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RELATIONSHIPS BETWEEN PLANT OPTICAL PROPERTIES AND CHLOROPHYLL CONTENT

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Abstract: *At a time of rising global concern about environmental issues remote sensing techniques acquire increasing importance in vegetation monitoring. Multispectral optical data have proved abilities in assessment of vegetation condition and health diagnostics. The visible and near infrared spectral regions reveal significant sensitivity to plant biophysical variables and pigment content. The spectral signatures of leaves in this wavelength range are mostly defined by the composition of photosynthetic pigments and their plant-maturing or stress-induced changes. As such, the spectral response provides valuable information about vegetation physiological status. Since chlorophyll content is the most important bioindicator of plant condition being responsible for the light absorption and photosynthetic processes, techniques for its non-destructive assessment are of prime interest. In our study, multispectral data of reflected, absorbed, transmitted and emitted by plants radiation have been used to reveal the performance of different spectral features for chlorophyll estimation. Vegetation indices, red edge shift, spectral transmittance characteristics, fluorescence parameters, and chromaticity properties have been related to plant chlorophyll in order to examine the statistical significance of the spectral response changes to plant chlorophyll variations. High correlations have been found and quantitative dependences established between chlorophyll and their spectral features. Empirical relationships have been derived that allow plant condition and stress assessment (in terms of chlorophyll inhibition) to be performed by using different spectral indicators.*

ВРЪЗКА МЕЖДУ ХЛОРОФИЛНОТО СЪДЪРЖАНИЕ И ОПТИЧНИТЕ СВОЙСТВА НА РАСТЕНИЯТА

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Ключови думи: *хлорофил, спектрално отражение, вегетационни индекси, червено отместване, пропускане, флуоресценция, цветови характеристики*

Резюме: *С нарастващата загриженост в целия свят относно проблемите на околната среда дистанционните методи придобиват все по-голямо значение за диагностика и оценка на състоянието на растителната покривка. Многоспектралните оптични данни са доказали възможностите си в растителния мониторинг. Видимата и близката инфрачервена област проявява значителна чувствителност към биофизичните параметри и пигментното съдържание на растенията. В този диапазон спектралните характеристики се определят предимно от състава на фотосинтетични пигменти и техните стрес-индуцирани изменения. Затова те предоставят ценна информация за физиологичното състояние на растенията. Хлорофилът е най-важният биоиндикатор, отговорен за поглъщането на светлината и за процеса на фотосинтезата. По тази причина неразрушителните методи за неговото определяне са от първостепенно значение. В нашия експеримент са проведени многоспектрални измервания на отразената, пропусната и излъчена радиация и е изследвана връзката между оптичните свойства на растенията и хлорофилното съдържание. За целта статистически са анализирани различни спектрални признаци като вегетационни индекси, червеното отместване, цветови характеристики, параметри на флуоресценцията. Установените значими корелации с хлорофила са позволили извеждането на количествени зависимости за неговото определяне чрез използване на различни спектрални показатели.*

Introduction

Advanced monitoring and on-time alerting techniques, information extraction, modeling and forecasting technologies are a preposition for successful data application and decision support in environmental studies. The increasing ecological and economical concerns at local, regional and global scales are much relevant to vegetation land covers. The ever growing pressure on the environment imposes the necessity of efficient means for monitoring and assessing the impact of natural and anthropogenic factors on this most vital component of the biosphere. Different human activities, biotic and abiotic stresses are problems related to croplands as well as to the issue of natural vegetation resources degradation. In agriculture, proper growth and high production of crops are conditioned by a variety of environmental parameters and human-induced factors. One third of the world's agricultural plant production is destroyed by pests and diseases. The possibility for timely crop diagnostics and identification of stress situations is of particular importance for the agronomic production and management practices. Early detection of the presence of pathogens in crops allows proper forecasting and preventive control measures to be taken and makes it possible to decrease losses. Further development and extension of new technologies for the early detection and identification of plant pathogens is therefore of the utmost economic importance.

