Evaluation of a portable chlorophyll meter to estimate chlorophyll concentrations in leaves of tropical wood species from Amazonian forest

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ABSTRACT - (Evaluation of a portable chlorophyll meter to estimate chlorophyll concentrations in leaves of tropical wood species from Amazonian forest). A portable chlorophyll meter can be an important tool to estimating chlorophyll contents in leaves of tree species under field conditions. The method is quick and non-destructive compared to the classic chemical methods of pigment extraction. The aim of this work was to evaluate the performance of a portable chlorophyll meter (CCM-200, Opti-Science) to estimate the concentrations of chloroplastid pigments and the fraction of photosynthetically active irradiance absorbed by leaves (α) in four tropical wood species from Amazonian forest. The results shown positive correlation between the chlorophyll contente index (CCI) readings and concentrations of Chl *a* ($P < 0.0001$; $r^2 = 0.904$), Chl *b* ($P < 0.0001$; $r^2 = 0.803$), Chl_{tot} ($P < 0.0001$; $r^2 = 0.923$) and α ($P < 0.0001$; $r^2 = 0.886$), suggesting that the portable chlorophyll meter can be an effective tool for the relative estimation of chlorophyll concentrations and the fraction of the photosynthetically active irradiance absorbed by the leaves of tropical tree species.
Key words: Chlorophyll contents, chloroplastid pigments, photosynthetically active radiation

RESUMO - (Avaliação de um medidor portátil de clorofila para estimar as concentrações de clorofilas em folhas de espécies lenhosas da floresta Amazônica). Os medidores portáteis de índice de conteúdo de clorofilas podem ser uma ferramenta importante para estimativa das concentrações de clorofilas de folhas de espécies arbóreas no campo, sendo este um método rápido e não destrutivo quando comparado com os métodos químicos clássicos de extração de pigmentos. O objetivo desse trabalho foi avaliar a capacidade do clorofilômetro portátil (CCM-200, Opti-Science) em estimar as concentrações de pigmentos cloroplastídicos e a fração da irradiância fotossinteticamente ativa absorvida pela folha (α) em folhas de quarto espécies arbóreas da floresta Amazônica. Os resultados indicam que houve boa relação entre os índices de conteúdo de clorofilas (ICC) e as concentrações de Chl *a* ($P < 0,0001$; $r^2 = 0,904$), Chl *b* ($P < 0,0001$; $r^2 = 0,803$), Chl_{tot} ($P < 0,0001$; $r^2 = 0,923$) e α ($P < 0,0001$; $r^2 = 0,886$), sugerindo que o clorofilômetro portátil pode ser uma ferramenta efetiva na estimativa relativa das concentrações de clorofilas e da fração da irradiância fotossinteticamente ativa absorvida pela folha de espécies arbóreas tropicais.
Palavras-chave: Pigmentos cloroplastídicos, conteúdo de clorofilas, radiação fotossintéticamente ativa

Introduction

Among the factors related to the photosynthetic efficiency of plants and, consequently, their growth and adaptability to environments of high luminosity, the concentration and composition of chloroplastid pigments are two factors of particular importance (Marini 1986, Thiele *et al*. 1998). Traditionally, chlorophyll contents are determined using chemical methods. These methods evolved a pigment extraction with organic solvents, spectrophotometric readings in order to obtain absorbance values of pigment extracted solution (Lichtenthaler & Wellburn 1983, Porra *et al*. 1989, Wellburn 1994) and conversion of the absorbance values to concentration using well known model equations in the literature (Hendry & Price 1993). Unfortunately, the chemical method of chlorophyll extraction is a destructive and rather painstaking process. Recently, non-destructive optical methods have been developed for measurement and estimation of chlorophyll concentrations in leaves. Optical methods express relative values of chlorophyll

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rather than absolute values per unit of area or leaf mass, however the values obtained through optical methods using portable chlorophyll meters are proportional to the concentrations of chlorophyll present in the leaves (Richardson *et al.* 2002). For this reason, some research have been done to establish the relationship between the differential transmittance of light through leaf and the concentrations of chlorophyll in several species from temperate regions (Cate & Perkins *et al.* 2003, Berg & Perkins 2004, Bìber 2007) or crops (Netto *et al.* 2002, Yamamoto *et al.* 2002). Nevertheless, there are few studies that analyze the relationship between the extractable chlorophyll content and the readings obtained with the chlorophyll meter measurements in tropical tree species. Therefore, this work aimed to evaluate the relationship between the portable chlorophyll meter readings and the chloroplastid pigments concentrations as well as the fraction of photosythetically active irradiance absorbed by the leaves (α) in tropical tree species from Amazonian forest. Four plant species (*Bombacopsis macrocalyx* (Ducke) A. Robyns, *Eugenia cumini* (L.) Druce, *Irianthera macrophyla* (Benth.) Baehni and *Senna reticulata* (Willd) Irwin & Barneby) belonging to representative families (Bombacaceae, Myrtaceae, Myristicaceae and Leguminosae-Mimosoideae respectively) from Amazonian forest and with high potential for forest management were chosen.

