

The use of a chlorophyll meter (SPAD-502) for field determinations of red mangrove (*Rhizophora mangle* L.) leaf chlorophyll amount.

Xana M. Connelly

Department of Marine Sciences University of Puerto Rico

The red mangrove *Rhizophora mangle* L., is a halophytic woody spermatophyte common to the land-sea interface of tropical and subtropical intertidal zones. It has been reported that 60 to 75% of the coastline of the earth's tropical regions are lined with mangroves (Walsh (1974) in Nybakken "1988). Mangroves help prevent shoreline erosion, provide breeding, nesting and feeding areas for many marine animals and birds. Mangroves are important contributors of primary production in the coastal environment, and this is largely proportional to the standing crop of leaf chlorophylls (Oswin and Kathiresan, 1994). Higher intensities of ultraviolet radiation, resulting from stratospheric ozone depletion, can lead to a reduction of chlorophyll in terrestrial plants (Tevini et al., 1980). Since the most common method for determining chlorophyll concentration is by extraction and this is labor intensive and time consuming, few studies on photosynthetic pigments of mangroves have been reported.

Chlorophyll meter readings have been related to leaf chlorophyll content in apples (Campbell et al., 1990) and maples (Sibley et al., 1996). It has also been correlated to nitrogen status in corn (Wood et al., 1992a) and cotton (Wood et al., 1992b). Peterson et al., (1993) used a chlorophyll meter to detect nitrogen deficiency in crops and in determining the need for additional nitrogen fertilizer. Efforts to correlate chlorophyll meter measurements to chlorophyll content of mangroves have not been reported. This paper describes the use of a hand-held chlorophyll meter (Minolta SPAD-502) to determine the amount of red mangrove foliar chlorophyll present in the field.

The chlorophyll absorption curve has peaks in the red (600-700 nm) and blue (400-500 nm) regions of the spectrum with little absorbance in the near-infrared region. The SPAD-502 measures transmittances in the red (650nm) where absorbance is high and unaffected by carotene and in the infrared (940 nm) region where absorbance by pigments is very low. Two light emitting diodes (LED's) (a red LED and an infrared LED) are built into the measuring head and emit light in sequence when the measuring head is closed. Light from these LED's goes through the emitting window, passes through the sample leaf in the measuring head, and enters the receiving window where it is converted into analog electrical signals. Then the ratio of the intensities of the transmitted light is calculated into a numerical SPAD value that is proportional to the amount of chlorophyll present in the leaf (Minolta Chlorophyll meter SPAD-502 Instruction Manual).

Healthy and mature leaves were obtained from mangroves growing at various localities along the southwest coast of Puerto Rico. SPAD measurements were taken by simply inserting a leaf and closing the measuring head, Six SPAD measurements were taken for each leaf and the average was calculated. Fresh leaf material was sampled using a cork borer (5mm diameter) and extracted immediately in pure acetone using an all-glass hand tissue grinder, following the procedure detailed by Corredor et al., (1995). Extracts were cleared by filtration (Gelman PTFE ACRODISC CR 0.2µm) and stored at -5°C until analysis. Absorbance of acetone extracts were measured at 644 and 662nm in a Hewlett-Packard 8452 spectrophotometer. The amount of chlorophyll was determined using the equations described by Lichtenthaler and Wellburn (1983). Regression analysis was used to assess the relationship between extracted and predicted chlorophyll values.

An absorption spectrum for *R. mangle* pigments extracted in pure acetone is shown in Figure 1, The maximum peaks characteristic of chlorophyll were observed at 430 and 662 nm.

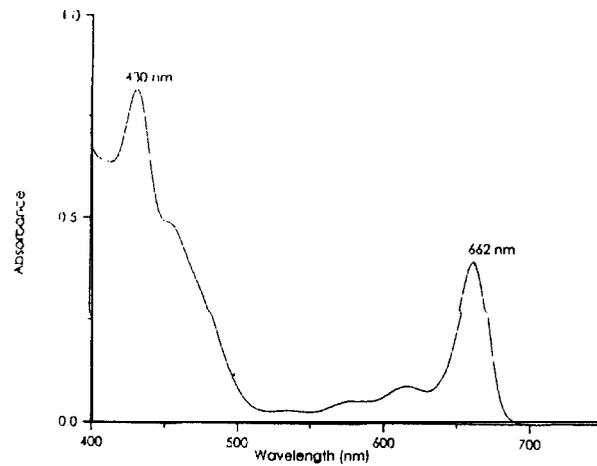


Figure 1. Red mangrove chlorophyll absorption spectrum in pure acetone extract

The chlorophyll meter, however, measures *in vivo* chlorophyll absorption which occurs at a slightly lower wavelength (650 nm) than *in vitro* extractions. The measured chlorophyll concentrations varied from 23.85 $\mu\text{g}/\text{cm}^2$ to 72.09 $\mu\text{g}/\text{cm}^2$, with an average of 43.74 $\mu\text{g}/\text{cm}^2$. The SPAD values ranged from 32.4 to 68.8. There is a high relationship between chlorophyll meter readings and total chlorophyll ($r^2 = 0.68$) (Figure 2). The equation used to predict chlorophyll concentration from SPAD-502 readings is:

