the hypothesis of this author that all reflectance differences in unvegetated soils (excluding surface roughness conditions) result from, and can be attributed to, observable and quantifiable soil properties. If this is true, geographic distance alone is not a factor which can disrupt organic matter - reflectance relationships. Therefore the differences in mineral reflectance of soils from different regions must be due to quantifiable soil properties which vary with geographic area because they are directly related to parent material, age, climate or other site-specific genetic factors. Examples of such soil properties are texture, mineralogy, and perhaps other factors not yet identified. In theory, if all such factors can be identified, and their relationships to reflectance and other factors can be understood and quantified, there should be no geographic limitation to the use of HIRIS data for monitoring soil properties worldwide.

In the case of the four soils used in this study, the reflectance differences between Clermont and the other soil mineral fractions were most likely due to the indirect influences of the two different parent materials on soil mineral properties. An attempt to confirm this, and to identify the other soil properties responsible for these reflectance differences, will be discussed in a later section. Given the fact, however, that parent material differences do exist in nature and complicate organic matter - reflectance relationships, one of the goals of this research was to investigate the feasibility of using reflectance data in HIRIS bands to estimate organic carbon contents of soils formed on different parent materials in spite of these differences. To this

end, the separability analysis, described above for the set of all four soils, was repeated for the set of the three northern soils (Chalmers, Raub, and Fincastle) which were formed on the same parent material.

Results of the separability analyses for both sets of soils (the northern, or "same parent material" set of three soils and the "different parent materials" set of all four soils) are presented in Table 14. Interpretations were first made on the separable bands for the three northern soils, to gain an understanding of the importance of the organic carbon content effect on reflectance without the complicating effects of parent material. The first three columns of the table list all the bands over which the unfractionated soil spectral curves for the three soils were separable. The atmospheric water absorption bands (1350-1450 nm and 1850-1950 nm) were excluded from the list whether or not they were separable, because accurate signals in these bands cannot be detected from space altitudes due to atmospheric interference. Only the separable bands are listed in the table. The soil curves in any bands not listed (mainly in the shorter middle infrared wavelengths) were inseparable, and therefore were not information-bearing for the purposes of identifying soils based on differing organic carbon content.

As discussed earlier, the fact that bands are separable does not indicate that they are responding to organic carbon content. For this reason, the separable bands for the three soils were divided into three bandsets, based on the nature of the reflectance response to organic carbon content and other soil properties. The first column in Table 14 lists the bands which responded to organic carbon content differences only. In these bands, the three mineral fraction curves were

Table 14. Importance of spectral bands based on response of soil reflectance to organic carbon (O.C.) content, parent material (P.M.) and other soil properties. The wavelength range of each band is given in nanometers (nm).

Within similar P.M. (Northern Soils)			Across different P.M. (All Soils)
Responds to O.C. content differences only	Responds to other properties, but O.C. content effect is dominant	Responds to other properties which mask O.C. content effect	Responds to O.C. content and minimizes P.M. effect
(nm)	(nm)	(nm)	(nm)
1065	405-1055	1775-1885	1955-1965
1085-1105	1075	1975-2205	2215
1125-1165	1115	2225-2255	2265
1955-1965	2325	2275	2285-2295
2215	2375		2315-2495
2265	2425		
2285-2295	2445-2485		
2315			
2335-2365			
2385-2415			
2435			
2495			

inseparable, so the unfractionated soil reflectance differences could not be attributed to mineral factors. The magnitudes of reflectance of the unfractionated soil curves in these bands were ordered according to organic carbon content, with Chalmers (1.72% O.C.) having the lowest reflectance, followed by Raub (1.46% O.C.) and Fincastle (0.99% O.C.) with the highest reflectance. This relative ordering of the soil spectral curves was evidence that organic carbon content controlled the reflectance response in these bands, an optimal condition for organic carbon content estimation. Four of these twelve optimal "column 1" bands were in the near infrared portion of the spectrum, with the rest in the middle infrared wavelengths.

The second column in Table 14 lists the bands in which reflectance of the unfractionated soils responds to both organic carbon content and other soil properties. These bands are still considered important for organic carbon content estimation, because the effects of organic carbon content on reflectance dominate or mask the effects of the other soil properties. In these bands, the three unfractionated soil curves were all separable, but the mineral fraction curves were also separable, for at least some combination of the three curves. The relative ordering of the mineral fraction reflectance curves was Fincastle (lowest reflectance) followed by Chalmers and Raub together. This indicated that some property of the inorganic fraction of the soil, which differed between Fincastle and the other two soils, was influencing the mineral fraction reflectance in these bands. However, the unfractionated soil reflectance in these bands was ordered according to organic carbon content, with Chalmers having the lowest reflectance and Fincastle, the highest, as in the column 1 bands. This indicated that in spite of the

influence of mineral soil properties, organic carbon content was the dominant factor controlling reflectance in the column 2 bands. These bands represent the entire visible range, as well as some near and middle infrared wavelengths.

The third set of bands for the three soils with similar parent material (Table 14, column 3) contained those bands for which some combination of the unfractionated soil curves were statistically separable, but the magnitude of the unfractionated soil reflectance did not correspond to organic carbon content. In most of these bands, Chalmers soil still had the lowest reflectance, but the Raub and Fincastle soils could not be distinguished despite the observed differences in their organic carbon contents. In some of these bands, the Raub soil actually had a slightly higher reflectance than Fincastle. The mineral fractions in most of the column 3 bands were not statistically separable. From examination of the spectral curves, it appears that reflectance in these bands responded significantly only to high amounts of organic carbon. For soils containing low or moderate levels of organic matter, other properties masked the effects of organic carbon content on reflectance. Use of data in these bands, all in the middle infrared wavelengths, would probably be detrimental to a classification of remotely sensed data according to soil organic carbon content.

The last column of Table 14 represents the optimal bands for separating the set of four soils (including Clermont, formed on the different parent material) according to organic carbon content levels. Throughout the spectrum, the Clermont mineral fraction curve was clearly separable from the mineral curves of the other three soils. This was

expected, due to the presumed mineral differences resulting from the different sources of parent material. In the bands listed in column 4, the mineral reflectance curves of at least some combination of the three northern soils were separable, indicating that both mineral factors and some factors related to parent material affected reflectance. Three unfractionated soil spectral curves in these bands were distinguishable. Chalmers had the lowest reflectance, followed by Raub, followed by the third curve which represented both Fincastle and Clermont. The Fincastle and Clermont unfractionated soil curves were not only inseparable, but were nearly identical in these bands (Figure 16). This indicated that, in spite of the parent material differences, reflectance in these bands corresponded very clearly with three levels of organic carbon content: high (Chalmers), moderate (Raub), and low (Fincastle and Clermont). The difference in organic carbon content between the Fincastle and Clermont soils was only 0.12% O.C., a small amount compared to the 0.35% O.C. difference between Clermont and Raub and the 0.26% O.C. difference between Raub and Chalmers. The capability to distinguish between the three organic matter levels in spite of parent material differences is considered valuable, and for practical purposes, the inability to detect differences on the order of 0.1% O.C. for low organic matter soils is not considered a problem. Therefore, the column 4 bands, all of which lie in the longwave middle infrared region of the spectrum, may have potential for the estimation of levels of organic carbon content in soils formed on different parent materials or sampled across large geographic areas. These results are not conclusive, however, as only one soil from a different parent material was tested. Further testing, using soils from many other parent materials and

geographic regions, will be necessary.

The locations in the spectrum of the four sets of separable bands are illustrated in Figure 18. Examination of this figure shows that both broad and narrow wavelength bands are significant. Narrow-band reflectance appears to be particularly important in the middle infrared portion of the spectrum. With the exception of one MIR band (1955-1965 nm), most of the bands in the 1200-2200 nm range are either insignificant or actually detrimental to the use of reflectance data for soil organic carbon content classification.

It is interesting to consider the relative importance of HIRIS bands for organic carbon content estimation in terms of the existing reflective bands on the Landsat Thematic Mapper. The TM bands 1-4 (all in the visible and near infrared regions) fall into column 2 of Table 14, or the second order of importance. TM band 5 (1550-1750 nm) appears not to contain any useful information about soil organic carbon content. Parts of this band were inseparable, and parts fall under column 3. TM band 7 (2080-2350 nm) includes some column 4 bands, optimal for observing soils over different parent materials, but also includes some "bad" bands from column 3, thus limiting its usefulness for soil organic matter studies.

Separability analyses or statistical distance measures are routinely used on multispectral remote sensing image data as criteria for band selection. However, it is clear from the above example that separability alone is not enough to determine optimal bands. In the separable bands, soil reflectance must be shown to respond to the soil

IMPORTANCE OF SPECTRAL BANDS

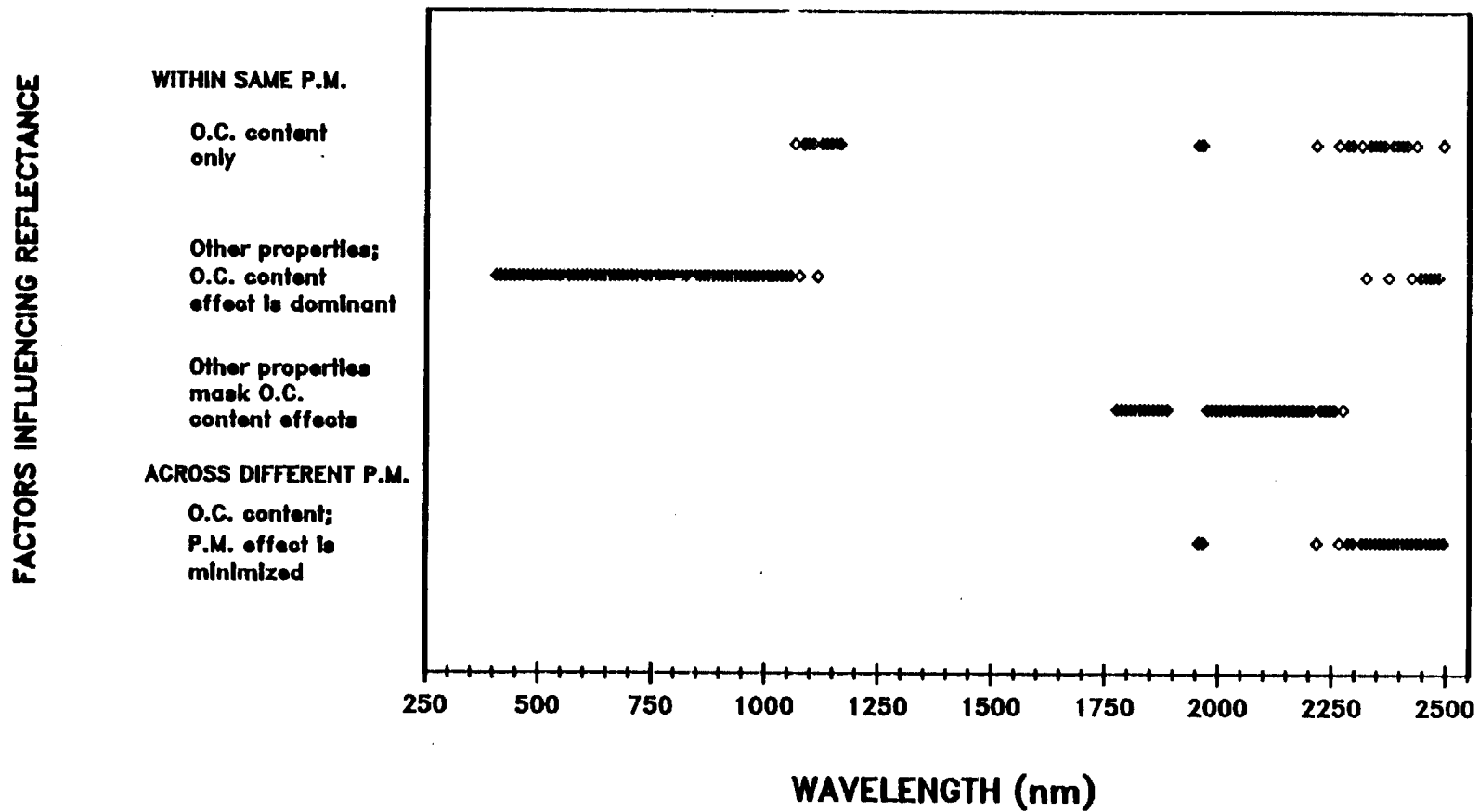


Figure 18. Importance of spectral bands for reflectance measurements as influenced by organic carbon content, parent material, and other soil properties.

property or characteristic in question to be considered information-bearing.

