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Quantification of leaf level chlorophyll of various tropical tree species with the help of laboratory reflectance spectra

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Abstract

Leaf chlorophyll content (LCC) is considered as one of the key indicators of photosynthetic activity and thus the overall health of the plant. In present study an attempt has been made to compare selected indices and identify best index for quantification of LCC using leaf level laboratory reflectance spectra. Leaf samples of teak, bamboo, and other mixed vegetation tree species were collected from the field and averaged readings were taken (n=15). Leaf thickness, pigmentation, and reflectance of entire electromagnetic spectrum were measured for the same leaf samples. Results showed that the R^2 values for estimation of total, a, b, were higher (> 0.70) for indices based on red edge effect and near infrared region (NIR). Results in present study clearly illustrated the relationship between number of palisade layer and reflection in NIR region.

Keywords: Chlorophyll, reflectance spectra, tropical trees

Introduction

Chlorophyll content is considered as one of the key indicators of photosynthetic activity and thus the overall health of the plant. It changes dynamically based on the age of the leaves, illumination and also the environmental conditions. The leaf chlorophyll content (LCC) changes due to several natural and anthropogenic stress conditions. Leaf chlorophyll content suggests the physiological status of plants and is closely related to plant photosynthetic capacity (Wang *et al*., 2020) [28]. Chlorophyll quantity can provide insights on the plant physiological condition (Carter and knapp, 2001; Lichtenthaler, 1998, Cervena *et al*., 2022) [5, 7, 18]. Chlorophyll content is also associated to nitrogen content (Niinemets & Tenhunen, 1997; Yoder & Pettigrew-Crosby, 1995) [19, 29]. Estimates of the foliar chemistry allow a better understanding of ecosystem functioning since many biochemical processes such as photosynthesis, respiration, and litter decomposition are related to the foliar chemistry of plants (Huber *et al*. 2008) [14]. Therefore, accurate quantification of LCC is of great significance for terrestrial carbon flux cycling and biomass estimation (Zhang at al., 2022).

The leaf chlorophyll content (LCC) can be determined by laboratory methods consisting of pigment extraction into an organic solvent followed by spectrophotometric evaluation (Porra *et al*., 1989) [21]. This method is considered to be precise and reliable but it is destructive and time-demanding. The non-destructive methods are based on the specific optical properties of the chlorophyll. Laboratory scale reflectance spectra of leaves and its applications have evolved as very useful tool for accurate estimation of LCC. Owing to its fast, non-destructive and relatively cheap categorization. Many researchers has already developed indices to calculate chlorophyll content from laboratory scale reflectance spectra (Huete, 1988., Gitelson and Merzylak, 1997, Zarco Tejada *et al*., 2001., Haboudane *et al*., 2004). [11, 12, 15, 31]

In present study an attempt has been made to compare selected indices and identify best index for quantification of LCC using leaf level laboratory reflectance spectra.

Materials and Methods

Laboratory spectra of teak and bamboo along with few important mixed vegetation tree species *Madhuca indica (Gmel.), Tectona grandis (L.) Mangifera indica (L.), Ficus glomerata(L.), Dendrocalamus strictus (Nees.)* were obtained using foliage samples collected from the field. All leaves collected in the field were placed in plastic bags and kept in cool condition until they were subjected for spectral measurements. Reflectance spectra coming from 10 leaves (young, middle and mature ones) of all the species were averaged to get standardized leaf reflectance spectra for further analysis (total 3 different standardized spectra for five species, n=15). All spectral measurements were made with a field portable spectrometer (ASD FieldSpec®3). The FieldSpec®3 has a spectral range from 350 to 2500 nm with 1 nm bandwidth. Leaf illumination was provided through halogen lamp. Leaf thickness readings and quantification of pigments (total chlorophyll and chlorophyll a, b) were done for the same leaf samples and averaged readings were taken (n=15). Leaves of selected species for laboratory spectra acquisition showed variation in various attributes (leaf thickness, pigmentation). Samples were immediately brought to the laboratory for biochemical analysis. Chlorophyll content per unit leaf area was determined following the procedure of Arnon (1949) [1].