$$\text{Chlorophyll in } \mu\text{g}/\text{cm}^2 = (\text{SPAD} - 22.70) / 0.57$$

To validate the regression curve 14 samples were selected at random from the total of 52 samples. The relationship between the chlorophyll values obtained by extraction in pure acetone and the values predicted from SPAD-502 measurements is presented in Figure 3. Chlorophyll meter readings had a higher correlation with chlorophyll a ($r^* = 0.77$) than chlorophyll b ($r^2 = 0.56$).

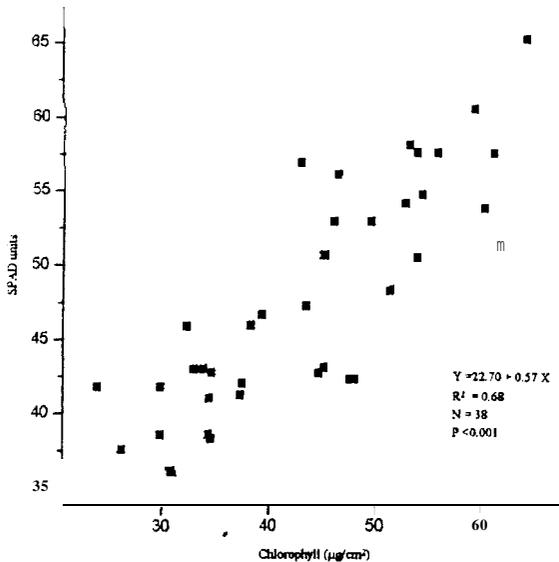


Figure 2. Relationship of Chlorophyll meter readings to chlorophyll extractions

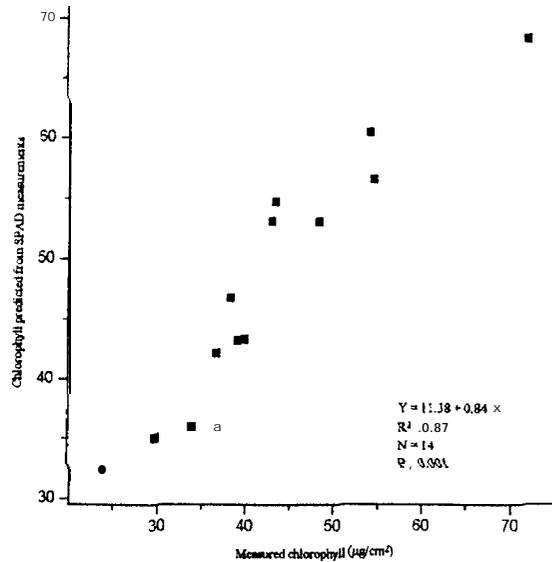


Figure 3. Relationship between predicted chlorophyll and extracted chlorophyll

Red mangrove leaves were collected from four stations in southwestern Puerto Rico in order to provide a wide range of chlorophyll values for the calibration. There were statistically significant differences between stations ($p < 0.001$). These differences can be related to variations in the nutrient input received at each station. Means were significantly higher for the Sewage Treatment Plant (STP) station, with a mean chlorophyll value of 55.07 $\mu\text{g}/\text{cm}^2$. Intermediate values were found in Bird Island (42.39 $\mu\text{g}/\text{cm}^2$) and Caballo Blanco (39.77 $\mu\text{g}/\text{cm}^2$). Cayo Enrique had the lowest values with a mean of 37.34 $\mu\text{g}/\text{cm}^2$. Anthropogenic effects are responsible for the higher nutrient levels at the STP station, Bird Island has a large population of

birds which contribute high level of nutrients while **Caballo Blanco** and **Cayo Enrique** receive the least amount of exogenous nutrient inputs.