Comparison of bands identified as separable for the two different sets of soils indicated that different bands may be optimal for providing information about soil organic carbon content, depending on the homogeneity of the parent materials of the soils under investigation. Theoretically, the bands in column 1 of Table 14 should be considered the optimal bands for organic carbon content estimation in soils formed on the same parent material, followed by the bands in column 2. To emphasize the effect of organic carbon content while minimizing mineral differences between soils, column 1 bands should be used. If mineral soil differences between the soils under study are very slight, column 2 bands should be used together with column 1 bands. For organic carbon content estimation across soils formed on different parent materials, or from different geographic locations, the bands in column 4 of Table 14 should be optimal. These optimal band determinations are based on the limited set of samples used in this study, and must be confirmed with more soils before they can be considered conclusive. The band determinations were based on the physical nature of the soil-reflectance response only, without regard to the relative strength of the organic matter - reflectance relationship in the different bandsets. This factor was considered next, during statistical evaluation of the optimal bands.

Evaluation of Band Selection

Statistical evaluation of the optimal bands was performed using both discriminant and correlation analyses. The purpose of the

discriminant analysis was to verify that the four soils could be differentiated and placed into four classes based on the spectral data in the separable bands. For this analysis, the original six 210-band scans in each sample block were used, rather than the average reflectance data files for each soil. One of the six scans for each unfractionated soil provided the training data, and the rest of the scans were used as test data. The discriminant analysis was performed on each of the four sets of separable bands as identified in Table 14. Results showed that each set of separable bands was capable of differentiating between the four different soils represented by the twenty test samples with 100% accuracy. While this statistical analysis did not give any information on the relationship of organic carbon content to reflectance in the different bandsets, it did provide a means to verify that the reflectance curves in the identified bands were separable, validating the results of the separability analysis.

The strength of the organic carbon content - reflectance relationship in the four separable bandsets was evaluated using correlation analysis. In each of the 210 bands, the correlation between reflectance values and organic carbon contents of the four soils was computed. The correlations were plotted against wavelength to compare the degree of correlation in the different bandsets. Next, correlations were computed again, this time for the set of three northern soils formed on the same parent material, and plotted on the same set of axes (Figure 19). This allowed comparison of the effects of parent material differences on the organic carbon content - reflectance relationship in different bands.

CORRELATIONS OF SOIL REFLECTANCE WITH O.C. CONTENT

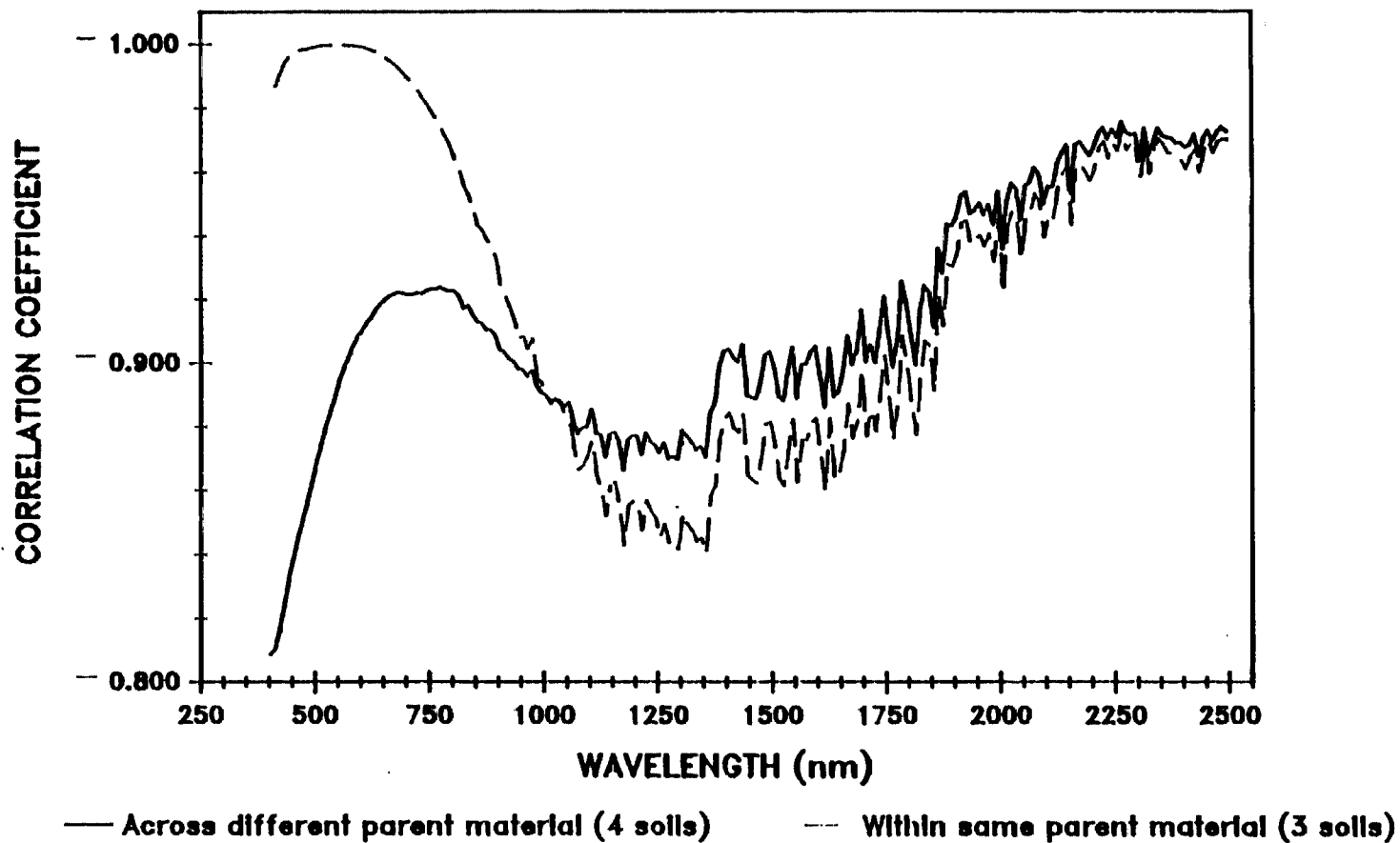


Figure 19. Correlation of soil reflectance with soil organic carbon content as a function of wavelength, comparing soils formed on two different parent materials with soils formed on the same parent material.

All of the correlations were extremely high ($r = -0.80$ or better) in this analysis, due to the limited number of samples used. If more samples were used, the correlations could be expected to decrease to some extent. For this analysis, it is the relative magnitudes of the correlations in different bands and for the two sets of soils which is of importance.

An examination of the correlation plots in Figure 19 shows that the highest correlation ($r = -0.99$) was obtained for the set of three northern soils in one broad visible band, approximately 500-750 nm. For the set of all four soils, the correlations in the visible bands were considerably lower, particularly in the 400-500 nm range. This indicates that the organic matter - reflectance relationship is stronger in the visible bands than in any other bandset, but only if the mineral differences between the soils are slight. The complicating effects of parent material greatly reduce the correlation in these bands.

Correlations for both sets of soils fell significantly in the NIR bands. Interestingly, the organic carbon content - reflectance relationship deteriorated much more rapidly in these bands for the northern soils than for the set of all soils. This supports the hypothesis that in these bands, reflectance is responding to other soil factors such as mineral differences as well as organic carbon content. In wavelengths longer than 1000 nm, correlations were consistently higher for the set of all soils, and in the long MIR bands, the correlations for both soil groups were almost as high ($r = -0.98$) as the maximum correlation for the northern soils in the visible bands. This suggests that reflectance data in the long MIR bands may prove to be very useful for soil organic carbon content estimation, regardless of

the parent material.

To aid in visual interpretation of the correlation plots in terms of the four separable bandsets identified in Table 14, rough approximations of bandset locations are given. The bands in column 1 (first set of optimal bands) correspond to the long MIR bands (2200-2500 nm) with several NIR bands; the column 2 bands correspond to the visible and short NIR range; column 3 bands are represented in the 1700-2200 nm range; and column 4 bands correspond to the 2200-2500 nm MIR wavelengths. Results of the correlation analysis support the selection of optimal bands presented in the previous section. For soils having the same parent material, column 1 and 2 bands together were optimal, since the mineral differences between these soils were very slight. However, when a soil from a different parent material or having different mineral reflectance characteristics was introduced, the correlation in the column 2 (visible and short NIR) bands was much lower. Many of the shorter MIR bands (the column 3 "bad" bands) gave low correlations between reflectance and organic carbon content. The optimal bands for soils formed on different parent materials were the long MIR column 4 bands. These results suggest that the MIR bands will be particularly important for soil organic matter studies using HIRIS data.

Narrow-band vs. Broadband Reflectance

The collection, transmission, and processing of high-dimensional satellite data will require a great deal of time and expense. Several means of data compression have been proposed for use with the HIRIS sensor. One is the collection of data in selected broad bands rather

than in all the narrow bands in regions of the spectrum where no advantage of high spectral resolution is apparent. In broadband reflectance, one reflectance data value represents the average of the reflectance values in several contiguous narrow bands. The current design of HIRIS will allow researchers to select the broad and narrow bands over which data will be collected, according to the specific requirements of the project. One advantage of broadband reflectance is a higher signal to noise ratio.

To compare the effects of broad- and narrow-band data on soil reflectance - organic matter relationships in the different band sets, correlations between organic carbon content and soil reflectance for the 210 narrow bands and for the significant broad bands (consisting of sets of contiguous narrow bands identified as significant in Table 14) were plotted on the same set of axes. Figure 20 shows the graph of narrow and broadband correlations for the three soils formed on the same parent material. Correlations for the set of four soils were plotted in Figure 21.

The graphs indicate that broadband reflectance may be used to represent the separable long MIR bands without a significant decrease in correlation. The reflectance in the single broad band in the visible-NIR region had a lower correlation with organic carbon content than reflectance in the best individual bands (approximately 500-700 nm), probably because of the sharp change in reflectance response over the broad band. If a smaller broad band were used instead, representing the best wavelengths (the flat part of the correlation curve), better results would have been achieved.

The sharp peaks in the correlation curves in the near and middle

CORRELATIONS OF SOIL REFLECTANCE WITH O.C. CONTENT
NARROW-BAND VS. BROADBAND EFFECT
(Within same P.M., 3 soils)

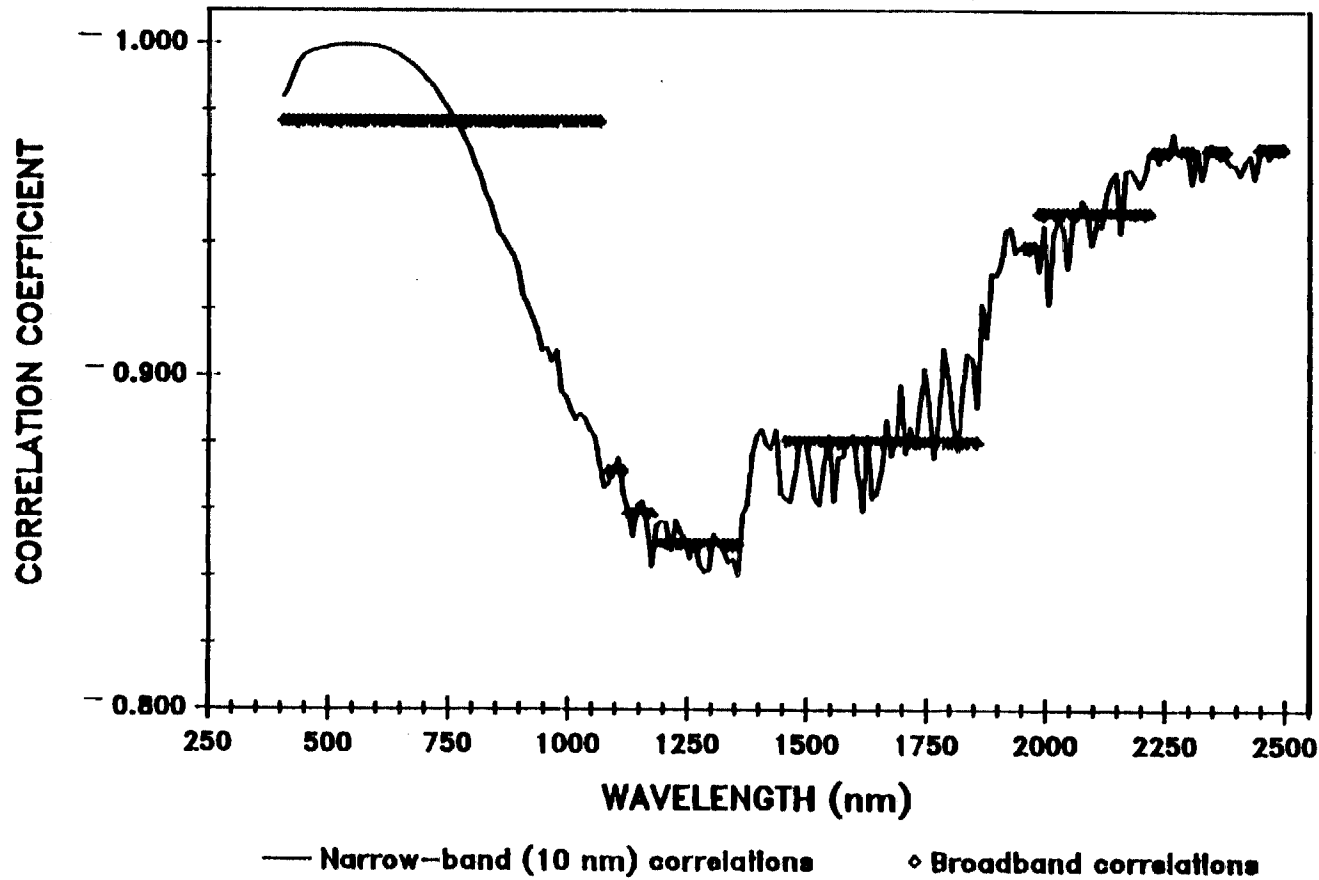


Figure 20. Correlation of soil reflectance with soil organic carbon content as a function of narrow and broad wavelength bands, for soils formed on the same parent material.

