Available online at www.elixirpublishers.com (Elixir International Journal)

Remote Sensing

Elixir Remote Sensing 58 (2013) 14771-14775

ISSN: 2229-712X

Non Destructive Assessment of Chlorophyll content in higher Plant Leaves from hyper spectral vegetation indices: Modeling and Validation

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ARTICLE INFO

Article history:
Received: 19 March 2013;
Received in revised form:
17 April 2013;
Accepted: 7 May 2013;

Keywords
Algorithm,
Cement dust,
Chlorophyll content,
Sensitivity,
Validation and Vegetation indices.

ABSTRACT

Leaf pigment content can provide valuable information about the physiological performance of leaves. Measurement of spectral reflectance provides a fast, nondestructive method for pigment estimation. In this paper, a set of vegetation indices belonged to two classes normalized difference vegetation index (NDVI) and modified simple ratio (MSR) index were tested to explore the potentials in chlorophyll content estimation. In the sensitivity study, two new formed indices NDVI [750,675] and MSR [750,675] were proved to have better linearity with chlorophyll content taking into account the effect of quick saturation at 675 nm with relatively low chlorophyll content. The results showed that the spectral indices NDVI [750,675] and MSR [750,675] are most appropriate for chlorophyll estimation with high correlation coefficients R^2 from 0.88 to 0.97 in control and polluted selected plant species even more disturbances such as shadow, soil reflectance, and non photosynthetic materials and cement dusted pollutant are taken into account.

Introduction

Chlorophylls are the most important pigments for photosynthesis (Evans, 1989; Yoder and Pettigrew-Crosby, 1995; Niinemets and Tenhunen, 1997). The amount of chlorophyll is an important indicator of the overall condition of the plant. Healthy plants capable of maximum growth are generally expected to have larger amounts of chlorophyll than unhealthy ones. Industries are emitting toxic substances which adversely affect man's food supply by polluting nearby growing plants (Saravanakumar and Sarala Thambavani 2012). The Cement industry plays a vital role in the imbalances of the environment and produces air pollution hazards (Stern, 1976; Sarala Thambavani and Saravanakumar, 2011). Vegetation indices have been developed as an attempt to reduce spectral effects caused by external factors such as the atmosphere and the soil background (Demarez and Gastellu- Etchegorry, 2000).

The chlorophylls have strong reflectance peaks in the red and blue wavelength regions. Blue peak is not used to estimate the chlorophyll content because it overlaps with the absorbance of the carotenoids. Maximal absorbance in the red region occurs between 660 nm and 680 nm. However, it is unknown if reflectance at these wavelengths can be used to predict chlorophyll content as reflectance at slightly longer or shorter wavelengths. This is because absorption in the 660–680 nm tends to saturate at low chlorophyll content, thus reducing the sensitivity of the spectral indices based on these wavelength to high chlorophyll content (Sims and Gamon, 2002). Empirical models to predict chlorophyll content are largely based on reflectance around the 550 nm or 700 nm regions where the absorption is saturated at higher chlorophyll. Indices formulated with these bands would thus have higher accuracy in estimating chlorophyll content. Increasing efforts have focused on understanding the relationships between vegetation optical properties and photosynthetic pigments concentrations within green leave tissues such as total chlorophyll.

Obviously, different pigments have specific absorption features at different wavelengths which have promoted the development of various approaches, based on model inversion or the use of empirical and semi-empirical methods, to estimate the chlorophyll content both at the leaf and canopy scales (Daughtry et al., 2000; Demarez and Gastellu-Etchegorry, 2000; Zarco-Tejada et al., 2001). Zarco-Tejada et al. (1999) performed a study of hyper spectral indices and model simulation for chlorophyll estimation in open-canopy tree crops which showed that crown-derived relationships between hyper spectral indices and biochemical constituents cannot be readily applied to hyper spectral imagery of lower spatial resolutions due to large soil and shadow effects. Our objective in this study was to develop spectral indices for prediction of leaf pigment content that are relatively insensitive to species.