Another source of variation in the measured leaf chlorophyll values had to do with their exposure to solar radiation. Although the chlorophyll amount is expected to be reduced in sun leaves due to increased UV-B exposure (Tevini et al., 1981), there was no statistically significant differences ($p=0.05$) between the chlorophyll content of shaded leaves and sun exposed leaves, except for **Caballo Blanco** station. At this station, the chlorophyll content for sun exposed leaves had a significantly higher mean ($44.57\mu\text{g}/\text{cm}^2$) than the chlorophyll content for shaded leaves (mean of $34.29\mu\text{g}/\text{cm}^2$). At the other stations sun leaf chlorophyll values ranged from $26.47\mu\text{g}/\text{cm}^2$ to $72.09\mu\text{g}/\text{cm}^2$ while shade leaf values ranged from $23.85\mu\text{g}/\text{cm}^2$ to $62.04\mu\text{g}/\text{cm}^2$.

The chlorophyll a to b ratio varied between 3.19 in shaded leaves to 5.62 in sun exposed leaves, which agrees with Tevini et al., (1981) who reported that the proportion of chlorophyll a to chlorophyll b rises in UV-irradiated higher plants. However Kathiresan and Moorthy (1993) found that the chlorophyll a to b ratio was higher in shaded leaves of *R. mucronata* in which they report a chlorophyll a to b ratio of 1.3 for sun exposed leaves and 1.80 for shaded leaves.

In summary, the **Minolta SPAD-502 Chlorophyll Meter** was useful in providing a large number of leaf chlorophyll measurements in the field. In red mangroves, chlorophyll meter readings compared favorably to extracted values in pure acetone. In remote areas where traditional chlorophyll extraction procedures are impractical and samples can degrade before they can reach laboratory facilities, this portable meter can provide reliable measurements of the chlorophyll status of mangrove forests.

Acknowledgments

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References

- Campbell, R. J., K.N. Mobley, R.P. Marini and D.G. Pfeiffer, 1990. Growing conditions alter the relationship between SPAD-501 values and apple leaf chlorophyll. *Hort Science* 25 (3): 330-331.
- Corredor, J. E., J.M. Morell, E.J. Klekowski and R. Lowenfeld, 1995. Mangrove genetics. HI. Pigment Fingerprints of chlorophyll-deficient mutants. *Int. J. Plant Sci.* 156 (1): 55-60.
- Kathiresan, K. and P. Moorthy, 1993. Influence of different irradiance on growth and photosynthetic characteristics in seedlings of *Rhizophora* species. *Photosynthetic* 29(1): 143-146.
- Lichtenthaler, H. K. and A. R. Wellburn, 1983, Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions* 603: 591-592.
- Minolta Chlorophyll meter SPAD-502 Instruction Manual 23pp.
- Nybakken, J. W., 1988. *Marine Biology: an ecological approach*. 2nd edition, Harper & Row, Publishers, New York. pp 415-428.
- Oswin, S.D. and K. Kathiresan, 1994. Pigments in mangrove species of Pichavaran. *Indian Journal of Marine Sciences*. Vol. 23: 64-66.

- Peterson, T.A., T.M. Blackner, D.D. Francis and J.S. Schepers, 1993. Using a chlorophyll meter to improve N management. *NebGuide*, Cooperative Extension, Institute of Agriculture and Natural Resources, University of Nebraska 4 pp.
- Sibley, J.L., D.J. Eakes, C.H. Gilliam, G.J. Keever, W.A. Dozier and D.G. Himelrick, 1996. Foliar SPAD-502 meter values, nitrogen levels, and extractable chlorophyll for red maple selections. *HortScience* 31 (3): 468-470.
- Tevini, M., W. Iwanzik and U. Thoma, 1980. The effects of UV-B radiation *on* higher plants. in Calkins, J. (Ed.), 1980. The role of ultraviolet radiation in marine ecosystems. Plenum Press, New York and London. pp 581-616.
- Tevini, M., W. Iwanzik and U. Thoma, 1981. Some effects of enhanced UV-B radiation on the growth and composition of plants. *Planta* 153: 388-394.
- Wood, C. W., D. W. Reeves, R.R. Duffield and K.L. Edmisten, 1992a. Field chlorophyll measurements for evaluation of corn nitrogen status. *Journal of Plant Nutrition* 15 (4): 487-500.
- Wood, C. W., P. W. Tracy, D.W. Reeves and K.L. Edmisten, 1992b. Determination of cotton nitrogen status with a hand-held chlorophyll meter. *Journal of Plant Nutrition* 15 (9): 1435-1448.