CORRELATIONS OF SOIL REFLECTANCE WITH O.C. CONTENT
NARROW-BAND VS. BROADBAND EFFECT
 (Across different P.M., 4 soils)

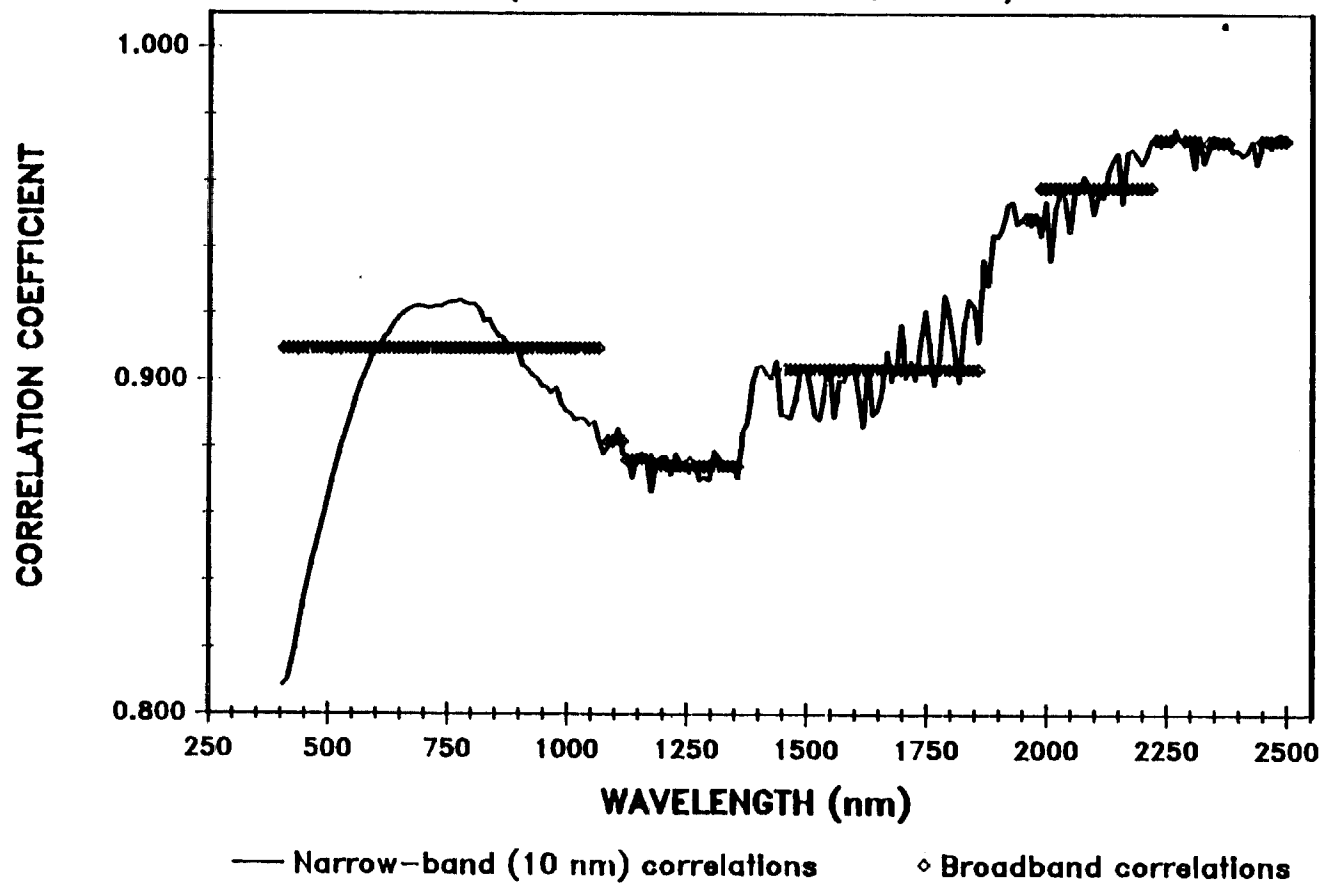


Figure 21. Correlation of soil reflectance with soil organic carbon content as a function of narrow and broad wavelength bands, for soils formed on two different parent materials.

infrared regions are most likely due to magnified effects of the noisier data with the lead sulfide detector, and are probably not significant in terms of changes in the organic matter - reflectance relationship over individual narrow bands. Thus, the results of broadband reflectance would probably not differ significantly from narrowband reflectance in bands where reflectance was known to respond similarly to organic carbon content. Comparison of the two graphs (Figures 20 and 21) showed no evidence of parent material effect on the information content of broad versus narrow bands.

Visual analysis of the correlation curves suggests that the loss of spectral detail due to averaging reflectance over broad bands may not be detrimental for soil organic matter studies if the broad bands are chosen carefully, since soil reflectance curves are relatively flat and contain few sharp features. However, further research using high-altitude data will be necessary before conclusions can be made about the use of broadband reflectance as a HIRIS data compression technique.

Effects of Other Soil Properties on Reflectance

It has been shown in previous sections that soil reflectance responds to other factors in addition to organic carbon content, and that some of these factors are properties of the mineral fractions of the soils. An examination of Figure 17 indicated that the Clermont mineral fraction had the highest reflectance and was separable from the other soils throughout the 400-2500 nm range. The Chalmers and Raub (two soils from the same toposequence) mineral fraction reflectance curves were nearly identical, except in the 550-650 nm range, where Chalmers had a slightly higher reflectance, and in the 2100-2500 nm

range, where Raub mineral reflectance was slightly higher. The Fincastle mineral fraction had the lowest reflectance in the 400-1350 nm range, after which it converged with the Raub and Chalmers curves until the long MIR wavelengths, where Fincastle reflectance became slightly higher than Raub.

The band separability analyses indicated that the factors causing these differences in mineral reflectance of the four soils also complicated the organic matter - reflectance relationships in certain sets of bands. For a thorough understanding of organic matter - reflectance relationships, the nature of these other factors must be known. Based on the premise that soil reflectance can be explained by a combination of quantifiable soil properties, an attempt was made to identify the properties of the mineral soil fractions which caused the different reflectance responses.

Stoner (1979) listed the soil properties which directly affect reflectance as organic matter content, particle size distribution or soil texture, soil moisture, surface conditions, and soil mineralogy, including the amount and kind of iron and manganese oxide pigments. Moisture content and surface conditions were controlled in this experiment. The texture effect could not be evaluated but was presumed to be of minimal importance for this experiment, because all the soils had to be ground to pass a 250 μm sieve before spectral measurements, resulting in a relatively uniform particle size for all samples. This left mineralogy or other unidentified factors to be investigated as the source of the reflectance differences.

Each of the four mineral fractions were examined under a microscope. Evidence of iron coatings appeared in all the soils, but

were much darker on the three soils (Chalmers, Fincastle, and Raub) from the northern part of the state. There was also some evidence of manganese concretions in the Fincastle soil. Results of laboratory analyses for soil iron and manganese oxides confirmed these findings (Table 9). The Chalmers, Raub and Fincastle soils had approximately the same iron oxide content (1.25% to 1.30%), which was much higher than that of Clermont (0.4%). Assuming a higher iron oxide content decreases reflectance, this fact would explain the higher reflectance of the Clermont mineral fraction throughout the spectrum. The small difference (0.05%) in iron oxide content between the Chalmers and Raub soils may also have been detected in the reflectance data, and may explain the slightly higher Chalmers mineral reflectance in the 550-650 nm range. Reflectance has been previously shown to be sensitive to iron in absorption bands in the visible portion of the spectrum.

Fincastle soil had the highest Mn oxide content. Since manganese concretions are black, they absorb light and can be assumed to lower reflectance, at least in the visible wavelengths. Therefore, this Mn oxide content provided an explanation for the lower reflectance of the Fincastle mineral fraction in the 400-1350 nm range.

To test the hypothesis that varying amounts of iron and manganese oxide pigments in the four soils caused the differences in mineral reflectance, two samples of each soil mineral fraction were treated with a citrate-bicarbonate-dithionite (CBD) solution to remove iron and manganese oxides. Although visually detectable color differences existed in the four mineral fractions prior to this treatment, after the extraction all the samples were gray in color (10YR/8/1) and could no longer be differentiated visually. Reflectance was measured on each of

the two samples from each soil, and the average spectral curves were plotted on the same set of axes (Figure 22). Examination of the spectral curves showed only very slight reflectance differences among the three soils formed on the northern parent material, once organic matter and the mineral pigments were removed. This indicated that iron and manganese oxide pigments were largely responsible for the differences between Fincastle mineral reflectance and that of Chalmers and Raub. However, the spectral curve of the Clermont mineral fraction after CBD treatment was still significantly higher than that of the other three soils, suggesting that some factor other than iron and manganese oxide pigments was causing the higher reflectance of the Clermont soil.

The contribution of the iron and manganese oxide pigments to overall soil reflectance is illustrated in Figure 23 for Fincastle soil. The unfractionated soil had the lowest reflectance. Removal of organic matter left the intact mineral fraction, which had a higher reflectance than the whole soil. Removal of the iron and manganese oxides caused an even higher reflectance, indicating that a decreasing content of these mineral oxides causes increased reflectance. From comparing the two mineral curves, it can be seen that the mineral pigments had the greatest effect on reflectance in the visible portion of the spectrum. The same trend was also evident for the other three soils.

The evidence presented here suggests that although iron and manganese oxides have a strong influence on soil reflectance in the visible and NIR regions, they affect the soils from the two parent materials in this study similarly, and therefore do not explain the higher reflectance of the southern (Clermont) soil. The major