Among different methods used for plant health assessment, spreading role become to play various radiometric techniques which are the basis of Earth remote sensing The radiation behavior of vegetation land covers and their spectral response to changing conditions lie at the root of vegetation remote sensing. Visible and near infrared measurements have proved abilities in vegetation monitoring. The reason is that this wavelength range reveals significant sensitivity to plant biophysical and biochemical properties. The information is carried by vegetation spectral characteristics which depend on such parameters as chlorophyll content, biomass amount, leaf area, etc. These are growth parameters associated with plant phenological development and physiological state, and are closely related to growing conditions. As thus, the specific spectral behaviour of healthy plants and plants subjected to short-term or long-term stress impacts has the potential to be used as vegetation state indicator. Vegetation is in the focus of various remote sensing investigations, research work and experimental studies. It is a subject of a big number of recent projects and programs related to vegetation resources management and preservation. Spectral data have been successfully used for vegetation phenology monitoring [1-3], spatial distribution assessment of vegetation types [4], ecosystem forecasting [5], and evaluating year-to-year spatial-temporal variations of vegetation seasonality and their dependence on environmental factors [6, 7]. Monitoring of cropland dynamics is carried out with the main goal to track the phenological development, assess the growth process and forecast crop production. Remotely sensed multispectral data have been applied for plant growth monitoring and state assessment [8-11]. These data are particularly effective in deriving plant biophysical parameters. In recent decades, a large amount of work has been published on the derivation of vegetation biophysical parameters (e.g. of biomass, chlorophyll content, leaf area index, and others) from optical data [12-20]. Such information is valuable in yield modeling and forecast. Various data and data processing algorithms are applied to provide quantitative crop information and make yield predictions [21-22] at different scales. Crop stress detection [23-30] plays a significant role in monitoring agricultural fields during the growing season.

Much research has been is carried out on vegetation health issues. In order to use remote sensing data collected over vegetative targets for vegetation health assessment, multispectral data are analyzed to estimate green phytomass and other physiological variables related to plant growth and indicative of vegetation health status. Such key characteristic is plant chlorophyll content. Chlorophyll is a vital physiological parameter and an important bioindicator of the performance of vegetation especially when it is growing under various unfavourable environmental conditions. Chlorophyll determines the photosynthetic capacity and physiological status of plants. Chlorophyll a is the primary pigment for photosynthesis in plants, but the range of light absorption is extended by chlorophyll-b, carotenoids and other accessory pigments. Chlorophyll b exhibits a blue-green visual color and absorption peaks at 453 nm and 642 nm. It occurs in all plants and is usually about half as much as the chlorophyll a variety. Chlorosis when not the result of natural phenological development (senescence or maturing) is a basic symptom of vegetation stress. That is why the assessment of plant chlorophyll as well as other pigment indicators (carotenoids, chlorophyll a to chlorophyll b ratio, and chlorophylls to carotenoids ratio) is essential for vegetation state monitoring. Many papers have the objective to investigate the relations of plant chlorophyll with different spectral data from different sources and the possibility of using these data as chlorophyll spectral predictor [31-36]. Fluorescence occupies a special place in the study of vegetation photosynthesis system and stress-induced chlorophyll inhibition [37-29]. The goal of this paper is to study plant spectral response to changing chlorophyll content, to examine and compare its relations with different spectral features, and quantitatively describe these relationships. Multispectral data of reflected, transmitted and emitted by plants radiation have been used to reveal the performance of different spectral signatures in chlorophyll estimation.

Materials and methods

The study comprised green-house and laboratory experiments. Agricultural species were cultivated in a greenhouse under different growing conditions and controlled combination of factors. Spring barley and alfalfa were grown on two soil types differing by their organic content and acidity (neutral chernozem soil with pH=7.0-7.5 and acid grey forest soil with pH=5.0-5.5),. Different nitrogen supply rate and fertilizer type were applied as well as heavy metal (Ni, Cd) contamination of the soils in different concentrations. The type of the fertilizer effected nitrogen access to plants. Besides, the two soils exhibited different behaviour to the heavy metals increasing their mobility and uptake by plants in the case of the acid soil treatments. A second set of experiments included spring barley and peas hydroponically grown in different media (water and algae supernatant) and subjected to Cd contamination. This variety of growing conditions and their interactive effects ensured a wide range of plant performance and physiological status thus causing considerable changes in plant pigments' content. Different spectral data and data processing techniques were applied to derive various plant spectral features (vegetation indices, red edge wavelength, transmittance, fluorescence parameters, and chromaticity characteristics). Empirical relationships have been established between a variety of spectral response features and plant pigment variables.