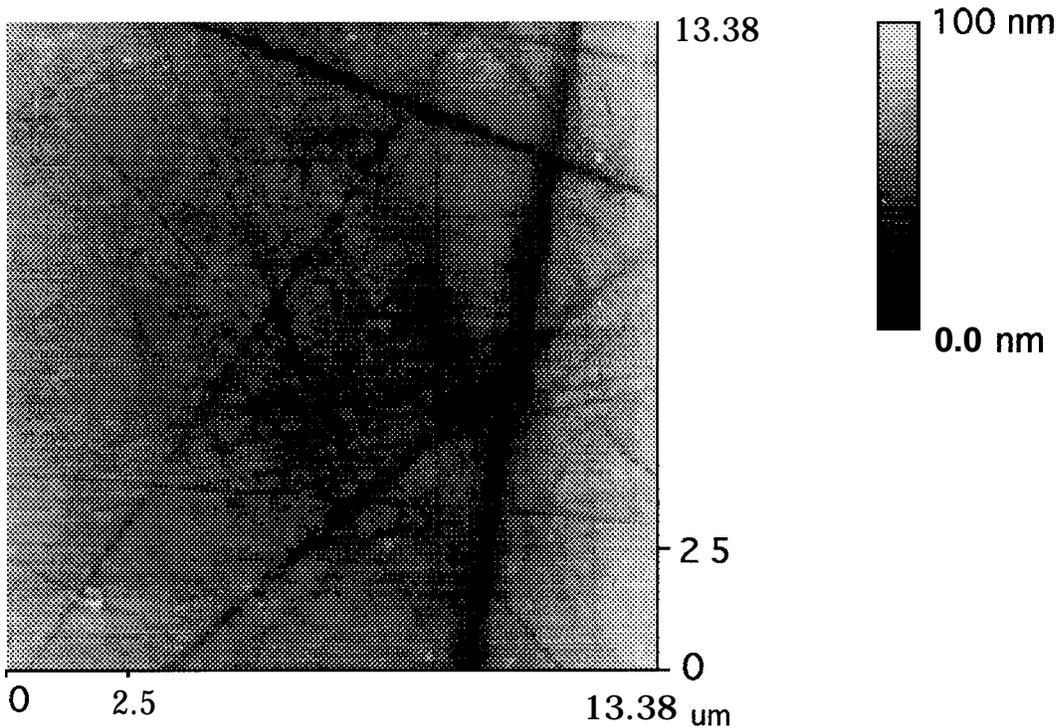


FIGURE 3: AFM IMAGE OF ILMENITE, THE BAR ON THE RIGHT INDICATES HEIGHT SCALE

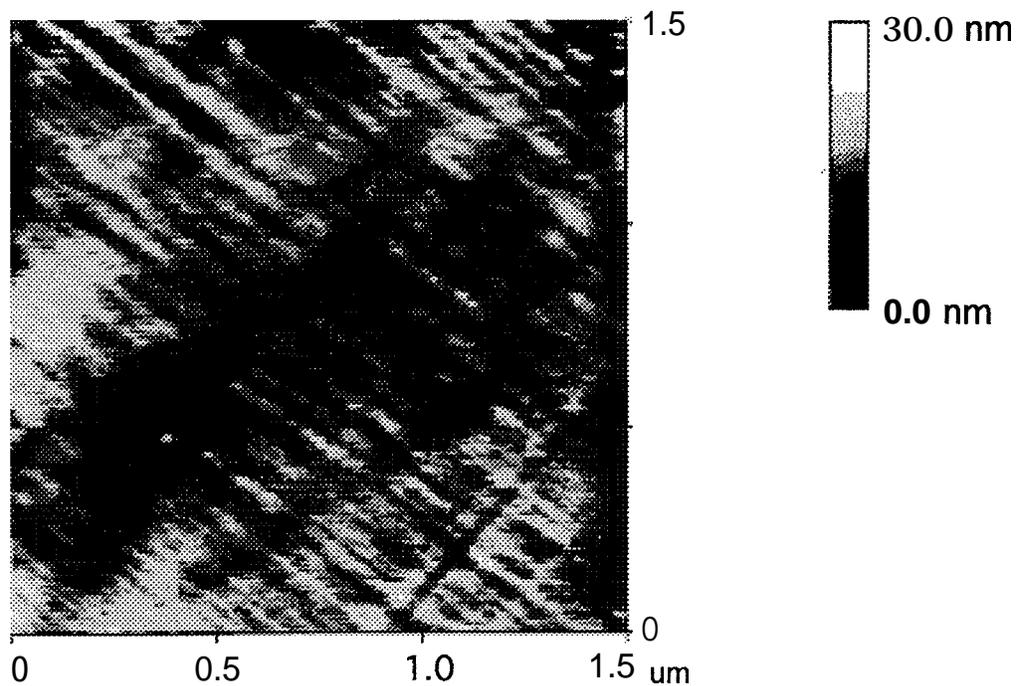


FIGURE 4: STM IMAGE OF ILMENITE, THE BAR ON THE RIGHT INDICATES HEIGHT SCALE