SPAD calibration in trees of Valencia city (Spain) and chlorophyll map of urban vegetation from hyperspectral index NAOC

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Abstract. The study of chlorophyll content in urban vegetation leaves provides important information about the plants health and ability for CO_2 absorption. Recent advances in optical remote sensing led to the development of improved methodologies for remote estimation of chlorophyll content. For instance, the hyperspectral index NAOC (Normalized Area Over reflectance Curve), proved to be a promising indicator.
In this work we studied tree chlorophyll content in the city of Valencia (Spain) based on measurement of chlorophyll taken from four representative tree species: plane tree (*Platanus x. acerifolia*), canarian palm (*Phoenix canariensis*), hackberry (*Celtis australis*) and mulberry (*Morus alba*). Measurements were made during summer 2011 within the European project BIOHYPE (BIOmonitoring of urban habitat quality with airborne HYPErspectral observation). For 320 leaf samples, chlorophyll content were measured both in the laboratory and by using a SPAD-502 chlorophyll meter. These data were correlated leading to a best fit with power type functions, with coefficients of determination R^2 above 0.86. During the field campaign an aircraft flew over the city with onboard a hyperspectral sensor CASI (Compact Airborne Spectral Imager) and obtained several images with a resolution of 1 m. From the CASI data we first calculated the NAOC index and secondly related it to the laboratory chlorophyll content measures. However, in order to adapt the index to remotely estimated chlorophyll content over all urban vegetation, extra data of low growing vegetation coming from earlier campaigns was added. It led to a high resolution chlorophyll content map over the study area of Valencia.

Keywords: Urban trees, chlorophyll, NAOC, SPAD.

1. Introduction

Chlorophyll is a natural pigment found in green plants, and its content is an important variable in assessing the plants physiological state and photosynthetic performance, the basic process for the vegetation life(Wang and Li, 2012; le Maire et al., 2008). Vegetation in urban areas, particularly trees, provides immense benefits to urban environment such as the main absorbers of CO_2. The quantification of Chl from urban vegetation allows us not only to gain insight in the plants' health status but also in their ability to regenerate the urban environment (McPherson et al., 2011).

However, traditionally the measurement of Chl is a laborious and destructive process that requires sampling and chlorophyll extraction in a particular area of the leaf by dissolving chemicals in the laboratory. Moreover, Chl concentration is known to be heterogeneously distributed across different leaves of the same plant, depending on sunlight exposure and the size and age of the leaves. And even within a single leaf the Chl concentration is not uniformly distributed. Therefore, in order to infer Chl in a particular tree or a large area of vegetation it requires the collection of large number of measurements, which cannot be performed in the laboratory for practical reasons. Alternatively, to overcome this limitation, instruments have been designed that are capable non-destructive and fast estimating of Chl, directly by measuring the leaf light absorption at certain wavelengths. These instruments, called chlorophyll meters, provide a numerical value which is related to the actual Chl through a calibration process that may depend on the vegetation type and its phenology. One of the widely used chlorophyll meters is the SPAD-502 and is used in this study.

However the study of Chl over large areas such as over a city can be quite challenging. Here, an attractive alternative is to make use of optical remotely sensed images (Gamon & Qiu, 1999). During the last decades various techniques have been developed that estimate Chl from images acquired from sensors mounted on an air- or spacecrafts (Wang & Li, 2012). These techniques are usually based on the characteristic chlorophyll spectrum with two peaks in the blue and red visible part. However, also other plants pigments absorb light in the blue region. This is why other optical Chl estimation techniques prefer to make use of bands located between 600 and 700 nm, which is the spectral region where chlorophyll is the main absorber. Thanks to the development of hyperspectral sensors, which can obtain a continuous spectrum in this region, or new superspectral sensors such as ESA's forthcoming Sentinel-2 mission, which also incorporates multiple bands in this region (Delegido et al., 2011b), new and more powerful Chl detection methods are being developed. One such example is the NAOC (Normalized Area Over reflectance Curve) index, a spectral index that was initially developed for Chl mapping over heterogeneous crops areas (Delegido et al., 2010).