**MINERAL SOIL FRACTION
(AFTER EXTRACTION OF FE AND MN OXIDES)**

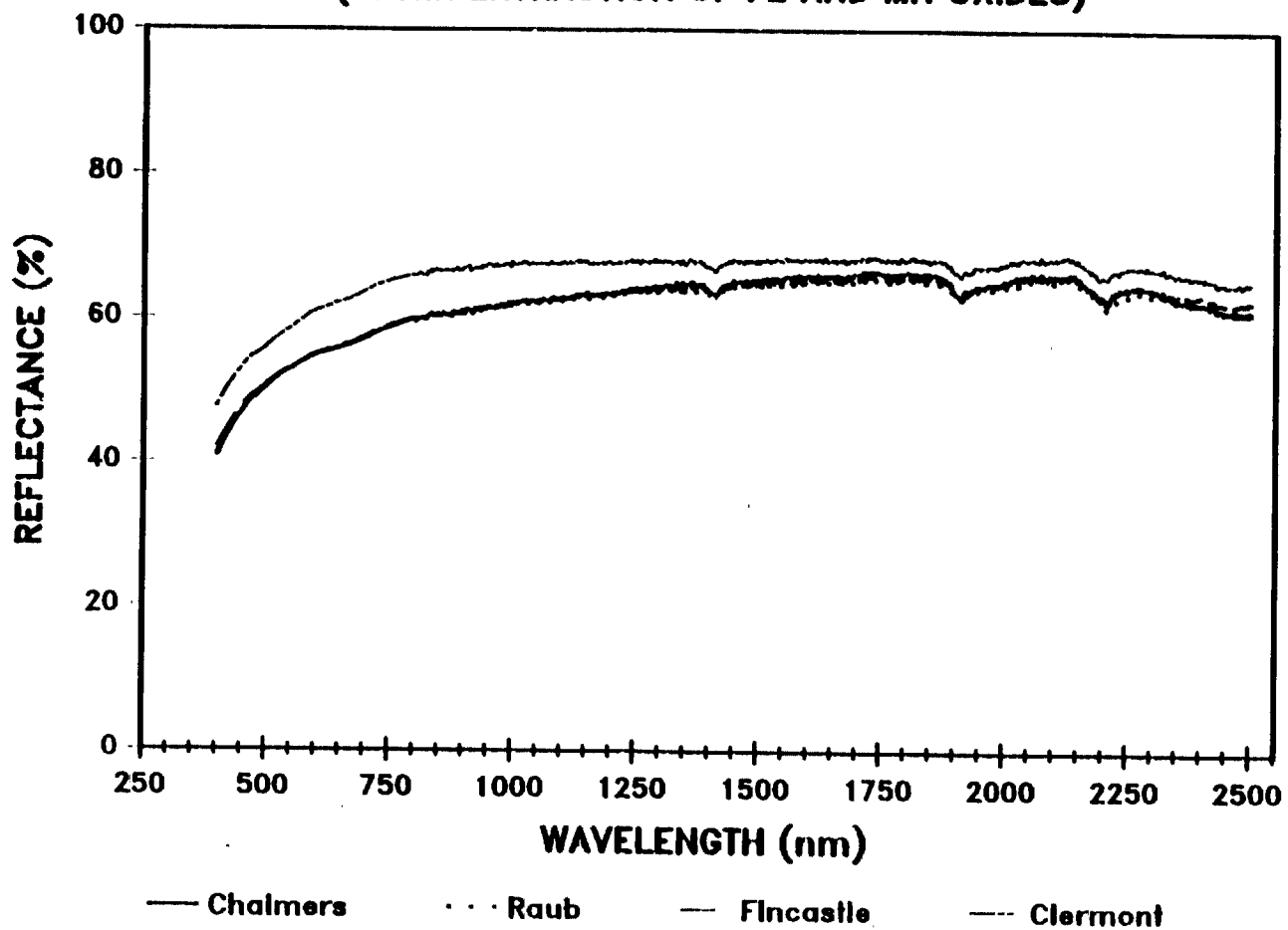


Figure 22. Average spectral curves of the mineral soil fraction of four soils, after extraction of iron and manganese oxides.

**EFFECT OF IRON AND MANGANESE OXIDE EXTRACTION
(FINCASTLE)**

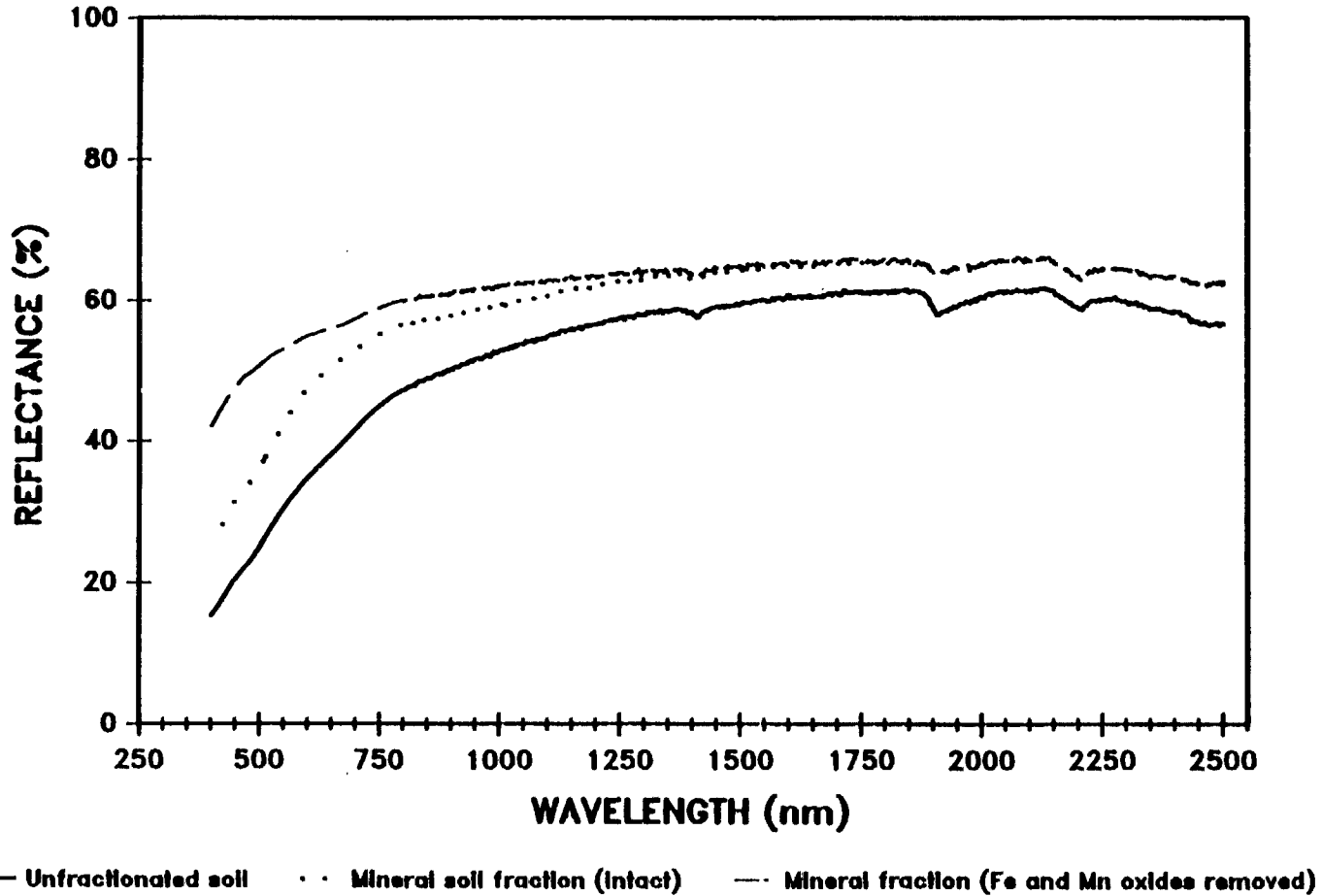


Figure 23. Average spectral curves of Fincastle unfractionated soil, intact mineral soil fraction, and mineral fraction after removal of iron and manganese oxides, illustrating the effect of iron and manganese oxide pigments on soil reflectance.

complicating factor when comparing organic matter - reflectance relationships for soils formed on different parent materials (or located in different geographic regions) has yet to be determined, but is probably related to the mineralogy, and perhaps the texture, of the soil matrix. If the specific factors can be identified, research can be undertaken to understand and quantify relationships between these factors and organic matter, mineral pigments, and reflectance. Such an understanding will bring routine utilization of HIRIS data for soil characterization over large areas a step closer to reality.

Limit of Organic Carbon Content Influence on Reflectance

In one of the earliest studies of organic matter - reflectance relationships, Baumgardner et al. (1970) found that organic matter masked the effects of other soil properties on reflectance when soil organic matter content exceeded 2%. Applying the 1.72 conversion factor from organic carbon content to organic matter content used by these researchers, this corresponded roughly to a lower cutoff point of 1.2% organic carbon, for organic matter to significantly affect soil reflectance. The soils they studied were taken from within a small and relatively homogeneous site in Tippecanoe County and were formed on the same parent material. The 2% organic matter cutoff point was based on correlation of organic matter content with reflectance values of the unfractionated soils in the field.

As part of the current research, an attempt was made to verify whether this 1.2% O.C. cutoff point, below which organic carbon content presumably did not have a dominant influence on soil reflectance, existed for the four soils studied in this project. Specifically, the

objective was to identify the lower limit of organic carbon content which was necessary to significantly influence the magnitude and relative ordering of the soil reflectance curves. The lowest soil organic carbon content (for Fincastle) was 0.99% O.C., only 0.2% O.C. lower than the previously identified limit. For this reason, the humin plus inorganic soil fractions were used to simulate very low organic matter soils.

The humin organic fraction consists of the humic substances left in the mineral soil residue after removal of the extractable organic matter, and is inseparable from the mineral soil. Historically, humin was thought to have a different chemical composition from humic and fulvic acids. Most organic matter chemists now believe that none of the humic substances are chemically distinct, and that the humin fraction actually consists of the same material as the extractable organic matter, but it is more tightly bound to clay particles. If this is true, humin in the soil can be considered to be a small amount of organic matter. For the purposes of this experiment, each humin plus mineral fraction was considered as a soil sample containing a very low amount of organic matter. Each of the four humin plus mineral soil fractions was analyzed for organic carbon content (Table 7), with values ranging from 0.44% O.C. for Clermont and Fincastle to 0.66% O.C. for Chalmers.

Comparison of the average spectral curves for the humin plus mineral fractions (Figure 24) with the unfractionated soil curves and the mineral fraction curves (Figures 16 and 17), showed that the humin plus mineral (simulated low organic matter soil) curves resembled the mineral curves much more closely than the unfractionated soil curves.

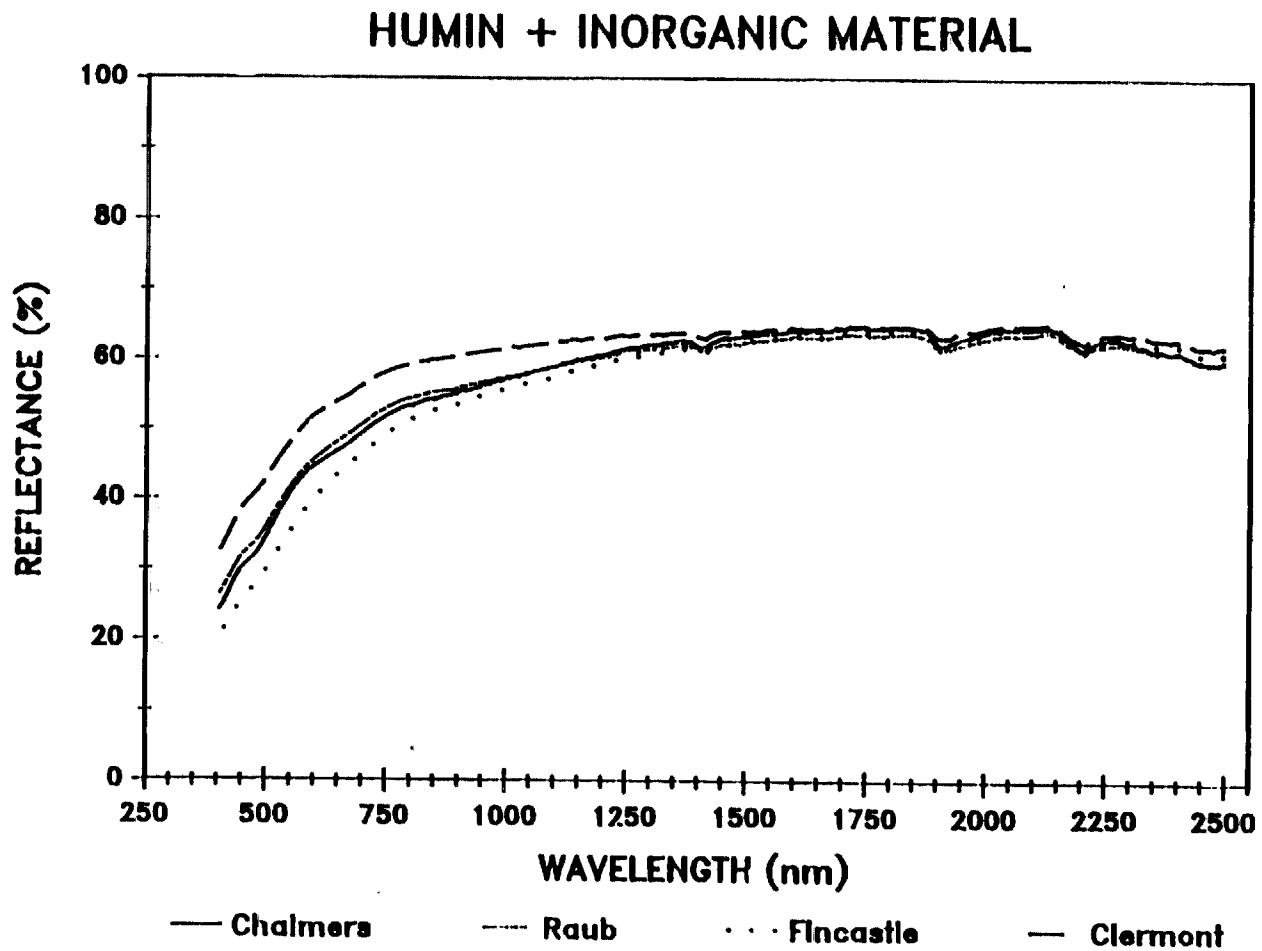


Figure 24. Average spectral reflectance curves of the humin plus inorganic fraction components of the four soils.