Materials and Methods

Description of study area

For development of algorithm validation for chlorophyll assessment two sites are selected in this paper where *Azadirachta indica(L)* and *Ficus religiosa(L)* leaves were collected from a park at Madurai city which is represented as control site and the same plant leaves also collected from the cement industry which is located at Virudhunagar representing as polluted site. The data set covered all stages of leaf development and senescence. The sampling scheme covered a wide variation of pigment contents in each experiment. Only leaves having homogeneous dark green colour was selected.

Extraction of Photosynthetic pigments

To determine chlorophylls and carotenoids contents about 1g of leaves were extracted with 100% acetone and measured spectrophotometrically using absorption coefficients reported by Lichtenthaler (1987). The pigment contents were expressed in

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milligram per gram. Absorption and Reflectance spectra for the selected plant species were measured in laboratory with Lambda-35 UV spectrophotometer equipped with an integrating sphere in the range of 400-800 nm with a spectral resolution of 2nm. The reflectance spectra were measured against barium sulphate as a reference standard. Absorption of the leaves was calculated as 100-T-R. Validation was done in the selected plant species the chlorophyll contents were calculated with regression coefficients for the model development data set equation. To examine the linearity of different indices to chlorophyll content, the chlorophyll content increased from 3mg/g to 34mg/g in the selected plant leaves. The selection of parameters values were based on the experiment on fresh leaves.

Results and Analysis

Estimation of chlorophyll contents

The present investigation has been undertaken to study the effect of cement dust pollutant on total chlorophyll content of selected plant species spectrophotometrically and it was observed pigment content decreased significantly in response to cement dust pollutants in polluted plant leaves compared with control of *Azadirachta indica(L) and Ficus religiosa(L).* The ranges of total chlorophyll content was observed from 12.7 mg/g to 34.3 mg/g of *Azadirachta indica(L)* in control site and it was reduced due to cement dust pollutant from 7 mg/g to 15 mg/g at polluted site at different period interval. Likewise the total chlorophyll content was observed from 13.3 mg/g to 25.7 mg/g of *Ficus religiosa(L)* in control site and it was reduced between 3.1 mg/g and 16.2 mg/g in polluted site at different period interval.

Reflectance spectra

The mean (n=6) reflectance spectra of each group of selected plant leaves are shown in the figure (1). It showed very little detail other than a maximum at approximately 550 nm and minimum at approximately 670nm the regions of minimal and maximal chlorophyll absorption respectively. It is only at these spectral bands that significant differences in reflectance as the result of cement dusted pollutant levels have been seen. To study the effect of chlorophyll and carotenoids on reflectance spectra, leaves were separated into two groups with different pigment content. The first group can be considered to represent healthy plant species and second group can be considered to represent unhealthy (cement dusted) plant species. In the blue range, reflectance was low (below10%). A sharp increase in reflectance took place at 550nm at longer wavelengths reflectance remained practically unchangeable in a wide spectral range including NIR. An increase in the total pigment content led to slight decrease of reflectance in the blue range and a significant decrease in the green and especially in the red. The only spectral ranges sensitive to pigment variation were between 530nm to 650nm and in the red edge around 700nm.

The data used the plant leaves of *Azadirachta indica(L) and Ficus religiosa(L)* to examine the relationship between total chlorophyll content and reflectance at four wavelength R670nm, R675nm, R750nm and R800nm. Chlorophyll content was best correlated with R675nm in control and polluted sites of the selected plant species compared with other spectral bands which is shown in the figure(2 to 5).

Vegetation indices used in this analysis

Indices of normalized difference

Vegetation Indices (VIs) are combinations of surface reflectance at two or more wavelengths designed to highlight a particular property of vegetation. They are derived using the reflectance properties of vegetation described in plant foliage. Each vegetation indices designed to accentuate a particular vegetation property. All vegetation indices require high-quality reflectance measurements from either multispectral or hyper spectral sensors. The vegetation indices that can be calculated on a specific dataset are determined by the spectral bands sampled in the input dataset. If all spectral bands required for a specific index are available, that vegetation index is available for the dataset. For example, an input dataset from a sensor that matches only the near-infrared and red spectral bands is only able to calculate two of the indices: the NDVI (Normalized Difference Vegetation Index) and SR (Simple Ratio).