Total chlorophyll = $[(27.8 \times A_{645nm}) / 1000] \times$ final volume in ml.

Chlorophyll a= $[(12.3 \times A_{663nm}) - (0.86 \times A_{645nm}) /1000] \times$ final volume in ml.

Chlorophyll b= $[(19.3 \times A_{645nm}) - (3.6 \times A_{663nm}) /1000] \times$ final volume in ml.

Indices derived by other researchers were tested for their performance in estimation of total chlorophyll, chlorophyll a and chlorophyll b from laboratory scale reflectance spectra

Table: 1 List of Indices tested for estimation of total Chlorophyll, chlorophyll a and b from laboratory spectra

Index	Computation	Reference
SAVI (Soil Adjusted Vegetation Index)	$(R_n - R_r)(1+L) / (R_n + R_r + L)$ where L = 0.5	Huete, (1988) [15]
MSAVI2 (Modified SAVI)	$R_n + 0.5 - ((R_n + 0.5)^2 - 2(R_n - R_r))^{0.5}$	Qi *et al.*, (1994)
OSAVI (Optimized SAVI)	$(1+0.16)(R_{800} - R_{670}) / (R_{800} + R_{670} + 0.16)$	Rondeaux *et al.* (1996).
MSR (Modified SR)	$MSR = ((R_{800} - R_{670})-1) / ((R_{800} + R_{670})^{0.5} + 1)$	Chen (1996)
RDVI (Renormalized Difference Vegetation Index)	$RDVI = (R_{800} - R_{670}) / (R_{800} + R_{670})^{0.5}$	Rougean and Breon, (1995)
MCARI (Modified CARI)	$MCARI = [(R_{700} - R_{670})-0.2(R_{700} - R_{550})] (R_{700}/R_{670})$	Daughtry *et al.*, (2000)
TCARI (Transformed CARI)	$TCARI = 3\ [(R_{700} - R_{670})-0.2(R_{700} - R_{550}) (R_{700}/R_{670})]$	Haboudane *et al.*, (2002) [12]
TVI (Triangular vegetation index)	$TVI = 0.5\ [120\ (R_{750} - R_{550}) - 200\ (R_{670} - R_{550})]$	Broge and Leblanc (2000)
SIPI (Structural insensitive pigment index)	$SIPI = (R_{800} - R_{445}) / (R_{800} + R_{680})$	Penuelas *et al.*, (1995)
NPCI (Normalized Pigment Chlorophyll Index)	$NPCI = (R_{680} - R_{430}) / (R_{680} + R_{430})$	Penuelas *et al.*, (1995)
MCARI1	$MCARI1 = 1.2\ [2.5\ (R_{800} - R_{670}) - 1.3\ (R_{800} - R_{550})]$	Haboudane *et al.*, (2004) [12]
MCARI2	$MCARI2 = 1.5\ [2.5\ (R_{800} - R_{670}) - 1.3\ (R_{800} - R_{550})] / [(2\ R_{800} + 1)^2 - (6R_{800} - 5\ (R670)^{0.5}) - 0.5]$	Haboudane *et al.*, (2004) [12]
Red edge 750~700	$R_{750} - R_{700}$	Gitelson and Merzylak (1997)[11]
Red edge 740~720	$R_{740} - R_{720}$	Vogelmann *et al.*, (1993) [25,26,27]
ZTM (Zarco Tejada and Miller)	$ZTM = (R_{750} / R_{710})$	Zarco Tejada *et al.*, (2001) [31]

Results

Average reflectance spectra acquired in the laboratory for teak, bamboo and few other species were shown in Figure 1. Leaf thickness readings along with number of palisade and spongy tissue layers for all the five species were shown in Table 2. Measured total chlorophyll, chlorophyll a and b values were given in Table 3. Leaf thickness clearly effected reflectance values of NIR region (700-1300 nm). Reflectance of NIR region was proportional to leaf thickness. Teak showed highest leaf thickness and maximum reflectance in NIR region.