Fincastle had the lowest reflectance, while Clermont had the highest. In the visible and NIR wavelengths, the Chalmers humin plus inorganic fraction had a slightly lower reflectance than the same Raub fraction, whereas the mineral curves of these soils were nearly identical. This indicates that where no mineral differences exist, an organic carbon content as low as 0.66% O.C. can lower soil reflectance to a degree that can be detected. However, where mineral differences exist, none of the organic carbon contents in the humin plus inorganic fractions was high enough to mask out the effects of the mineral pigment contents on reflectance. Therefore, for the three soils on the same parent material, the lower limit of organic carbon content for dominant effects on reflectance was somewhere in the 0.66% O.C. to 0.99% O.C. range, slightly lower than the cutoff point identified by Baumgardner et al. For the four soils formed on two different parent materials, the cutoff point lies somewhere above 1.11% O.C., as this amount of organic carbon in the Clermont unfractionated soil was not enough to mask out the effects of its low mineral pigment content. A more conclusive lower limit could not be identified with only four soils.

The effect of very small amounts of organic carbon on soil reflectance also depends on the nature of the mineral soil material to be masked. A small amount of organic carbon will cause a greater reflectance difference on a lighter mineral fraction than on a darker one. Figures 25 and 26 show the effect of the humin fraction on reflectance for Chalmers and Clermont soils. Although the Chalmers humin plus mineral sample had a considerably higher organic carbon content, the magnitude of the decrease in reflectance it caused was approximately the same as that caused by the Clermont humin in the

EFFECT OF HUMIN ON REFLECTANCE (CHALMERS)

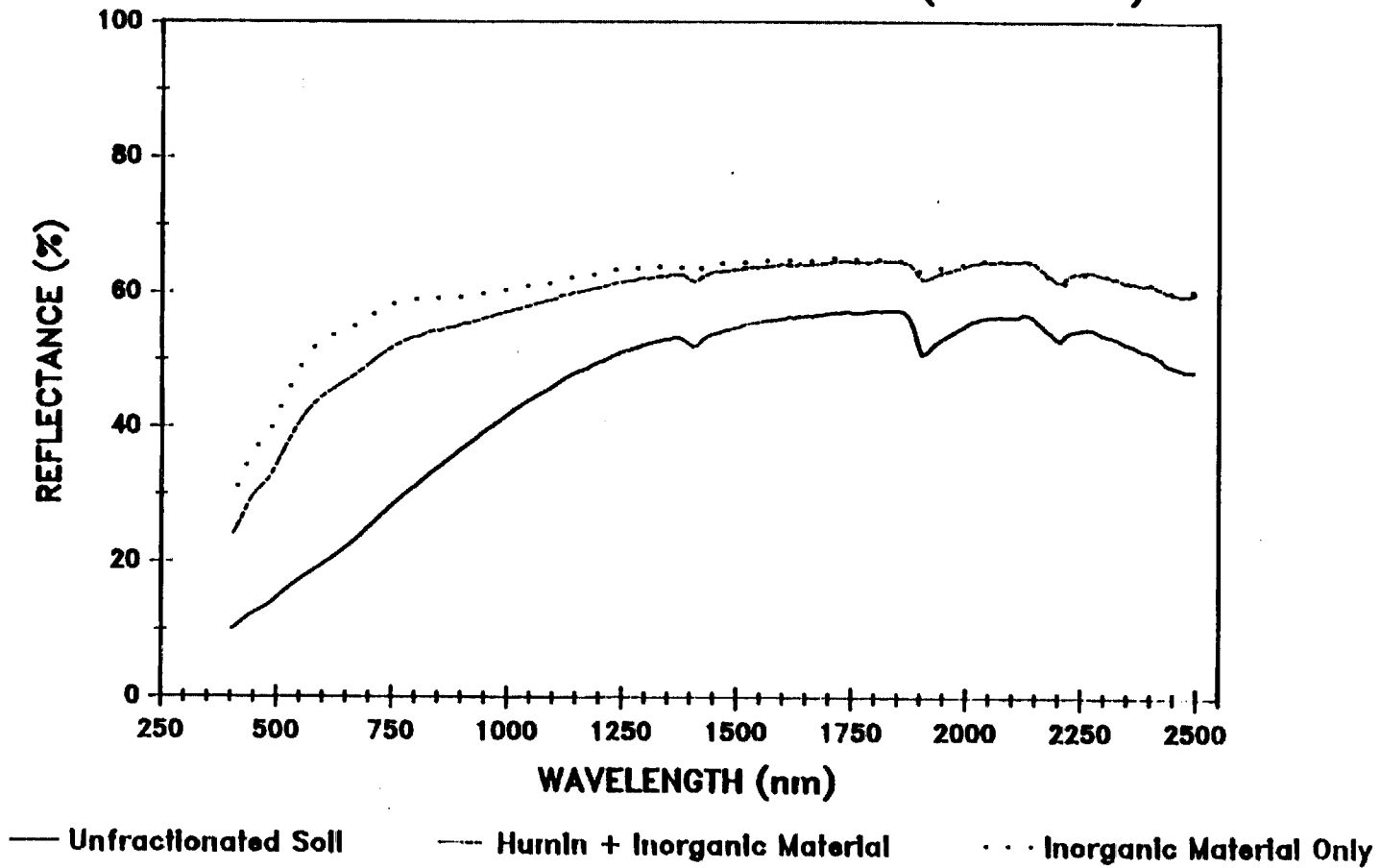


Figure 25. Average spectral curves for Chalmers unfractionated soil, humin plus inorganic component, and inorganic only fraction, illustrating the effect of humin on soil reflectance.

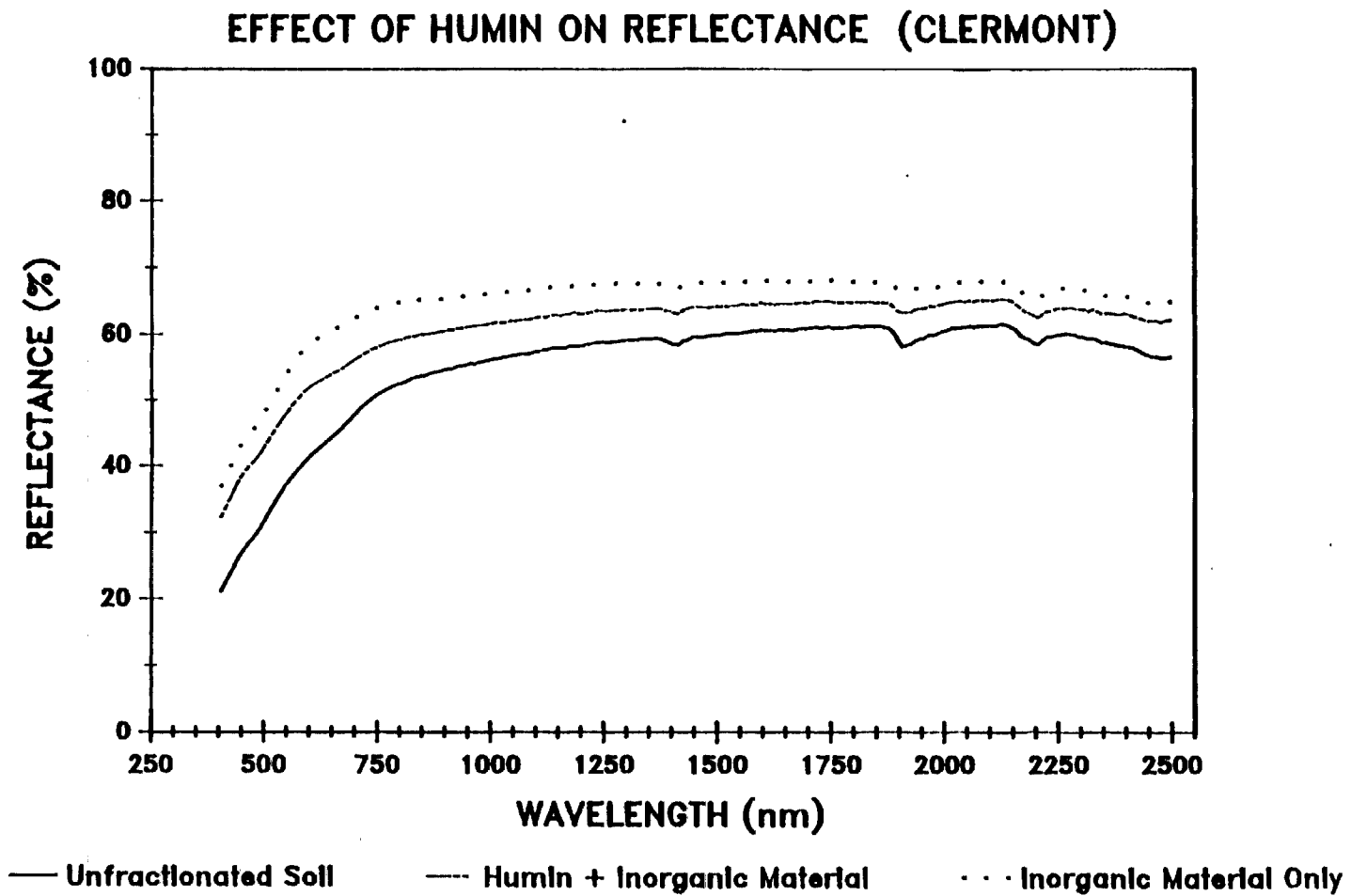


Figure 26. Average spectral curves for Clermont unfractionated soil, humin plus inorganic component, and inorganic only fraction, illustrating the effect of humin on soil reflectance.

visible wavelengths. In the NIR and MIR regions, the smaller amount of Clermont humin actually had a stronger lowering effect on reflectance than the Chalmers humin. This observation may suggest that the cutoff point for organic carbon content influence on soil reflectance could be lower in soils with lighter mineral fractions or lower mineral oxide pigment contents.

Effect of Organic Matter Composition on Soil Reflectance

Visual Analysis of Spectral Curves

There is some evidence from previous research that organic matter composition, as well as organic matter content, may influence soil spectral reflectance properties. However, only one study was identified in which the reflectance of individual humic substances was measured, and data were collected in the visible wavelengths only (Obukhov and Orlov, 1964). That experiment was conducted before modern organic matter fractionation and purification techniques had been developed, and a review of the methods used indicated that a large amount of impurities was likely present in both the humic and fulvic acid fractions. In the present study, extractable organic matter was removed from each of the four soils, and individual organic fractions were isolated for spectral analysis using currently recommended purification techniques.

Although soil reflectance curves are usually relatively flat or gently sloped, with no sharp peaks outside the water absorption bands, it was thought that perhaps certain individual organic fractions had more spectral character than the unfractionated soils. The presence of sharp peaks in infrared absorption spectra of humic substances led to the question of whether or not reflectance spectra would show similar

responses. Distinctive features in the organic fraction reflectance curves were of interest, and it was hypothesized that if sharp peaks or strong absorption bands were present in specific bands of the reflectance spectra of humic substances, examination of soil reflectance data in these bands might provide information about the composition of the soil's organic matter. To be of any practical use, however, such diagnostic features would have to affect the reflectance of the unfractionated soil to a degree that could be detected by a sensor.

To examine the effects of organic matter composition on soil reflectance, the reflectance curves of each of the four organic fractions (crude extractable organic matter, crude humic acid, purified humic acid, and purified fulvic acid) were first analyzed visually. As previously noted in the plots of the unfractionated soil plus six components for each soil (Figures 12-15), each soil organic fraction had a distinctive, easily identified curve shape which remained consistent across the four soils. For example, all the fulvic acid curves had a very steep slope in the 500-1000 nm range, reaching a maximum reflectance at approximately 1250 nm before gradually declining. For all soils, the purified humic acid fraction had the lowest reflectance and the flattest curves.