Narrowband greenness vegetation indices are a combination of reflectance measurements sensitive to the combined effects of foliage chlorophyll concentration, canopy leaf area, foliage clumping, and canopy architecture. Similar to the broadband greenness vegetation indices, narrowband greenness vegetation indices are designed to provide a measure of the overall amount and quality of photosynthetic material in vegetation, which is essential for understanding the state of vegetation. These indices use reflectance measurements in the red and near-infrared regions to sample the red edge portion of the reflectance curve. The red edge is a name used to describe the steeply sloped region of the vegetation reflectance curve between 690 nm and 740 nm that is caused by the transition from chlorophyll absorption and near-infrared leaf scattering. Use of near-infrared measurements, with much greater penetration depth through the canopy than red measurements allows estimation of the total amount of green material in the column.

Narrowband greenness vegetation indices are more sophisticated measures of general quantity and vigor of green vegetation than the broadband greenness Vegetation indices. Making narrowband measurements in the red edge allows these indices to be more sensitive to smaller changes in vegetation health than the broadband greenness vegetation indices particularly in conditions of dense vegetation where the broadband measures can saturate. Narrowband greenness vegetation indices are intended for use with high spectral resolution imaging data, such as that acquired by hyper spectral sensors. The most well known and widely used vegetation index is the normalized difference vegetation index (NDVI) developed by Rouse et al. (1974). It is based on the contrast between the maximum absorption in the red due to chlorophyll pigments and the maximum reflection in the infrared caused by leaf cellular structure. Using hyper spectral narrow wavebands, this index is quantified by the following equation, where Rx is the reflectance at the given wavelength (nm).

$$\text{NDVI }[670,800] = \frac{(R800 - R670)}{(R800 + R670)} \qquad (1)$$

Indices of Simple Ratio

Simple ratio vegetation indices directly compare signals between the reflection and absorption peak of chlorophyll pigments which mean they are sensitive to changes in chlorophyll content changes. However, compared to NDVI, simple ratio indices are more influenced by environmental factors, such as cloud and soil (Slater and Jackson, 1982). In this analysis, the modified simple ratio (MSR) was used instead of SR to avoid these disturbances.

$$\text{MSR }[670,800] = \frac{\left(\frac{R800}{R670}\right) - 1}{\sqrt{\left(\frac{R800}{R670}\right) + 1}} \qquad (2)$$

Bands used in this paper

Note that the aim of this paper was to find indices that can be used to predict chlorophyll content with high precision (better linearity). Since most of vegetation indices become saturated with increasing chlorophyll content. In this sensitivity study, two problems were faced band selection (chlorophyll prediction) and improvement of saturation limits. Spectral reflectance of vegetation from 400 nm to 700 nm region is primarily governed by the abundance of chlorophyll and other pigments absorbing most of the incident radiation (Thomas and Gausman, 1977). In this paper, bands at 670 nm, 675 nm, 750 nm, and 800 nm were selected. Reflectance of 670 nm is the maximum absorption in the red region. Sims and Gamon (2002) found that there were reliable relationships between leaf total chlorophylls and reflectance at 680 nm and 705 nm with correlation coefficients R^2 of 0.84 and 0.92, respectively. They also found a strong correlation between different combinations of reflectance at 700 nm, 705 nm, 750 nm, and 800 nm and leaf chlorophyll content. Therefore, reflectance of these bands was selected to formulate vegetation indices which may have potentials in estimating chlorophyll content. Several published spectral indices were tested using this data set, and developed a new set of indices that were less sensitive to leaf structural variation. Specifically, reflectance at 800 nm and 670 nm were replaced by that of 750 nm and 675 nm to form the revised indices. Two indices were tested in this study to calculate total chlorophyll content of the selected plant species (table 1).