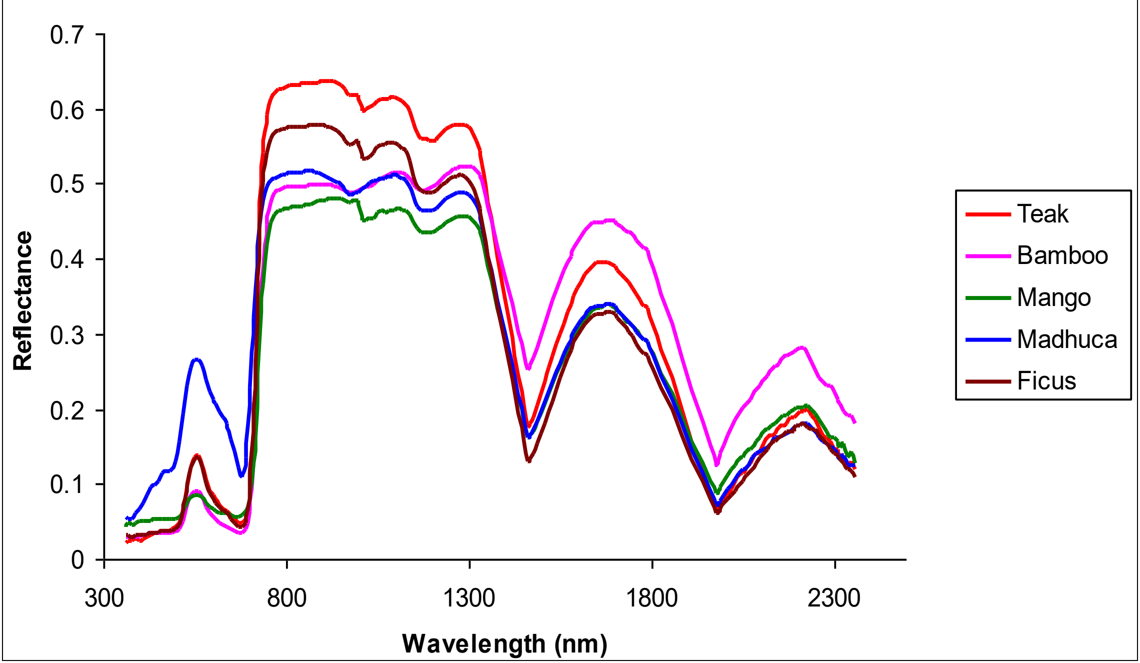

Fig :1 Average Laboratory reflectance spectra for selected species

Longitudinal section (L.S) of *Tectona* and *Ficus* leaf showed 2 layers of pallisade tissue. L.S of *Madhuca* leaf showed single layer of pallisade tissue. However, pallisade layer in *Madhuca* leaf was relatively longer. L.S of *Mangifera* leaf showed single layer of palisade tissue