This brings us to the aim of this study, which is to recalibrate the NAOC index so that it enables Chl estimation over urban trees. To do so, four most representative trees species in the Valencia city (Spain) were first sampled by using SPAD-502 measurements and through destructive Chl extraction used for calibration. The Chl estimates were subsequently linked to NAOC and then applied to an airborne image for upscaling Chl mapping over Valencia city.

2. Material and methods

2.1. Test site

The data used in this study were obtained within the BIOHYPE project (BIOmonitoring of urban habitat quality with airborne HYPErspectral observation) which has the objective of developing, testing and validating a passive biomonitoring methodology based on hyperspectral observations from an aircraft level and with an experimental field approach.

Within the BIOHYPE project there have been different field campaigns organized in order to obtain a series of measures of different biophysical parameters, in addition to their radiometric and hyperspectral imaging obtained by a sensor airborne. One of these campaigns took place in Valencia from May to October 2011. This work was carried out with data obtained during the Valencia campaign.

Valencia (39°28' N, 0°22' W, 15 m a.s.l.) is the third largest city by population in Spain, with 798,033 inhabitants (INE, 2012), and an area of 134.65 km^2. It is located on the Mediterranean coast of the Iberian Peninsula, on the banks of the Turia River. The climate is typical Mediterranean with mild, wet winters and hot dry summers, and a yearly-average temperature around 18 °C. Seasonal rainfall is minimal in summer and maximal in autumn and spring. The city is divided by a large park called Gardens of Turia which are built in the old Turia riverbed. Another large park, Viveros, is closely situated near the Gardens of Turia (Figure 1). For the whole city of Valencia 116,745 trees are counted in May 2010. Tree selection was made based on the species distribution maps provided by the City Garden Council Service, taking into account the distribution and occurrence of different species and leaf characteristics. Four dominant tree species were selected: London plane (*Platanus x. acerifolia (Aiton) Willd*), the Canary Island date palm (*Phoenix canariensis Chabaud*), European nettle tree (*Celtis australis L.*), and White mulberry (*Morus alba L.*). The city counts respectively 8.922, 3.513, 2.935 and 2.193 trees of *P. x acerifolia*, *M. alba, C. australis* and *P. canariensis*, presenting in total more than15% of all city trees.

In order to sample trees growing, ten city locations were chosen with different environmental conditions. In total 40 trees were sampled, ten for every species. Twelve trees at three of those ten locations were selected in order to take full crown measurements by crane at the low, middle and top layer of the crown. These three sites (Turia, Viveros and Glorieta) are three parks located relatively close to each other (Figure 1), but differ in area and traffic intensity surrounding them. The trees at Glorieta are surrounded by high buildings, Viveros, on the other hand, is one of the largest city parks where trees are somehow more isolated from traffic, and Turia park is the old riverbed of the river Turia and now planted with trees, forming an important recreation zone of the city.

Figure 1. Test site location. 1. Península Ibérica; 2. Valencia (with flight line); 3. a. Viveros; b. Turia; c. Glorieta.

2.2 Chlorophyll extraction

From each of the twelve trees a branch was cut at each of the three canopy levels (bottom, middle, top). These branches were immediately transferred to the laboratory in buckets filled with water. In the laboratory 5 leaves of each branch were chosen. Relative chlorophyll measurements were made with SPAD-502 (Konica Minolta Optics Inc., Japan) before samples were cut with a metal cylinder and stored in a freezer at -80° C. The samples were cut into the center of the distance between the stem and the apex of the leaf, and between the edge of the blade and median nerve. Upon chlorophyll extraction, samples were drawn from the freezer to measure the chlorophyll content by spectrophotometry in the laboratory. Each sample was ground in a porcelain mortar with 5 ml acetone and kept cold in an ice bin. After the sample was centrifuged for 5 minutes at 10000 rpm and then measurements were made with a spectrophotometer. The absorbances were measured according to Lichtenthaler and Buschmann (2001). Later, after an initial inspection of results it appeared that SPAD values were generally above 30 or 40 $\mu g*cm^{-2}$, new measures were taking from 140 leaves (35 of each tree species) to complete the solid curve for SPAD-Chl calibration. In total 320 samples were measured and used for calibration.