Sensitivity to chlorophylls effects and saturation limits

The sensitivity study was based on models rather than on the ground truth data. In sensitivity comparison, indices that can estimate chlorophyll content more accurately (better linearity with increasing chlorophyll content) will be the best choice especially for high content of chlorophylls. Since most of vegetation indices become saturated with increasing chlorophylls, they will have low sensitivity to high chlorophylls. The main purpose of this sensitivity test is to explore the performance of different indices and band combinations and to find indices which can overcome the saturation limits. To compare with other indices, the sensitivity results between 0 and 1 scaled as shown in Figure (2 to 5).

The simulation results showed that the normalized difference indices saturated with chlorophylls increasing from 12.7 mg/g to 34.3 mg/g in *Azadirachta indica(L)* and from 13.3 mg/g to 25.7 mg/g in *Ficus religiosa*(L) at control site(Fig. 2a and 4a). The curve of NDVI [670,800] saturated when chlorophyll content exceeded 34 mg/g in *A.indica* at control site and 15 mg/g at polluted site due to stress and effect on physiological status by cement dust pollution (Fig.2a and 3a). Similarly, in *F.religiosa*, the curve of NDVI [670,800] saturated when chlorophyll content exceeded 25.8 mg/g and 16.3mg/g in control and polluted sites respectively (Fig.4a and 5a). The reason is that relatively low chlorophyll content is sufficient to saturate absorption in 670 nm region.

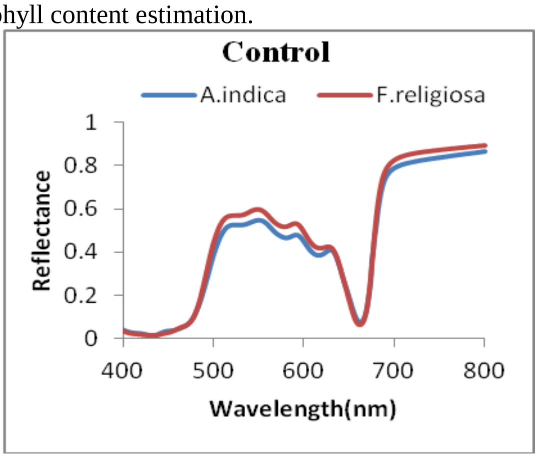
Therefore, different wavelength, such as 675 nm, and 750 nm are more suitable for the prediction of chlorophyll content. Similar situation occurs for indices MSR [670,800] and MSR [675,750]. MSR was expected well than NDVI in terms of sensitivity to leaf biophysical parameters due to its combination with the simple ratio (SR = NIR/Red; Jordan, 1969). MSR[670,800] and MSR[675,750] becomes saturated when chlorophyll content exceeds 16 mg/g and 33.5mg/g respectively in control site and polluted site from 10.35 mg/g and 14 mg/g respectively in *A.indica* (Fig.2b and 3b). Similarly in *F.religiosa*, the specified spectral bands of MSR becomes saturated when chlorophyll content exceeds 22 mg/g and 24mg/g respectively in control site and polluted site from 16.28 mg/g respectively (Fig.4b and 5b). When reflectance at 675 nm was introduced, as MSR [675,750], a better liner relationship can be obtained with the increase of chlorophylls. Fig. 2 to 5 showed the replacement helped to improve the linearity.

Conclusion

In this paper, two hyper spectral vegetation indices from two classes were tested to explore their potentials in the chlorophyll content estimation. As reflectance at 670 nm will quickly become saturated with relatively low chlorophyll content, different bands combinations were applied to derive the new indices that may have better linearity with the chlorophyll content. Index that is composed of reflectance at 750 nm and 675 nm NDVI[675,750] proves to have better linearity in control and polluted sites of the selected plant species than index composed of reflectance at 800 nm and 670 nm NDVI[670,800] and MSR[670,800] especially at high chlorophyll content values.