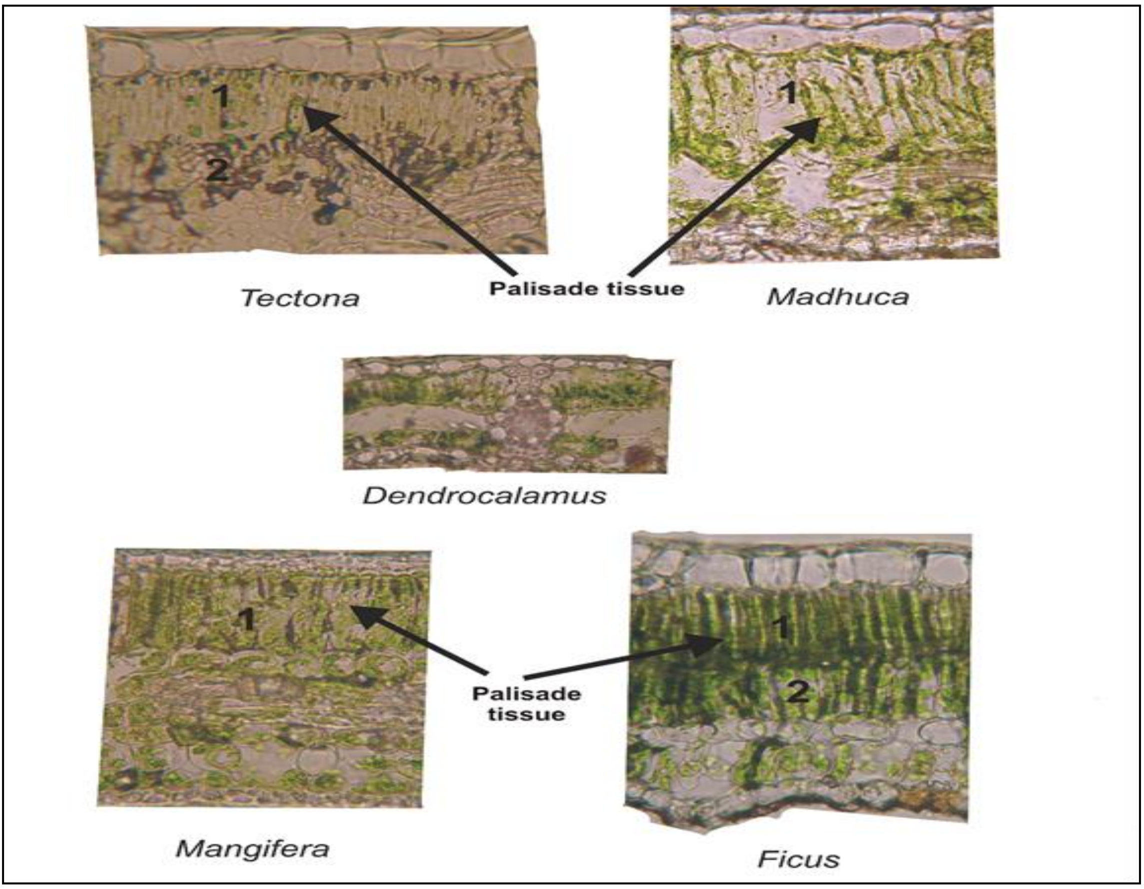

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Fig 2: Leaf anatomical structures for species selected for Laboratory spectra acquisition

Table :2 Thickness of leaves for species selected for Laboratory spectra acquisition

Species name	Leaf thickness (µm)
Tectona	266.35
Dendrocalamus	90.46
Madhuca	235.25
Mangifera	177.51
Ficus	233.67

Table: 3 Measured biochemical attributes for species selected for Laboratory spectra acquisition

Leaf biochemical attributes		n=15		
		Minimum	**Maximum**	**Mean**
Total Chlorophyll	µg cm^{-2}	18.20	53.95	37.29±10.95
Chlorophyll a	µg cm^{-2}	15.50	43.20	27.65±8.11
Chlorophyll b	µg cm^{-2}	1.35	9.00	2.58±2.37

Table: 4 Performance of different indices for estimation of total chlorophyll, Chlorophyll a and b from laboratory spectra

	Total chlorophyll (µg cm^{-2})	Chlorophyll a (µg cm^{-2})	Chlorophyll b (µg cm^{-2})
		(R^2)	
RE740-420	0.77	0.60	0.31
ZTM	0.77	0.60	0.29
RE750-700	0.73	0.57	0.32
743/692 (From present study)	0.64	0.45	0.24
MSAVI	0.56	0.40	0.26
SAVI	0.55	0.40	0.27
RDVI	0.55	0.40	0.27
MSR	0.54	0.41	0.19
OSAVI	0.54	0.39	0.27
SIPI	0.54	0.37	0.24
NDVI	0.53	0.38	0.27
NPCI	0.52	0.41	0.23
MCARI2	0.49	0.34	0.31
MCARI1	0.47	0.34	0.31
TVI	0.45	0.33	0.29
TCARI	0.00	0.00	0.13

Measured total chlorophyll values are ranging from minimum of 18.20 to maximum of 53.95 µg cm^{-2}. Measured chlorophyll a values are ranging from minimum of 15.50 to maximum of 43.20 µg cm^{-2}. Measured chlorophyll b values are ranging from minimum of 1.35 to maximum of 9.00 µg $cm^{-2.}$ Regression coefficients (R^2) generated by all tested indices for measurement of total chlorophyll, chlorophyll a and b were shown in Table 4. Results indicate that red edge index developed by Vogelmann *et al.* (1993) [25,26,27] that is Red edge 740~ 720 gave highest R^2 vales for estimation of total chlorophyll (R^2 0.77) and chlorophyll a (R^2 0.60). Followed by ZTM index developed by Zarco Tejada *et al.* (2001) [31] and Red Edge index 750~700 developed by Gitelson and Merzylak (1997) [11]. All tested indices were failed to achieve high R^2 values for estimation of chlorophyll b.