In spite of the distinctive curve shapes, however, no diagnostic features were identified in the organic component curves for which the effect on reflectance was strong enough to mask the effects of soil organic carbon content and soil mineral factors. In other words, although the humic and fulvic acid components probably had some raising or lowering effects on overall soil reflectance, none of the distinctive organic fraction curve shapes were manifested in the spectral curves of

the unfractionated soil, or at least were detectable visually. Thus, the results indicated that for the four soils considered, the effects of organic carbon content were far stronger than any effects of organic matter composition on reflectance. This conclusion was based on visual analysis only, so it is possible that some statistical method designed to extract information from very subtle curve features could identify effects of organic matter composition on the soil reflectance curves that were not visually detectable. It is also possible that due to the relatively homogeneous nature of the four soils, significant differences in their organic matter composition did not exist. If this is the case, a repeat of this experiment, comparing organic fractions from a wider variety of soils such as a peat, a high-silica volcanic soil, and an older tropical soil may produce very different results. In addition, better means of identifying significant factors in organic matter composition are needed to adequately interpret the spectral data. For example, the HA:FA ratio commonly used in the early days of humic substances research is no longer considered a valid means of characterizing organic matter composition differences, due to uncertainties about exactly which materials should be included in these two fractions. The more recent hypothesis that humic and fulvic acids are not chemically distinct fractions introduces more uncertainty into how organic matter composition is best described. Although it appears that organic carbon content masks the effects of compositional differences on soil reflectance, further research will be necessary before more definite conclusions can be reached.

Separability of Organic Fraction Spectral Curves

Differences in the reflectance spectra of the four soils for each individual organic fraction were also of interest, to evaluate possible effects of observable soil properties on the reflectance of the organic components. To examine and compare the effects of soil properties on the reflectance of specific organic fractions, the four spectral curves corresponding to the four soils were plotted on one set of axes for each individual organic component (Figures 27-30). The two organic fractions of main interest for this part of the study were purified humic and fulvic acid, because they contained known substances. The other two components (crude organic matter extract and crude humic acid) contained impurities such as ash (mineral impurities consisting mainly of small clay particles) and polysaccharides. The presence of these impurities strongly influenced the reflectance of the organic fractions. For example, all purified humic acid samples had very low reflectance. This was expected due to their black pigments which absorbed most of the incident radiation. The crude humic acid had higher reflectance than purified humic acid, and more closely resembled unfractionated soil, due to the higher reflectance of the ash. The crude organic matter reflectance curves were higher still, due to the influence of the highly reflective fulvic acid and polysaccharides included in this material. Since the exact nature of all the materials in the two crude fractions was not known, the use of separability analysis to identify significant differences in organic fraction reflectance over the four soils was confined to the two purified components.

Visual comparison of the spectral curves indicated the likelihood that the four purified humic acid samples (Figure 29) could be separated

CRUDE ORGANIC MATTER EXTRACT

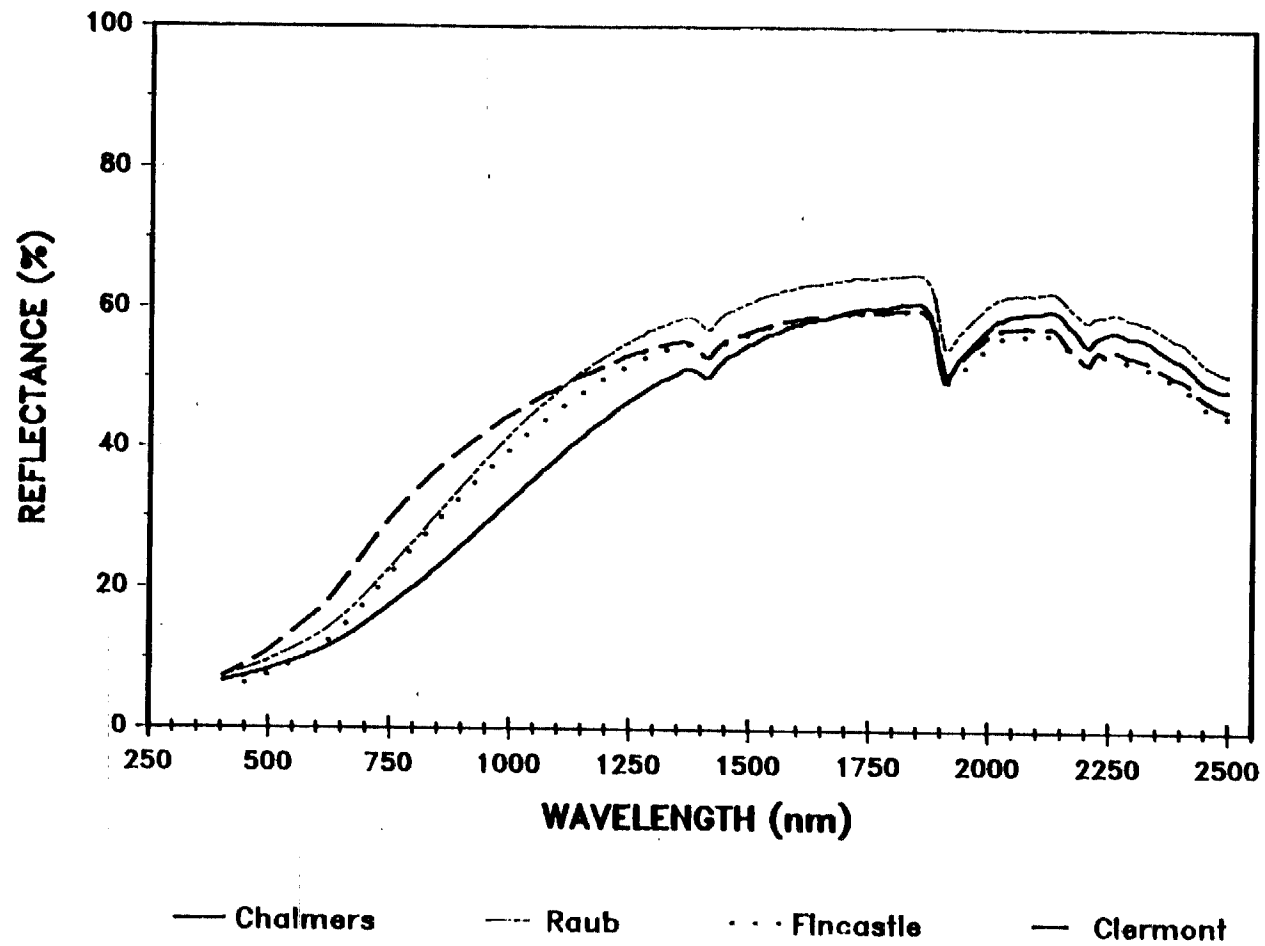


Figure 27. Average spectral reflectance curves of the crude organic matter extracts of the four soils.

HUMIC ACID (NOT PURIFIED)

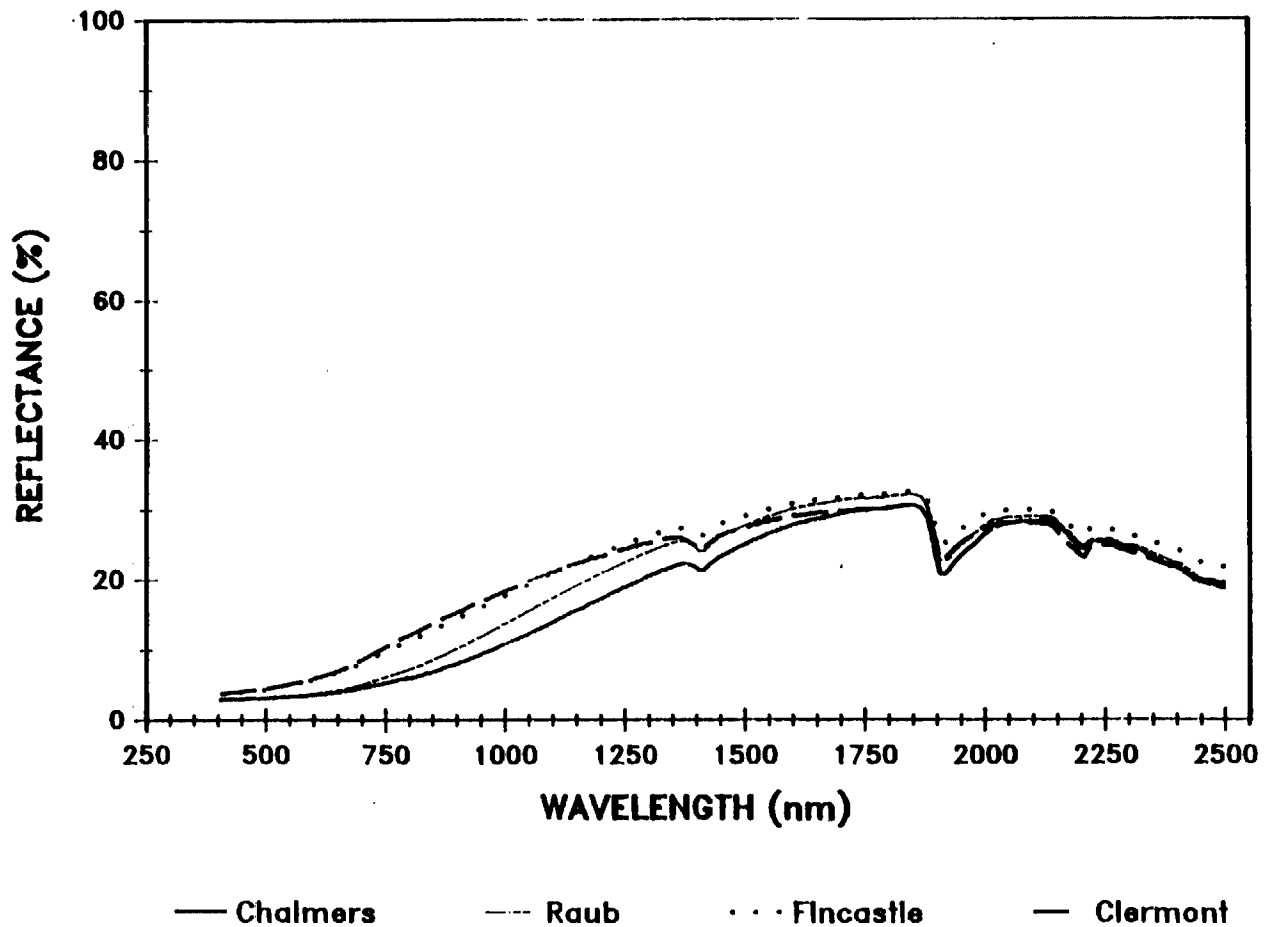


Figure 28. Average spectral reflectance curves of the crude humic acid components of the four soils.

HUMIC ACID (PURIFIED)

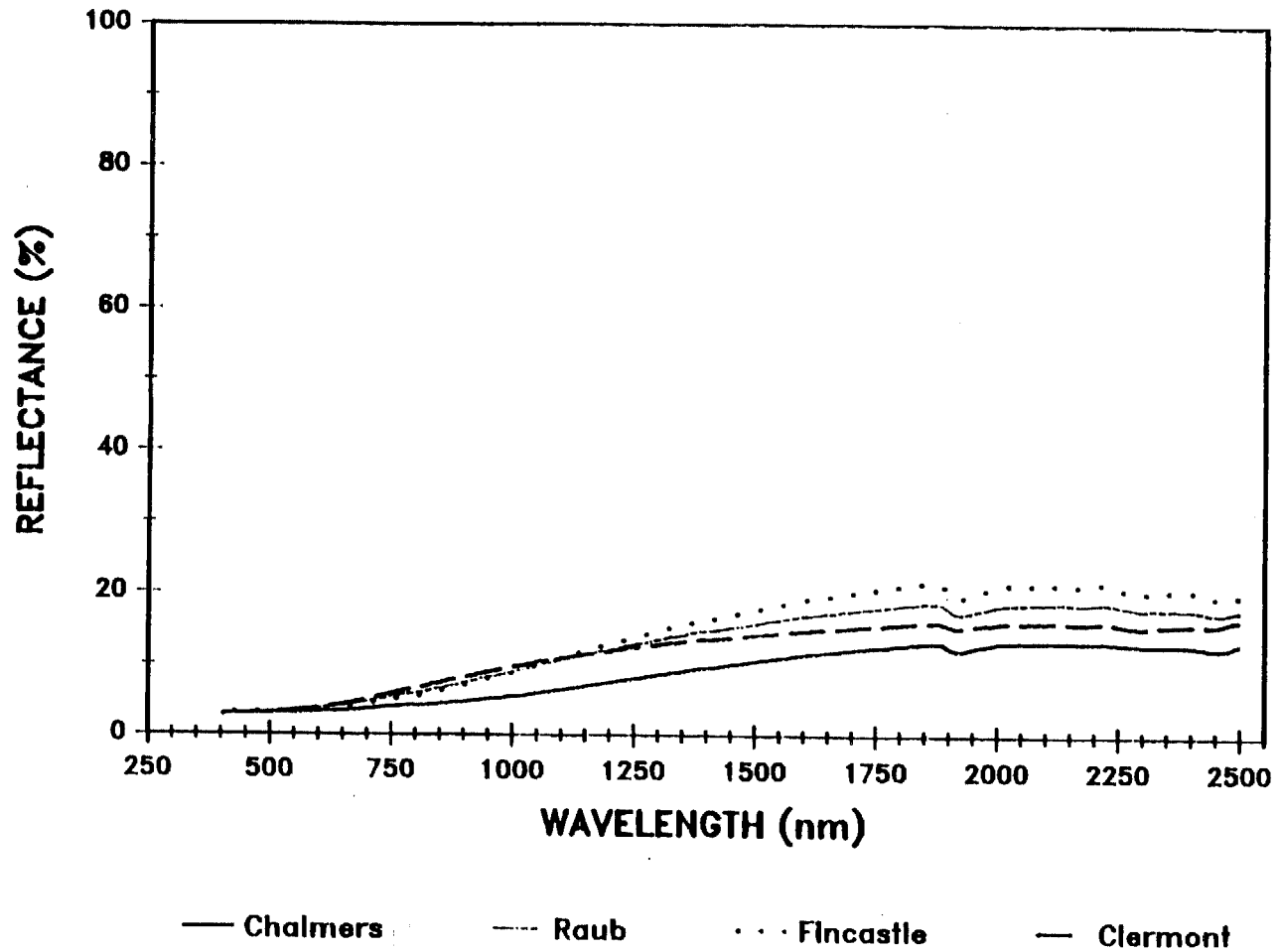


Figure 29. Average spectral reflectance curves of the purified humic acid components of the four soils.