The result of the sensitivity study demonstrated that the two new derived vegetation indices MSR [675,750] and NDVI [675,750] are appropriate candidates for chlorophyll content estimation. Validation study was also conducted with the ground truth data the two integrated vegetation indices MSR [675,750], and NDVI [675,750] were proved to be the best for chlorophyll content estimation with correlation coefficients R^2 of 0.88 and 0.97 in control and polluted plant leaves respectively.

This is because these integrated indices accounts more effects of disturbances, such as the soil reflectance, non photosynthetic materials and cement dusted pollutant. The assessment of the feasibility of certain indices in estimating chlorophyll content at canopy level with ground truth data can be viewed as a first step of remote estimation of chlorophyll content from satellite data (Weiss et al., 2001). Haboudane et al., (2002) pointed out that one thing of particular importance in chlorophyll content estimation was the analysis of right spectral bands and combination which could enhance sensitivity to chlorophyll content variations and reduce responsively to background and canopy structure effects. In this paper, two specific bands at 675 nm and 750 nm were introduced to propose new indices MSR[675,750] and NDVI[675,750] to explore the potentials in chlorophyll. The primarily results of this study may provide some reference to the further research of chlorophyll content estimation.



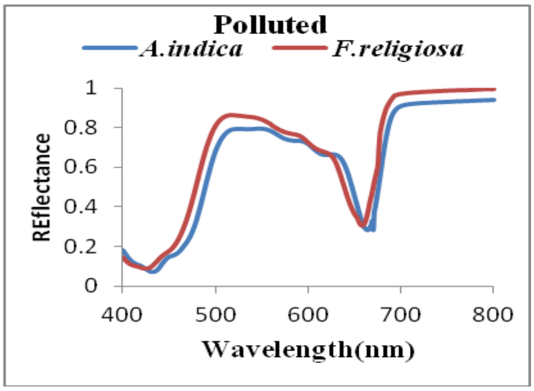


Fig-1 The Mean reflectance value of the selected plant species in control and polluted sites

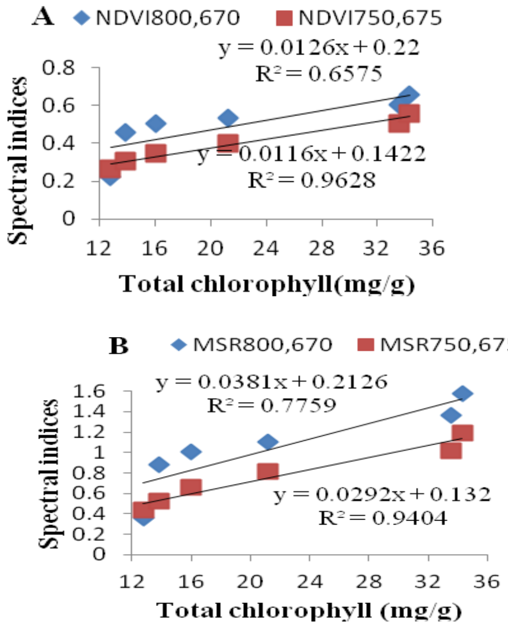


Fig-2 Relationships between different vegetation indices and chlorophyll content of *Azadirachta indica* at control site.

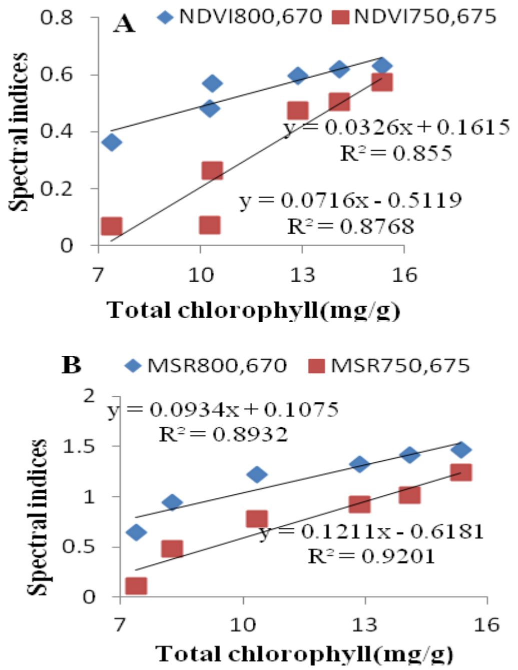


Fig-3 Relationships between different vegetation indices and chlorophyll content of *Azadirachta indica* at Polluted site