2.3. SPAD calibration

The SPAD instrument provides a relative value for chlorophyll content. The objective was to calibrate the SPAD with measurements from chlorophyll extraction in the laboratory. Several authors have addressed this problem and a wide variety of calibrations of SPAD for different plant species can be found in literature. Most of them provide calibrated linear, exponential or polynomial equations. Often, a linear equation was encountered especially when chlorophyll concentration ranges are narrow (Richardson et al., 2002). However, when Chl ranges are broader, the trend can be better described by an exponential or polynomial relationship. Overall, Chl values for tree species can vary between 0 and 150 $\mu g*cm^{-2}$ (Marenco et al., 2009), while Chl values for crop species vary between 0 and 70 (Uddling et al., 2007; Delegido et al., 2011a).

2.4. Remote sensing data

On 7 September 2011, a flight campaign was made by INTA (National Institute of Aerospace Technology) with the CASI sensors on board. 1500i CASI (Compact Airborne Spectral Imager) is a hyperspectral sensor in the range of 350-1200 nm that can work in several acquisition modes. CASI images were obtained during 4 flightlines over different parts of Valencia covering the 10 sample sites. For this work we used the CASI flightline that covered the three sites where measurements were conducted at three crown levels in the trees, configured with 144 spectral bands with 2.4 nm and a pixel size of 1.6 m along the flight track and 1.0 m across. This is the best configuration for the NAOC calculation because providing a high bands number in the region of interest that allows calculating the reflectance integral and also provides high spatial resolution which enables distinguish individual trees. The image was atmospherically corrected by the method of Guanter et al. (2007) and georeferenced.

During image acquisition, two ground teams conducted spectral measurements and measured various biophysical parameters. A team with a mobile crane sampled Glorieta and Viveros, while another was stationed in Turia.

2.5. Chl remote sensing estimation from NAOC

The aim of this study was to extend the application range of the hyperspectral index NAOC (Normalized Area Over reflectance Curve) for trees with high chlorophyll content. This index was initially developed for remotely detecting Chl over crop types and here it is evaluated whether this index enables remote estimation chlorophyll content over urban trees.

The NAOC index is calculated by the formula (Delegido et al., 2010):

$$NAOC = 1 - \frac{\int_a^b \rho \, d\lambda}{\rho_{max}(b - a)} \qquad (1)$$

Where ρ is the reflectance, λ the wavelength, ρ_{max} is the maximum far-red reflectance, corresponding to reflectance at the wavelength “b”, and “a” and “b” are the integration limits surrounding the Chl well centered at ~670 nm. In a previous work (Delegido et al., 2010), we obtained that this index reaches a maximum linear correlation with the chlorophyll content of leaves in a wide variety of crops, when the selected bands (a and b) are 643 and 795 nm. The method has been successfully validated against Chl data coming from a wide variety of crop types (Delegido et al., 2011a). The NAOC index tends to be more sensitive to changes in Chl than other remote sensing indices, because of the relationship is more linear than other indices that have the saturation problem at high values.

3. Results and discussion

3.1. SPAD calibration

The 320 chlorophyll values measured in the laboratory according to the method Lichtenthaler and Buschmann (2001) were plotted against the SPAD-502 recordings for all measurements in the four tree species in Figure 2. It led to a maximum correlation when using an exponential function according to:

$$Chl = 0.021752\ SPAD^{2.1129} \qquad R^2 = 0.8921 \qquad (2)$$

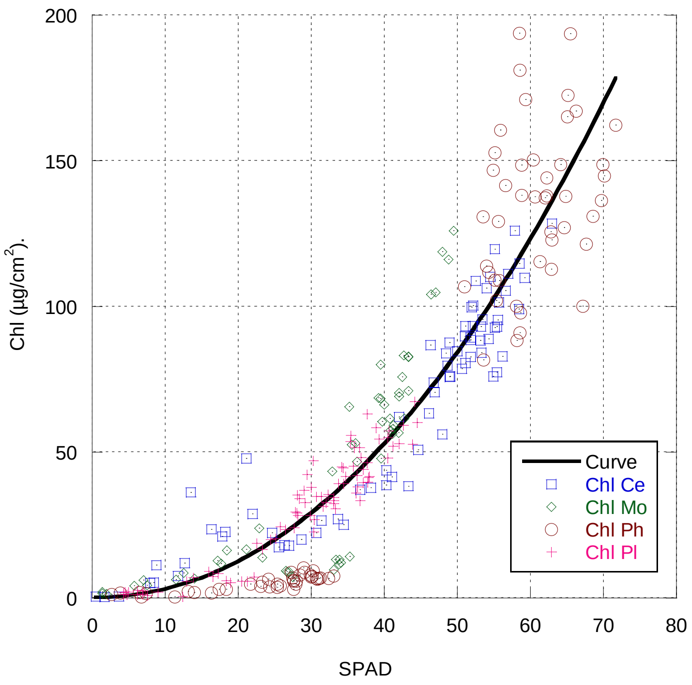


Figure 2. Total chlorophyll measured in the laboratory as function of SPAD measurements of the four species selected with the fitted curve using equation 2.

When looking closer to Figure 2, it can be observed that the curve is marked by high Chl values exclusively coming from Phoenix (*P. canariensis*) leaves. It should be noted that Phoenix species are characterized by a specific physiology with a pronounced hardness and thickness of the leaf width. Hence, because of this distinct leaf physiology, a more correct approach would be to seek for correlations for each tree species independently. It was further noted that in each of these graphs the lowest Chl values, e.g. up to 35 SPAD adjusts well to a linear relationship, which may explain why many authors have proposed a linear fit especially for narrower ranges of Chl. It can be observed that both Celtis and Morus span a similar Chl range, from 0 to 130 $\mu g*cm^{-2}$, while Platanus measurements never exceeded 70 $\mu g*cm^{-2}$. Remarkably, Phoenix Chl distribution appeared to be clustered towards two cloud points, with a first cloud for Chl <15 $\mu g*cm^{-2}$, and then a second cloud spanning values of 80 $\mu g*cm^{-2}$ to higher up until 200 $\mu g*cm^{-2}$. The Palm species behaved differently than the other species because of being an evergreen tree with steady chlorophyll content throughout the seasons. Here, low Chl values came rather from leaves that are approaching the end of its vegetative period and start to senescent.

3.2. Chlorophyll content mapping over Valencia

A following step was to calculate the NAOC index from airborne spectral data and relating it to Chl in-situ measurements for Chl mapping of over the city of Valencia. Prior to be able retrieving actual chlorophyll content the NAOC index must be calibrated. To do so, we used with the reflectance spectra of the atmospherically corrected CASI image, from which we calculated NAOC. These values were correlated with corresponding chlorophyll content measured in-situ at the 12 trees during the flight campaign (Figure 3a). For the measurement of chlorophyll from each tree, we took the arithmetic mean of all chlorophyll measures in the different sheets and calculated the standard deviation of each mean.

