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# Spectral Analysis of Chlorophyll, Water Content within Fresh and Water Stressed Leaves Using Hyperspectral Data

Rahul T. Naharkar<sup>1</sup>, Ratnadeep R. Deshmukh<sup>2</sup>

P.G. Student, Department of Computer Science and Information Technology, Dr.Babasaheb Ambedkar Marathwada

# University, Aurangabad, Maharashtra, India<sup>1</sup>

Professor, Dr.Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra, India<sup>2</sup>

**ABSTRACT**: Chlorophyll is the most important content that is required for the photosynthesis process as well as one of the most important biochemical parameters of plants and is generally an indicator of plants nutritional status, photosynthetic capacity and the health status of plants; that is why, it is an important information parameter in research on crop quality monitoring, ecosystem productivity estimation, carbon cycles, etc.

Water deficits can cause chlorophyll reduction which reduces the total concentration of chlorophyll. Water enables the incorporation of CO2 carbon into carbohydrates. It also produces the hydraulic pressure required for opening stomata and maintaining structural integrity. Thus, evaluating leaf water content is a key element in determining the health and productivity of vegetation. In this experiment spectral indices are used for estimating the chlorophyll and water content. The result shows high fluctuation of values between day 1 and day3. With these results health status of the plant can be estimated.

KEYWORDS: Chlorophyll, Spectroradiometer, FieldSpec, Spectral Signature, Carotenoids.

# I. INTRODUCTION

Photosynthesis is the most large-scale synthetic process on earth. There are many kinds of photosynthetic pigments, i.e., chlorophylls, carotenoids, and phycobilins in the plant leaves, but chlorophylls are considered to be the important factor because the photochemical reactions take place only at the trapped chlorophyll molecules. Light absorbed by chlorophyll provokes electrons in the molecules, enabling them to be transferred to other molecules for glucose production and thus enabling growth of vegetation. Chlorophyll content can directly determine photosynthetic potential and primary production [1].

Water enables the incorporation of CO2 carbon into carbohydrates. It also provides the hydraulic pressure needed for opening stomata and maintaining structural integrity. Thus, evaluating leaf water content is important in diagnosing the health and productivity of vegetation [2].

Reflectance measurement makes it possible to quickly and non-destructively assess, the chlorophyll and water content in leaves [3].

Estimating chlorophyll concentration has always been one of the most important issues of research using remote sensing techniques for vegetation. Typical reflectance of vegetation in the visible–infrared region will increase as water deficit occurs. As leaves dehydrate or vegetation undergoes water stress, leaf water potential becomes increasingly negative and the rate of photosynthesis is reduced because water deficit can cause chlorophyll abasement and thus significantly reduces foliar chlorophyll concentration especially, the magnesium ion (Mg2C) of the chlorophyll will be removed. As a result, chlorophyll becomes pheophytin (chlorophyll without Mg2C) and disables the photochemical reaction furthermore leaves decrease the absorptance of blue and red light while increasing the reflectance at the corresponding wavelength bands.



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The ability to accurate estimation of plant chlorophyll content and concentration may give growers useful information to allow estimation of crop yield potential and to make decisions in fertilizer management [4].

Spectroscopic technology offers fast, convenient and non-destructive detection, so it has long been used in fields, such as crop yield assessments, vegetation ecology assessments, light and effective radiation, land productivity, wetland management and tree species identification. Imaging spectroscopy can be separated into two categories according to the carrying platform of the sensors and applicable fields: spectroscopy that is build on remote sensing platforms, such as satellites and aircrafts, makes use of aerospace remote sensing and is suited for large-scale regional studies and applications; and spectroscopy that is depended on small ground application platforms, such as ground-based remote sensing systems, has a packed size and is flexible, mobile and largely used in agriculture applications, such as the retrieval of plant chlorophyll content and other biochemical parameters, differentiation of crops/weeds, monitoring of crop pests and disease and quality control of agricultural products and meat [5].

Water molecules swallow radiation in near- and midinfrared (NIR and MIR) wavelengths near 970, 1240, 1400, and 1900 nm. As Because the amount of absorption is related to total water content, these wavelengths are traditionally used in non-destructive and remote estimation of vegetation water content [e.g., Ceccato et al., 2001; Hunt and Rock, 1989]. However, infrared reflected radiation is affected largely by plant architecture, vegetation density and leaf structure, thus growing estimation uncertainty [Elachi, 1987] [6, 7].