FULVIC ACID (PURIFIED)

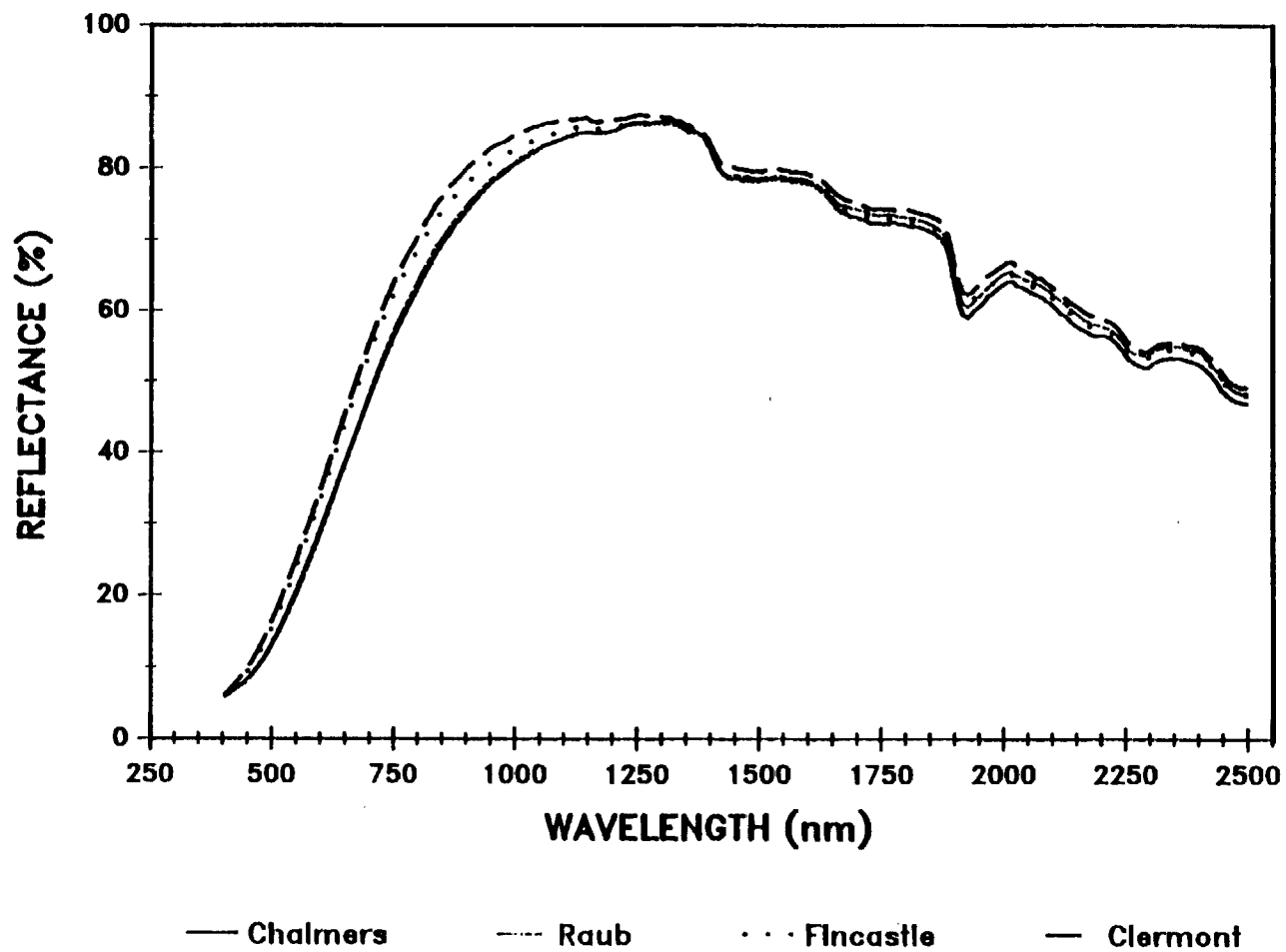


Figure 30. Average spectral reflectance curves of the purified fulvic acid components of the four soils.

by reflectance in the middle infrared region. No significant reflectance differences in the fulvic acid spectral curves (Figure 30) were apparent visually. Separability was analyzed statistically using Tukey multiple means comparison as described in a previous section. The fulvic acid curves for the four soils were not statistically separable in any part of the 400-2500 nm spectral range. This was due in part to the large sample-to-sample variation in the reflectance of the original three samples, before averaging to create the composite spectral curves. The high sampling error within each soil masked any reflectance differences that may have existed between fulvic acid samples of the four soils.

Results of the separability analysis for the pure humic acid fraction proved that the reflectance differences between the four curves were statistically significant. Six separable bands were identified, all in the middle infrared region: 1605 nm, 1645 nm, 1675-1685 nm, 1705-1895 nm, 2005-2375 nm, and 2395-2405 nm. No organic matter composition factor was identified which could explain the reflectance response of the four humic acid samples in these bands. Analysis of the ash content of the samples indicated that the reflectance differences could not be attributed to ash impurities. The ash content of all the purified humic acid samples was below 8%, with most samples below 5%. Since ash content has been shown to cause higher reflectance, the magnitude of the reflectance values of the four soils in the separable bands would have been ranked directly according to sample ash content if ash were responsible for the reflectance differences. The order of the spectral curves (Fincastle, Raub, Clermont and Chalmers, from highest to lowest reflectance) was unrelated to the ash content of the samples. The very

low degree of sample-to-sample variation for the purified humic acid fraction also suggested that reflectance in the significant bands responded to some factor other than ash. The ranking of the humic acid spectral curves was unrelated to the reflectance of the unfractionated soil and the crude organic matter extract. However, the four crude humic acid samples were ranked in the same order as the pure humic acid curves in this range, based on visual inspection. This provided further evidence that some aspect of the humic acid composition which differed between the four soils could be detected by middle infrared reflectance measurements.

It can be noted that the two somewhat poorly drained soils (Fincastle and Raub) have higher-reflecting HA fractions than the two poorly to very poorly drained soils (Clermont and Chalmers), and within each drainage class, the soil formed under forest vegetation has higher-reflecting humic acid than the soil formed under prairie vegetation. Whether these relationships are significant or coincidental cannot be determined with humic acid from only four soils. However, if similar results are achieved with many more soils having different internal drainage characteristics and native vegetation, attempts should be made to relate these soil genetic factors to a quantifiable property of humic acid composition. Until a physical or chemical cause of the pure humic acid reflectance response in the separable bands can be identified, it cannot be concluded that reflectance data can provide useful information about humic acid composition. If, as humus chemistry research progresses, an explanation for this spectral response is identified, middle infrared reflectance may have potential as a non-destructive laboratory technique for humic acid characterization.

Prediction of $E_4:E_6$ Ratios Using Reflectance Data

The $E_4:E_6$ ratio, based on spectral absorbance measurements in two narrow visible bands (465 nm and 665 nm), is a common means of characterizing and comparing soil humic and fulvic acids. $E_4:E_6$ ratios provide information about the particle size and molecular weight of humic substances. An attempt was made to determine whether the information contained in $E_4:E_6$ ratios, based on light absorbance, could be reproduced using visible reflectance data in the same bands. Use of reflectance to calculate $E_4:E_6$ ratios would be a non-destructive technique, since reflectance is measured on dry samples. To measure light absorption of humic substances, the dry samples must be dissolved in a buffer solution at a uniform concentration.

The two reflectance-to-absorbance data transformations used to calculate absorbance values and the resulting $E_4:E_6$ ratios were presented previously in Table 12. Analysis of these results showed no relationship between the measured and calculated $E_4:E_6$ ratios. The difference between samples was very slight for the ratios based on reflectance data, and they were not ranked in the same order as those calculated from absorbance measurements. It was therefore concluded that $E_4:E_6$ ratios of purified soil humic and fulvic acids could not be predicted using reflectance data. The poor results obtained using reflectance data may be explained by the fact that reflectance measurements are sensitive to particle shape, surface characteristics, and arrangement in addition to particle size. The first three factors are not as important for absorbance measurements, because the samples are in the form of a clear solution.

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary and Conclusions

The HIRIS sensor, currently scheduled for launch in 1996, will generate large quantities of high-dimensional reflectance data from satellite altitudes. This research was conducted to provide insight into how HIRIS data can best be used to monitor the status of organic matter in soils. High-resolution spectra of four soils and seven constituents of each soil were analyzed to evaluate soil reflectance in terms of the contributions of organic and mineral soil components in specific wavelength bands.

A method was developed to study organic matter - reflectance relationships in soils, using simulated HIRIS data to characterize the spectral properties of individual soil components. The CARY 17D spectrophotometer met all the criteria for laboratory simulation of HIRIS bands. With proper adjustment to extend the sensing capability into the middle infrared wavelengths, this instrument maintained a high integrity of wavelength calibration, even over the point of detector change, with excellent repeatability of reflectance measurements. The capability of the CARY 17D to measure reflectance of very small samples in a dry powder form made it particularly suitable for use with purified soil humic and fulvic acid samples, which take a great deal of time to prepare in large quantities.

Both narrow and broad wavelength bands containing significant information about soil organic carbon content were located in all three major regions of the spectral range of HIRIS: visible, near infrared and middle infrared. Spectral curve separability, based on reflectance of various combinations of the mineral fractions and the four unfractionated soils, was analyzed statistically to identify the information-bearing bands. Discriminant analysis of reflectance data for twenty test samples, representing the four soil series, confirmed the separability of data in the identified bands. Optimal bands were chosen based on examination of the reflectance response to organic carbon content and mineral soil constituents in each of the separable bands. Four bandsets were identified and ranked according to the significance of their reflectance response to soil organic carbon content.

Correlation analysis confirmed the selection of optimal bands for organic carbon content estimation based on curve separability and spectral response. For soils formed on the same parent material with little variation in the mineral fraction, reflectance in eight bands, including the entire visible range, gave the best correlation ($r=-0.991$) with organic carbon content. These bands also responded strongly to the presence of iron and manganese oxides. Twelve optimal bands were identified for soils having greater variation in mineral reflectance, but still formed on the same parent material. These twelve bands were in the near and middle infrared regions, and appeared to respond only to organic carbon content for soils with similar parent material. For soils formed on different parent materials, reflectance in six middle infrared bands gave the best correlation ($r=-0.964$) with organic carbon

content. Several other separable infrared bands were identified in which the reflectance response was dominated by soil properties other than organic matter, indicating that the choice of wavebands should not be based on spectral curve separability alone.

Parent material differences influenced soil reflectance in all bands. Some of these differences were explained by the effect of iron and manganese oxide contents on reflectance. However, these pigments only affected reflectance in the 400-1200 nm range, and their removal did not result in the elimination of all reflectance differences between soils formed on different parent materials. This fact indicated that other mineral factors not identified in this study were primarily responsible for the complicating effects of parent material on the observed organic carbon content - reflectance relationships. The cutoff point for the masking effects of other soil properties on the organic carbon content - reflectance relationship was found to lie in the 0.66% to 0.99% O.C. range.

The potential of HIRIS to provide information about soil organic matter composition does not appear promising at this time. Each individual organic fraction had a distinctive spectral curve shape which was easily identified in each of the four soils, indicating that individual organic components have unique reflectance properties. However, no diagnostic features were identified in the spectral curves of the unfractionated soils which provided information about organic matter composition, or about any particular organic fraction. Attempts to predict the $E_4:E_6$ ratios of purified humic and fulvic acids, using reflectance data rather than the usual spectral absorbance measurements, were unsuccessful.