Discussion

Considerable variation in reflectance NIR and SWIR regions of electromagnetic spectrum was observed between average leaf level laboratory reflectance spectra of teak, bamboo and other mixed vegetation tree species. Similar pattern of variation was observed in leaf level spectra of seven tropical tree species by Clark *et al.* (2005). They stated that several factors can cause leaf spectral variation within a given species, including leaf thickness, necrosis, maturation of the mesophyll, and the concentration of chlorophyll and water. In present study it was observed that increase in leaf thickness values cause increase in reflectance of NIR region. Many studies reported that the NIR spectral range is dominated by variation in leaf water content and leaf thickness, related to specific Leaf area (SLA) (Thomas *et al*., 1971; Hunt *et al*., 1987; Jacquemoud and Baret, 1990; Ceccato *et al*., 2001) [6, 16, 24]. Thin leaves are compact and have fewer air-cell wall refractive discontinuities causing lower NIR–SWIR reflectance (Gausman, 1985) [10]. Results in present study clearly illustrated the relationship between number of palisade layer and reflectance in NIR region. Earlier, Ourcival (1999) reported that palisade mesophyll and total thickness were strongly correlated with reflectance spectra. Vogelmann and Martin (1993) [25,26,27] showed that long, cylindrical palisade mesophyll cells propagate visible wavelengths deeper into the leaf interior, whereas the more spherical spongy mesophyll cells tend to scatter radiation. Present study tried to establish relationship between leaf structure and reflectance. Recently, Ban *et al*., 2022 [2] and Shi *et al*., 2023 also reaffirmed the importance of reflectance in NIR regon for estimation of leaf level chlorophyll in rice plant. Earlier, Slaton *et al*., (2001) [23] stated that this relation between leaf structure and reflectance may be useful in the interpretation of remote sensing data measured from satellite or aircraft, or with standard field and laboratory instrumentation. Variation in the SWIR region is caused by leaf water concentration, with important contributions from protein N, cellulose and lignin (Curran,1989) [9].

Range of total chlorophyll (leaf level) values tested in this study is similar to the ones published by le Maire *et al.* (2004) and Zarco-Tejada *et al.* (2004) [31], for different broad leaf tree species and few open canopy tree crops respectively. Range of chlorophyll a and b values (leaf level) is similar to range provided by Cao (1999) from leaves of few tropical woody tree species. Results indicate that red edge index developed by Vogelmann *et al.* (1993) [25,26,27] ZTM index developed by Zarco Tejada *et al.* (2001) [31] and Red Edge index 750~700 developed by Gitelson and Merzylak (1997) gave better results for estimation of both total chlorophyll and chlorophyll a. All the three vegetation indices were calculated from the reflectance coming from red edge region (680–750 nm) (Horler *et al*., 1983; Filella and Penuelas, 1994) [13]. Recently, Brown *et al*., 2022 [3] reported various indices for leaf level Chlorophyll estimation. Range of R^2 and accuracy values obtained in present studY are comparable to range reported in above-mentioned research article. Furthermore, R^2 values obtained in present study for various indices is also comparable to R^2 values obtained by Shi *et al*. (2023) for LCC measurement through different indices. In this study testing of different indices reaffirmed the importance of REP for estimation of total chlorophyll and chlorophyll a.

Conclusion

The study were able to highlight the significant relationship between leaf structure and reflectance in the NIR and SWIR regions, emphasizing that variations in leaf thickness and water content are critical for understanding leaf spectral characteristics. Additionally, the effectiveness of specific vegetation indices for estimating total chlorophyll and chlorophyll a is reaffirmed, aligning with previous research findings.

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