A number of spectral indices were developed for chlorophyll estimation. In this experiment the chlorophyll indicators that have been examined Blackburn (1998), Datt (1998), Gitelson et al. (2003), Sims and Gamon (2002), Carter (1994). Those combines integrate a couple of particular signatures of visible and near-infrared bands, for example, the reflectance at 445, 550, 680, 700, 705, 710, 720, 750, 780, 800, 860 nm for chlorophyll estimation. Chlorophyll is the important components, which is present in plant leaf. Deficiency of this may cause lower or unhealthy productions. To avoid this, different fertilizers are available in market which proportionate the quantity of this component. These fertilizers are applied to plants. However there may be higher or lower quantity of fertilizer applied by farmers without considering the actual need of the plant. If we measure the chlorophyll content and apply fertilizer according to actual need of the plant; then it will be more beneficial to crop as well as to farmer [8].

## II. RELATED WORK

CaranthesusC. Lin et. Al. designed three independent experiments to collect data from three leaf sample sets for the construction and validation of Chls estimation models. First, a reflectance experiment was conducted to collect foliar Chls and reflectance of leaves with varying water stress using the ASD FieldSpecspectroradiometer. Second, a chlorophyll meter (SPAD-502) experiment was carried out to collect foliar Chls and meter readings. These two data sets were separately used for developing reflectance-based or absorptance-based Chls estimation models using linear and nonlinear regression analysis. Suitable models were suggested mainly based on the coefficient of determination ( $R^2$ ). Finally, an experiment was conducted to collect the third data set for the validation of Chls models using the root mean squared error (RMSE) and the mean absolute error (MAE). They derived a model with an  $R^2$  of 0.90 (P <0.01) from the training samples and evaluated with RMSE 0.35 and 0.38 mg  $g^{-1}$  for the validation samples of fresh and water-stressed leaves, respectively. The average prediction error was within 14% of the mean absolute error [1].

Acording to Arthur I. Zygielbaumet. al., they identified a strong, systematic, and repeatable relationship between photosynthetically active radiation (PAR, 400–700 nm) albedo and leaf RWC. They have shown that visible spectrum reflectance provides a means to detect early stages of plant stress and estimate leaf RWC [2].

Bo Liu et. al. compared FISS (Field Imaging Spectroscopy System) with ASD The results revealed that the derivative reflectance was a more sensitive indicator of chlorophyll content and could extract content information more efficiently than the spectral reflectance, which is more significant for FISS data compared to ASD (analytical spectral devices) data, reducing the corresponding RMSE (root mean squared error) by 3.3%–35.6%. Compared with the spectral features, the regression methods had smaller effects on the retrieval accuracy [5].



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### III. MATERIAL AND METHODOLOGY

### a. Study Area

Caranthesus Roseus plant is been selected for the experiment. The samples were collected from the Dr. BAMU Universities botanical garden located in the Aurangabad district, of Masharashtra state, India, on 14 march 2016 at 10: 45 am. The latitude 19.9027° N and longitude 75.3105° E are the geocoordinate of the BAMU University of Aurangabad.

## b. Experiment Design and Data Collection

Five leaf samples were detached from the Catharanthus Roseus plant at 10:45 am in the morning and the immediately measured for spectral reflectance at 11:00 am the leaves are then kept at24<sup>o</sup>C temperature and next two days spectral measurements were taken at same time.

Spectral data were obtained from the FieldSpec Pro FR spectroradiometer manufactured by Analytical Spectral Devices (ASD). This instrument measures spectra over a spectral range of 350–2500 nm and offers 1 nm wide narrowband spectral data. Specifically, the full-width at half-maximum (FWHM) spectral resolution of the FieldSpec Pro FR spectroradiometer is 3 nm for the region 350–1000 and 10 nm for the region 1000–2500 nm (Hatchell, 1999) which meets the nominal sampling and resolution requirements for hyperspectral remote sensing applications (Curtiss and Goetz, 1994).

## c. Spectral Indices :

Out of different spectral Indices available we have selected five indices which are useful for detecting chlorophyll content (listed in table 1) and three indices(listed in table 2) which are useful for detecting plant water stress.

Index	Formula	Source
Normalized Difference Vegetation Index	$NDVI_{680} = (R_{800} - R_{680}) / (R_{800} + R_{680})$	Blackburn (1998)
Weighted Simple Ratio	wSR = $R_{860} / (R_{708} * R_{550})$	Datt (1998), Gitelson et al. (2003)
Modified Red Edge NDVI	mNDVI = $(R_{750}-R_{705})/(R_{750}+R_{705}-2R_{445})$	Sims and Gamon (2002)
Simple Ratio Stress Index	$SR_{420} = R_{695} / R_{420}$	Carter (1994)
Modified Red Edge Simple Ratio	$mSR = (R_{750} - R_{445}) / (R_{705} - R_{445})$	Sims and Gamon (2002)

Table 1:	Spectral	Indices for	Chlorophyll	Estimation
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The given table 1 shows the spectral indices used for estimating the chlorophyll content in the Catharanthus Roseus plant. The table shows the formula for indices with their spectral range number. After putting the values of the respected spectral range, we will have the results.