Material and methods

The experiment was carried out in a degraded area situated at the Base Operacional Geólogo Pedro de Moura (Petrobras-BR) in Urucu, Amazonas, Brazil, 4°53'S and 65°11'W. The species chosen for this study were *Bombacopsis macrocalyx* (Ducke) A. Robyns (Bombacaceae)*, Eugenia cumini* (L.) Druce (Myrtaceae), *Iryanthera macrophyla* (Benth.) Baehni (Myristicaceae) and *Senna reticulata* (Willd) Irwin & Barneby (Leguminosae-Mimosoideae). The readings of chlorophyll content index (CCI) in leaves were carried with a portable chlorophyll meter (Opti-Science model CCM-200) in healthily and fully expanded leaves. Five plants from each species were randomly selected. The individuals were of the same age and height. Between species, however, age varied but the individuals were of the same height at the time of planting (between 20 and 30 cm tall). In each of these individuals was measured four times in the middle of the leaves with the chlorophyll meter. The chloroplastid pigments were extracted in the same leaves used in the measurements with the portable chlorophyll meter. Leaves were collected from the middle third of the plant, wrapped in aluminum foil, placed in coolers with ice, and taken to the Fisiologia e Bioquímica de Plantas (MCT-INPA) laboratory, where the chloroplastid pigment concentrations were quantified following the method of Lichtenthaler and Wellburn (1983). For this samples with 0.1 g of leaves (fresh material) were grounded in 10 mL of 80% acetone and 0.5% (w v^{-1}) magnesium carbonate ($MgCO_3$) and immediately afterwards 10 ml of 100% acetone was added. The suspension was filtered and the absorbance was determined using a spectrophotometer (Jenway 6105 UV/VIS) in the wavelengths of 663 nm (chlorophyll *a* – Chl *a*), 645 nm (chlorophyll *b* – Chl *b*) and 480 nm (carotenoids – C_{x+c}). The chlorophyll concentrations were calculated using the equations described by Hendry & Price (1993). The fraction of photosynthetically active irradiance absorbed by the leaf (α), was depended on the chlorophyll content (μmol m^{-2}) and it was calculated as $\alpha = Chl_{tot}/(Chl_{tot} + 76)$ following Evans & Poorter (2001). The statistical arrangement was entirely randomized with four treatments (species) and 5 replications. The data obtained were subjected to an analysis of regression using the SigmaPlot 8.02 package for Windows.

Results and Discussion

The concentrations of chlorophyll *a*, *b*, and *total*, which were extracted using the chemical method, ranged from 37 to 347, 44 to 179, and 83 to 519 µmol m^{-2}, respectively, within the sample group obtained from the leaves of the four tree species. The values of α ranged from 0.523 to 0.872, while the values for the chlorophyll content index (CCI) measured with portable chlorophyll meter ranged from 2.425 to 51.5 in the samples. This range of values for the CCI was greater than those found in other studies which obtained values varying approximately between 2 and 25 units of CCI (Richardson *et al.* 2002, Berg & Perkins 2004). The relationship between the chlorophyll concentrations extracted using the chemical method with acetone and the readings using the portable chlorophyll meter were better explained by the non-linear equation $y = a + b\ (CCI)^c$ (figure 1, table 1). Moreover, the determination coefficients (r^2) among the readings from the portable chlorophyll meter and the chlorophyll concentrations and α ranged from 0.803 to 0.923 (table 1). The differences in the range of values for the CCI could have been influenced

Table 1. Regression equations obtained to describe the relationship between the average reading from the portable chlorophyll meter (CCI) and the contents of chlorophyll *a* (Chl *a*), *b* (Chl *b*), and total (Chl_{tot}) and the fraction of photosynthetically active irradiance absorbed by the leaf (α) in four tropical tree species.