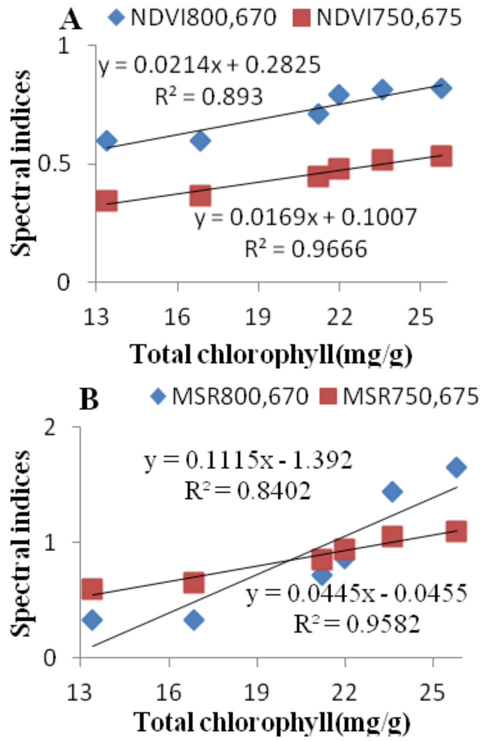


Fig-4 Relationships between different vegetation indices and chlorophyll content of *Ficus religiosa* at control site.

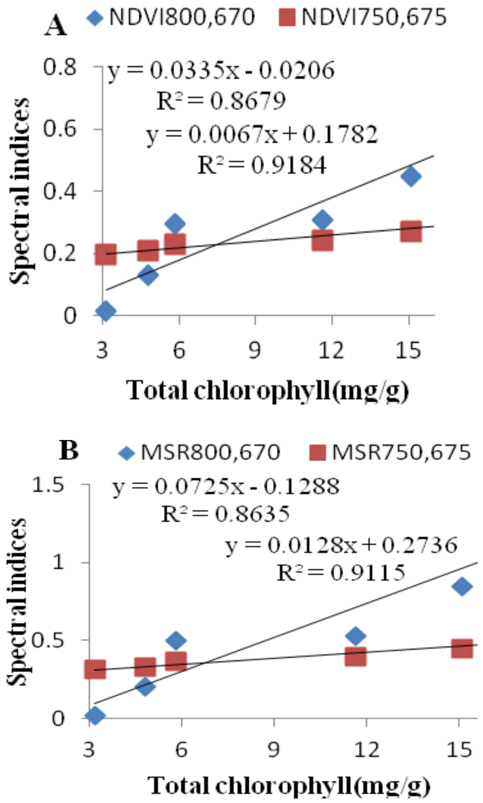


Fig-5 Relationships between different vegetation indices and chlorophyll content of *Ficus religiosa* at Polluted site.

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Table 1: Band used in the sensitivity analysis

Indices	Wavebands (nm)	Formula	References
NDVI [670,800]	670,800	$\frac{(R800 - R670)}{(R800 + R670)}$	Rouse et al., 1974
MSR [670,800]	670,800	$\frac{\left(\frac{R800}{R670}\right) - 1}{\sqrt{\left(\frac{R800}{R670}\right) + 1}}$	Chen, 1996
Revised indices			
NDVI [675,750]	675,750	$\frac{(R750 - R675)}{(R750 + R675)}$	----
MSR [675,750]	675,750	$\frac{\left(\frac{R750}{R675}\right) - 1}{\sqrt{\left(\frac{R750}{R675}\right) + 1}}$	----

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