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# **Table 2: Spectral Indices for Water Estimation**

Index	Formula	Source
Moisture Stress Index	$MSI = R_{1599} / R_{819}$	Hunt Jr. et. Al.(1989), Ceccato, P., et al(2001)
Water Band Index	$WBI = R_{970} / R_{900}$	Penuelas, J., et al.(1995), Champagne, C., et al.(2001)
Normalized Difference Water Index	NDWI = $(R_{857} - R_{1241}) / (R_{857} + R_{1241})$	Gao, B.(1995)

The given table 2 shows the spectral indices used for estimating the water content in the Catharanthus Roseus plant. The table shows the formula for indices with their spectral range number. In order to calculate the results, we will put values at the respected spectral range numbers.

# IV. RESULT AND DISCUSSION

# a. Spectral Signature Analysis

Figure 3a shows the spectral signature obtained by the ASD Field spectroradiometer over the range of 350nm-2500nm. The spectral profile corresponding to the healthy tree follows a typical spectral signature of plant leaves: a small peak at the green band (550 nm), a small drop at the red band (650 nm), and a rising plateau at the near-infrared (NIR) band (750 - 1000 nm).



Figure 1: Spectral signature of Catharanthus Roseus of day 1, day 2, day 3



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The spectral signature shows the three different stages of the same plant leaf. The spectral signature represented in green color the healthy leaf. The spectral signature shows the strong reflectance and absorbance in the range of chlorophyll and water. The spectral signature with red color was taken on day 2 after detaching it from plant. The leaves were kept at  $24^{\circ}$  temperature from day 1 to day 2. The difference between the spectral behavior of day 1 and day 2 clearly indicates a drop in a chlorophyll and water range as compared to day 1 (which in this experiment is referred to as Healthy leaf). The brown spectral signature shows the reading of day 3. After taking the reading at day 2 leafs were kept in  $30^{\circ}-40^{\circ}$  temperature (kept in sunlight) in order to make them dry. The day 3 readings in this experiment are considered as completely dry leafs. The day 3 reading shows the big difference in the spectral behavior between day 1 and 2, as spectral signature drops drastically in the chlorophyll region and remains flat in the water region

### b. Statistical Analysis

The tables which are listed below shows the values which were obtained after applying the spectral indices. As the experiment aims at finding chlorophyll and water content two categories of indices were used those are vegetation indices and water indices.

INDEX	DAY 1	DAY 2	DAY 3
NDVI <sub>680</sub>	0.679	0.690	0.755
wSR	13.797	16.814	28.287
mNDVI	0.609	0.587	0.358
SR <sub>420</sub>	1.222	1.359	2.278
mSR	4.113	3.840	3.030

Table 3: Result of Chlorophyll Indices for Day1, Day2, Day3

The table 3 shows the values obtained after putting the values of reflectance and wavelength in the respected formula for estimating chlorophyll content in the Catharanthus Roseus plant.. The table shows the comparisons of values observed at day1, day 2, day 3.

 Table 4: Results of Water Indices for Day 1, Day2, Day3

INDEX	DAY 1	DAY 2	DAY 3
MSI	0.669	0.819	1.121
WBI	0.969	0.987	1.031
NDWI	0.035	0.003	-0.046

The table 3 shows the values obtained after putting the values of reflectance and wavelength in the respected formula for estimating water content in the Catharanthus Roseus Plant. The table shows the comparisons of values observed at day1, day 2, day 3.

In this experiment five vegetation indices were used that are  $NDVI_{680}$ ,  $SR_{420}$ , mSR, wSR, mNDVI for estimating chlorophyll content. For estimating the water content three water indices were used which are MSI, WBI, and NDWI. The values obtained during this experiment show a slight difference between day 1 and day 2 whereas there is a big



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fluctuation in the values between day 1 and day 3. The gap between day 2 and day 3 values is not as big as day 1 and day 3.

## V. CONCLUSION

As water stress occurred, the reflectance over the visible and infrared area will increase. The estimation of chlorophyll concentration using only the remotely sensed reflectance will be seriously affected by the reflectance changes caused by a departure of water content from the non-water-stressed conditions. The significant uncertainty for the estimation of chlorophyll concentration is caused by the reflectance changes induced by variations of the water content.

Plant growth and productivity are mostly affected by water shortage. This stress condition induces plant cell dehydration and then causes the decreased chlorophyll in older leaves. With this experiment we can have good knowledge about the status of the plant (regarding the chlorophyll and water) and thus we can apply fertilizers and water in controlled manner.

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