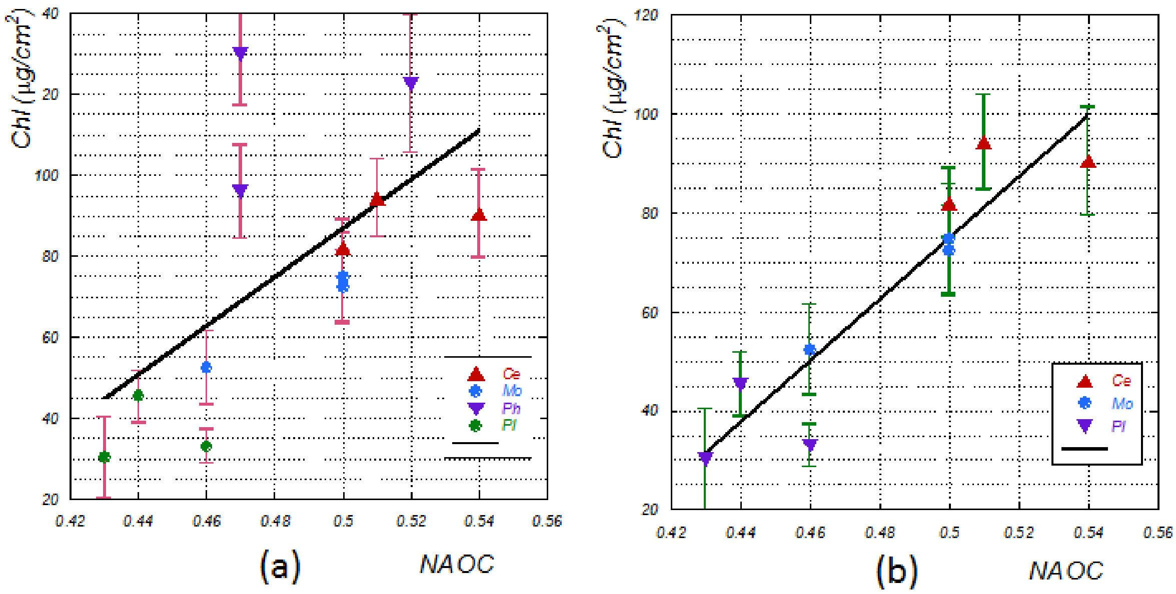


Figure 3. Chlorophyll content according NAOC. The bars indicate the standard deviation of the Chl measures. (a) All species of trees (b) Without Phoenix

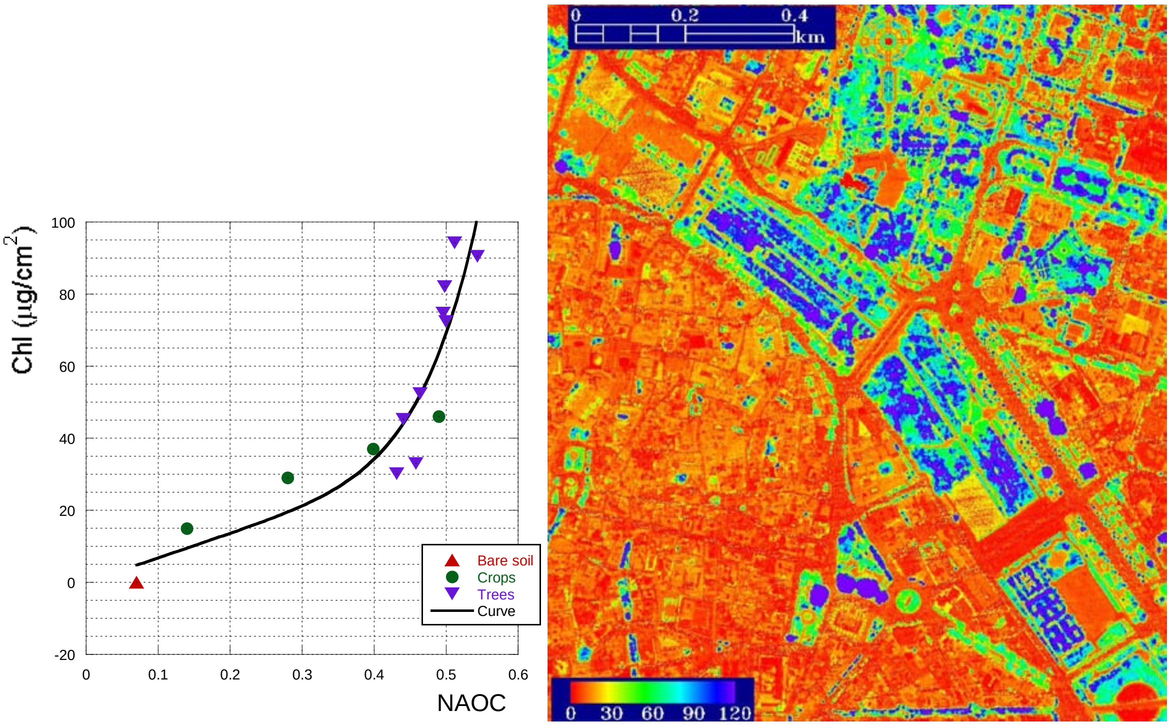
It appeared that the three canopy class values of Phoenix species deviated from the general trend. This might be due to the fact that Phoenix leaves are very narrow and sparsely organized on top of the trunk, so the fractional cover is very low for the majority of the crown area as seen from above. From a pixel point of view, this results into a mixture of tree crown and background for the pixels covering Palm trees. In addition, Phoenix leaves are much thicker than other tree leaves so underestimates the reflectance and chlorophyll content is difficult for a single spectral index present similar behavior in palm and other trees. For these reasons we have eliminated the three mean values of Palm in further processing (Fig. 3 b). The Fig. 3b data shows a linear trend that can be fitted to the following equation:

$$\text{Chl } (\mu g * cm^{-2}) = -234.76 + 619.32 * \text{NAOC} \qquad (R^2 = 0.857) \qquad (3)$$

Equation 3 would estimate the chlorophyll content for the great majority of trees in the Valencia city. However, this fit did neither include low vegetation (e.g., grass, herbs, bushes...) nor non-green targets (e.g., roads, buildings), so it would inevitably wrongly estimate chlorophyll content on those targets, e.g. when mapping Chl over a city. In order to resolve this situation, we have incorporated a calibration from previous ESA´s campaigns (SPARC, CEFLES2 and Sen3Exp, see details in Delegido et al., 2011a) over agricultural targets that show similar chlorophyll levels that those. It was found possible to find a single function relating the Chl and the NAOC will both low plant and trees, however the equation was no longer linear. Figure 4a shows the values of NAOC for all targets: trees, grass and low vegetation (similar to crops) and a non-vegetated surface (with Chl = 0). These values can be fitted according to an exponential equation:

$$\text{Chl } (\mu g{*}cm^{-2}) = 67.74{*}\text{NAOC} + 5071.3{*}\text{NAOC}^{7.169} \quad (R^2 = 0.854) \qquad (4)$$

To end with, by applying Eq. 4 on the CASI image a chlorophyll content map has been generated (Figure 4b). In the map, trees with high chlorophyll content such as Celtis (about 100 μg*cm^{-2} in purple) can be clearly distinguished from the lowest content trees (Morus and Platanus in green and blue, over 60 μg*cm^{-2}). Light green and yellow areas represent low vegetation and orange-red correspond to non-vegetation surfaces.



(a) (b)

Figure 4. (a) Chlorophyll content according NAOC from crops and low vegetation, trees and bare soil. (b) Chlorophyll content map (in μg*cm^{-2}) of test site.

4. Conclusions

The measurements with the SPAD-502 meter over different tree species show a strong correlation with Chl obtained in the laboratory. Equation 2 provides the calibrated empirical relationship as obtained by four representative species trees of in the Valencia city and many other Mediterranean climate cities. The best performing correlation was obtained by an exponential-type function, but for lower values also a linear calibration would be acceptable. Due to distinct physiological characteristics between *Phoenix canariensis* and other species (*Platanus hispanica*, *Celtis australis* and *Morus alba*), there were however clear differences observed in Chl. The leaf chlorophyll content of the palm tree was mostly sampled between 100 and 200 μg*cm^{-2} while Chl did not exceed 140 μg*cm^{-2} in the other species. The pronounced thickness of the palm leaves leads that both the SPAD measurements as the reflectance NAOC underestimated Chl. This requires a more in-depth study on these kinds of thick leaves, such as analysis on both the reflectance as well transmissivity through the leaves.

As a next step, the hyperspectral NAOC index was calculated from the CASI sensor and successfully validated against the in situ measured Chl. Again there appeared to a pronounced different behavior between the palm and other species due to its aforementioned characteristic physiology. Besides, in contrast to the other 3 tree species, the specific shape, size and distribution of palm leaves causes that the fractional cover of the palm tree is low, which implies a relatively greater influence of soil background contamination. For this reason, the palm data have been removed from the fit between Chl and NAOC data. Alternatively, by incorporating averaged data from various agricultural sites originating from earlier campaigns, a robust empirical relationship between Chl and NAOC estimates was obtained. This relationship can serve Chl mapping over low vegetation types (e.g., grass, shrubs.), bare soil as well trees. Finally, the developed method allowed us generating a Chl map over the Valencia city.

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