Spectral curves of the purified humic acid fractions of the four soils were separable in six middle infrared bands in the 1600-2400 nm range. No organic matter composition factor was identified which could explain this reflectance response, but it was established that ash impurities were not responsible. The significance of the information contained in these bands remains to be demonstrated, but if future research in organic matter chemistry can provide a link between spectral response and humic acid properties of interest, middle infrared reflectance may have potential as a non-destructive laboratory technique for humic acid characterization.

Recommendations

The results of this research indicate the potential of HIRIS data to provide information about soil organic carbon content, but not organic matter composition. However, many problems must be solved before HIRIS data can be used directly to identify and classify soils according to their organic carbon content. The portion of this study involving organic carbon content - reflectance relationships (using the unfractionated soil and mineral components) should be extended, using many more soils from a wider distribution of geographic regions and representing a greater variety of parent materials. The soil factors responsible for the complicating effects of different parent materials on organic matter - reflectance relationships must be identified and quantified.

Similar studies should also be conducted to determine the optimal bands for estimating other soil properties from reflectance data. Once the specific influences of soil mineralogy, texture, moisture content,

and perhaps other unidentified soil factors on narrow-band reflectance are understood, attempts can be made to quantify the relationships of each of these soil factors, as well as organic carbon content, to soil reflectance. Only then will prediction of these soil properties from reflectance data be possible.

Results of laboratory-based studies must be confirmed in the field using portable field spectroradiometers. Research must then be conducted to quantify the effects of soil surface conditions and vegetative cover on reflectance in relation to the soil physical and chemical properties listed above. The final step will be the extension of such studies for use with data collected from satellite altitudes.

Although a great deal of work must be accomplished before HIRIS-generated reflectance data can be routinely converted to accurate data on soil properties, the information provided by this sensor promises to be extremely valuable to soil and environmental scientists. Therefore, a continuation of the current research effort on the use of HIRIS data to monitor soil properties, including organic carbon content, should be encouraged and supported.

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Appendix A. Citrate-Bicarbonate-Dithionite Extraction
of Iron Oxide Minerals from Soil

(modified from D.G. Schulze, Purdue University, 1986)

Supplies:

- a. 50 ml centrifuge tubes and stoppers.
- b. Na-citrate/Na-bicarbonate solution. First prepare:
 - i. 0.3 M Na-citrate solution. Prepare by dissolving 88.23 g of $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7\text{-H}_2\text{O}$ in 1 L of deionized water.
 - ii. 1 M Na-bicarbonate solution. Prepare by dissolving 16.8 g of NaCHO_3 in 200 ml of deionized water.

Then:

Pour 125 ml of 1 M Na-bicarbonate solution into 1 L of 0.3 M Na-citrate solution and mix well. This provides enough solution for approximately 25 samples. Scale up the amounts accordingly for larger numbers of samples.

- c. 0.1 M MgCl_2 .
- d. $\text{Na}_2\text{S}_2\text{O}_4$ powder.

Procedure:

Organic matter had been extracted from soil samples prior to this procedure. Each sample should be analyzed in duplicate.

1. Weigh 1 g of soil into the 50 ml centrifuge tube.
2. Add 40 ml of Na-citrate/Na-bicarbonate solution.
3. Add 1 g of $\text{Na}_2\text{S}_2\text{O}_4$ powder, stopper carefully, and place on a reciprocating shaker overnight.
4. The next morning, remove the samples from the shaker. Remove the stoppers and rinse any liquid hanging on the stoppers into the tube with deionized water. Centrifuge at 1000 rpm for 5 min. Decant and discard supernatant.
5. Wash the precipitate once with about 15 ml of 0.1 of MgCl_2 . Centrifuge as before; then decant and discard the supernatant.
6. Repeat step 5 one additional time.

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7. Wash the precipitate once with 20 ml of deionized water and centrifuge for 30 minutes.
8. Repeat step 7. After centrifuging, if the supernatant is clear, decant and discard supernatant. If the sample has dispersed and the supernatant is turbid, do not decant the supernatant.
9. The sample may now be taken to dryness in an oven at 50°C.

Appendix B. Organic Matter Extraction, Fractionation
and Purification Procedures Used to Prepare Soil
Component Samples for Spectral Analysis

(Modified from the unpublished IHSS paper "Outline of Extraction Procedures" by P. MacCarthy of the International Humic Substances Society, 1987, obtained by D.E. Stott, Purdue University, through personal communication with the author.)

Equipment and Supplies:

- a. 250 ml centrifuge bottles with caps
- b. Centrifuge
Note: The centrifuge used for this procedure was a Beckman model J2-21 with a JS-7.5 rotor head (Beckman Instruments, Inc., Palo Alto, CA). At 6000 rpm, the speed used throughout these procedures, the RCF values were as follows: RCF at r_{max} = 10,400 x g; RCF at r_{min} = 3210 x g. Calculations can be made to adjust the centrifugation speed according to centrifuge specifications. The calculation is explained in the Beckman bucket rotor instruction manual (Beckman, 1980.)
- c. Oscillating shaker
- d. pH meter
- e. Vacuum pump
- f. 50 ml chromatography columns
- g. Peristaltic pump with adjustable flowrate
- h. Dialysis tubing
 1. Spectra-por 6000-8000 MWCO
 2. Spectra-por 1000 MWCO
(Spectrum Medical Industries, Inc., Los Angeles, CA)
- i. Amberlite XAD-8 nonionic polymer adsorbant resin, 20-60 mesh (Sigma Chemicals, St. Louis, MO)
- j. Biorad AG-MP-50 cation resin, hydrogen form, 50-100 mesh (Biorad Laboratories, Richmond, CA)
- k. Freeze-dryer

Chemicals:

- a. 0.1 M HCl
- b. 0.1 M NaOH
- c. N₂ gas
- d. 6 M HCl
- e. Deionized water at pH 2, pH 4, and neutral pH
- f. AgNO₃
- g. 0.3 M HF - 0.1 M HCl

Procedures:

Initial soil preparation consists of air-drying soil and crushing to pass a 2 mm sieve. The procedure described here is for one 20 g soil sample, in one 250 ml centrifuge bottle. In this project, the procedure was performed on batches of 8 samples at a time.

EXTRACTION OF ORGANIC MATTER

1. Weigh out approximately 20 g air-dry soil and place in a labelled 250 ml polyethylene centrifuge bottle.
2. Add 200 ml 0.1 M HCl to each bottle. Shake in air for 1 hour. Centrifuge (6000 rpm for 30 min) and decant. Discard supernatant.
3. Repeat step 2, using deionized water (neutral pH) instead of acid.
4. Under an atmosphere of N₂, add 200 ml 0.1 M NaOH and cover quickly. Shake overnight.
5. The next morning, centrifuge at 6000 rpm for 45 min. If preparation of crude organic matter is desired, proceed to steps 5.a-d. If further fractionation is desired, immediately proceed to step 6.

For preparation of crude organic matter extract:

- a. Decant and immediately neutralize the supernatant to pH 7 by dropwise addition of 6 M HCl.
 - b. Repeat steps 4 through 5.a. Combine the supernatants.
 1. If retention of the humin plus inorganic material (material remaining after organic matter extraction) is desired, save the precipitate and follow the procedure in step 8.a-d.
 - c. Dialyze in 1000 MWCO dialysis tubing against frequent changes of deionized water until there is no reaction with AgNO₃.
 - d. Freeze dry and store in glass vials in a desiccator.
6. Immediately acidify the supernatant to pH 2, by the following procedure: Decant the supernatant into a large beaker containing the amount of 6 M HCl necessary to neutralize the NaOH (approximately 10 ml). Add 6 M HCl dropwise until pH 2 is reached.
 7. Repeat NaOH extraction and centrifuge as before (steps 4 and 5). Neutralize this supernatant with NaOH as before (step 6). Add the two supernatants together and acidify to pH 2.

8. For fractionation of humic and fulvic acids, save the supernatant and proceed to step 9. If the humin plus mineral soil component (material remaining after extraction of organic matter) is to be retained, save the precipitate and follow steps 8.a-d.

To retain the humin plus mineral soil component:

- a. Wash the soil residue (precipitate from step 5 and/or 7) with 50 ml deionized water (neutral pH). Centrifuge at 6000 rpm for 45 min. Discard washings.
- b. Acidify the precipitate to pH 5.5 by dropwise addition of 3 N HCl.
- c. Dialyze precipitate in 6000-8000 MWCO dialysis tubing against frequent changes of deionized water at pH 4 for 2 days. Then dialyze against frequent changes of neutral pH deionized water until there is no reaction with AgNO_3 .
- d. Freeze dry and store in glass vials in a desiccator.

FRACTIONATION OF HUMIC AND FULVIC ACIDS

9. Stir acidified supernatant (from step 7) and let stand overnight. Siphon all but 30 - 40 ml solution off the top with a small siphon under a weak vacuum. Pour the rest into 250 ml centrifuge bottles and centrifuge at 6000 rpm for 45 min. Decant. Save precipitate (HA) and supernatant (FA).
10. Wash the precipitate from step 9 with 50 ml deionized water at pH 2. Centrifuge at 6000 rpm for 45 min. Add washings to supernatant from step 9. Save precipitate (HA) and supernatant (FA).
11. If crude (unpurified) humic acid is desired, take precipitate and follow steps 11.a-b.
If purified humic acid is desired, take precipitate and proceed to step 12.
If purified fulvic acid is desired, take supernatant and proceed to step 17.

To retain the crude humic acid:

- a. Dialyze the untreated humic acid (precipitate from step 10) in 6000-8000 MWCO dialysis tubing against frequent changes of deionized water at pH 4 for 2 days. Dialyze against frequent changes of neutral deionized water until there is no reaction with AgNO_3 .
- b. Freeze-dry and store in glass vials in a desiccator.
(Note: This material contains ash which can be considered a contaminant.)

PURIFICATION OF HUMIC SUBSTANCES

Humic Acid

12. In each 250 ml centrifuge bottle containing untreated humic acid (precipitate from step 10), add 0.3 M HF - 0.1 M HCl solution to humic acid at a volume ratio of 10:1.
13. Shake overnight at room temperature and centrifuge (6000 rpm for 45 min.) Discard supernatant.
14. Repeat steps 12 and 13 five to ten times, until desired ash content (<5%) is achieved.
15. Dialyze (using 6000-8000 MWCO dialysis tubing) against frequent changes of an equal volume of deionized water at pH 4 for 2 days. Then dialyze against frequent changes of neutral deionized water until there is no reaction with AgNO_3 .
16. Freeze-dry for spectral analysis. Store the purified HA in glass vials in a desiccator.

Fulvic Acid

Note: The solution quantities and flowrate presented in this section of the procedure are for purification and concentration of 1 L of fulvic acid-containing solution (supernatant from step 10). The quantities were calculated using the equation presented by Thurman and Malcolm (1981). Appropriate quantities for starting solution volumes other than 1 L may be calculated using the same equation.

50 ml chromatography columns were used, with a peristaltic pump to control flowrate. Resins used were Amberlite XAD-8 and Biorad Ag-Mp-50 (H form). The XAD-8 resin must be cleaned and prepared prior to use according to the procedure outlined by Thurman and Malcolm (1981). The AG-MP-50 resin is prepared according to instructions by the manufacturer.

17. Pass 1000 ml of fulvic acid solution (supernatant from step 10) through a 15 ml (bed volume) column of XAD-8 resin at a flowrate of 175 ml/hr. The XAD-8 resin column must remain saturated at all times. When the liquid level reaches the top of the resin column, pass 10 ml (1 void volume) of deionized water (neutral pH) through the column. Discard the solution which passes through the column (which contains polysaccharides, proteins, and other organic contaminants). The purified FA is retained on the column.

18. Forward elute the FA (retained on column) with 0.1 N NaOH until the dark ring passes out of the column. Follow with 45 ml (3 bed volumes) deionized water. Collect the eluate in a beaker containing a small volume of the AG-MP-50 resin in the H form. (This neutralizes the concentrated FA solution, preventing oxidation). When all the eluate has been collected, immediately pass it through a column (approx. 10 ml) of the AG-MP-50 resin in H-form. The AG-MP-50 column should contain twice the amount of resin necessary to neutralize the NaOH.
19. Transfer the solution coming off the AG-MP-50 column to evaporating dishes, covered with paper wipes to prevent FA loss. Freeze-dry. The freeze-dried fulvic acid should appear fluffy and light-textured, but not spongy. Pulverize in a smooth glass dish. Store in glass vials in a desiccator. Do not expose to air during storage.