Parameters	Equation: $y = a + b(CCI)^c$				
	a	b	c	r^2	*P*
Chl *a*	-421.35	375.02	0.1863	0.904	< 0.0001
Chl *b*	38.23	4.03	0.88	0.803	< 0.0001
Chl_{tot}	-283.20	269.96	0.277	0.923	< 0.0001
α	-3.50	3.96	0.027	0.886	< 0.0001

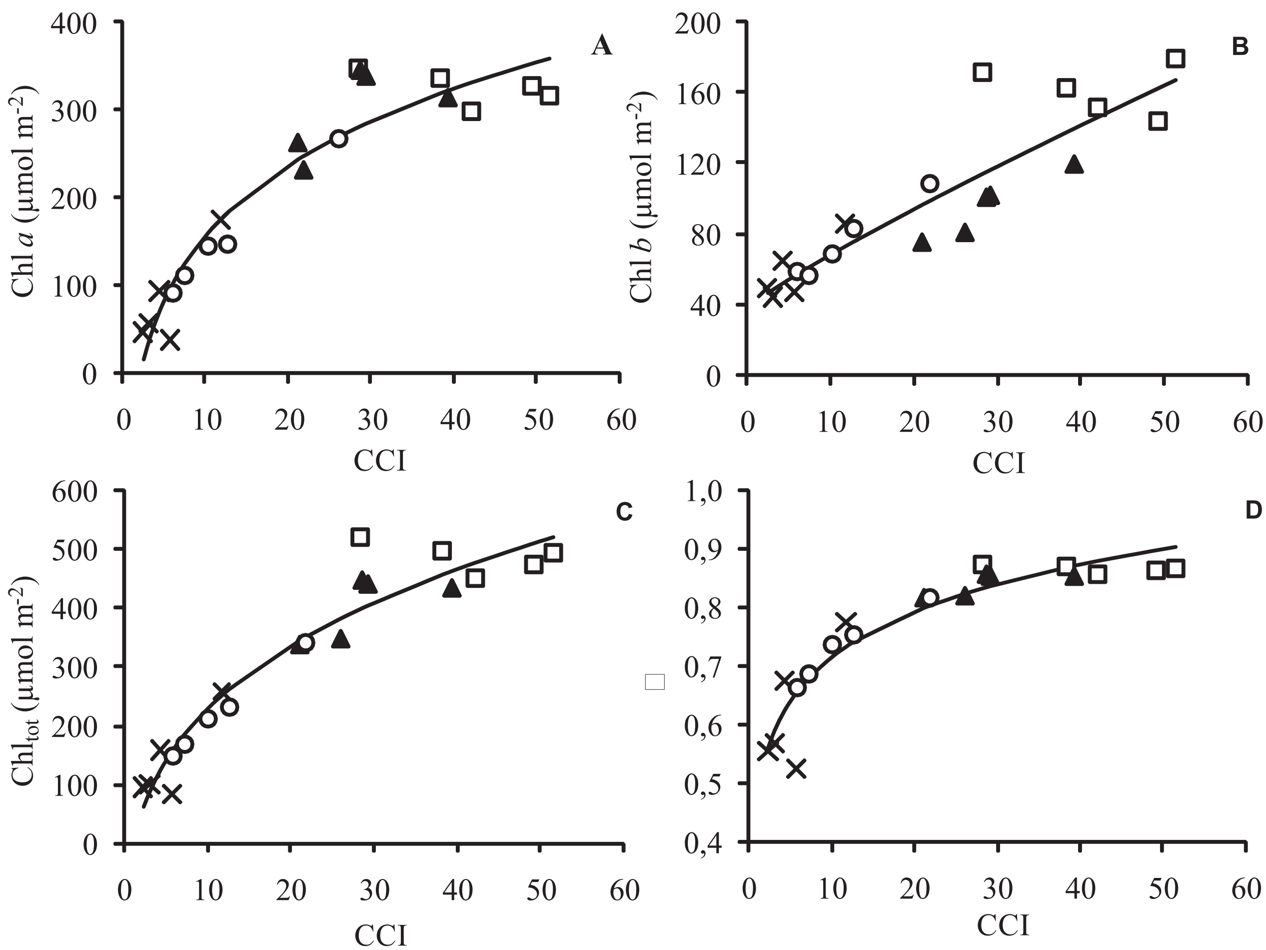

Figure 1. Relationship between chlorophyll content index (CCI) obtained from the portable chlorophyll meter and the concentrations of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl_{tot}) and the fraction of photosynthetically active irradiance absorbed by the leaf (α) in tree species of *B. macrocalyx* (✕), *E. cunini* (□), *I. macrophyla* (○) and *S. reticulate* (▲) growing in a degraded area in Central Amazonian.

by the differences in the adjustment models used by Richardson *et al*. (2002) who used a second degree polynomial, and Berg & Perkins (2004) who used a linear model.

The correlation coefficients observed between the readings from the portable chlorophyll meter and the concentrations of chlorophyll suggests that the portable chlorophyll meter can be used to determine chlorophyll concentrations in tropical tree species with appropriate adjustment equations. Portable chlorophyll meter provides a fast, convenient, and non-destructive way to estimate the concentrations of chlorophyll, making it a useful tool for the evaluation of physiological behavior as related to the capture of energy by plants in the field.

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