Spectral reflectance measurements were conducted at canopy level over plants grown in soil. A multichannel radiometer in the visible and near infrared region (400-820 nm) with a 10 nm step was used. Along with spectral measurements, the content of chlorophyll a, that of chlorophyll b, total chlorophyll and carotene content were measured and the ratios of chlorophylls a/b and of total chlorophyll to carotenoids (a+b)/car were determined.

Various spectral indices were calculated exploiting the specifics of vegetation reflectance and absorbance. A common technique for multispectral data analysis is the implementation of spectral transformations called vegetation indices (VIs). They represent various combinations of the measured reflectance factors $r(\lambda)$ at two or more wavelengths λ, usually in the form of various simple $r(\lambda_i)/r(\lambda_j)$ or other ratios $[r(\lambda_i)-r(\lambda_j)]/r(\lambda_i)$, $r(\lambda_i)/[r(\lambda_i)+r(\lambda_j)+r(\lambda_k)]$, differences $r(\lambda_i)-r(\lambda_j)$, weighted sums $ar(\lambda_i)+br(\lambda_j)+cr(\lambda_k)$, and normalized differences $[r(\lambda_i)-r(\lambda_j)]/[r(\lambda_i)+r(\lambda_j)]$. The wavelengths were selected corresponding to vegetation high reflectance and absorption bands in the blue (450 nm), green (550 nm), red (670 nm) and near infrared (800-900 nm) range or were located within the red edge interval (690÷760 nm) of steep reflectance increase. By derivative analysis of the spectral reflectance curves determination of the red edge position (wavelength) was performed and related to plant chlorophyll. From spectral reflectance curves, vegetation canopy tristimulus values X, Y, Z and color coordinates x, y, z (relative X, Y, Z stimulus) were calculated according to the CIE 1964 method and for D_{65} light source. The efficiency of each colorometric variable, their ratios, and sums was examined in respect to their sensitivity to plant chlorophyll variations. The sum of the tristimulus values X+Y+Z showed highest correlation and was chosen for further regression analysis. Multispectral data of transmitted irradiance in the 540-800 nm spectral range was gathered from detached leaves of the hydroponically grown plants. These data were used to detect plant stress and discriminate between healthy and depressed plants as well as to assess plant physiological state associated with chlorophyll inhibition. Chlorophyll fluorescence is a measure of the efficiency of photosynthesis and is used, therefore, as an indicator of vegetation health and vitality. In our study, fluorescence emission spectra were also acquired from detached plant leaves. Chlorophyll red and far red fluorescence was excited by a blue light source at 470 nm. Empirical approach was used to test the performance of spectral data in response to pigment changes. Regression analysis was performed to reveal the relationships between different spectral variables and plant chlorophyll and carotenoid pigments.

Results and discussion

Since the objective of the work is to assess the capability of different spectral features to effectively distinguish plant health condition and monitor the physiological status defined by the amount of chlorophyll and carotenoid pigments, the presented here results illustrate some of the findings. Vegetation indices, red edge shift, spectral transmittance, fluorescence, and chromaticity features, were related in a statistical manner to plant chlorophyll in order to examine the statistical significance of spectral response changes to chlorophyll variations. High correlations were found permitting quantitative dependences to be established between chlorophyll in plants and their spectral properties. The assessment of plant stress (in terms of chlorophyll inhibition) from the derived empirical relationships with different spectral indicators was in good correspondence with the objective plant state.

We examined a great number of spectral indices for their correlation with plant photosynthetic pigments chlorophylls and carotenoids. Many of the VIs were significantly correlated with the concentration of chlorophyll *a* (C_a), chlorophyll *b* (C_b), total chlorophyll *a+b* (C_{a+b}), carotene (Car) as well as with the chlorophylls to carotene ratio. Some of the results for canopy level measurements of spring barley are given in Table 1 and Table 2.

Table 1. Linear correlation coefficients between spring barley Vis and chlorophyll a+b concentration at different phenological stages

Growth stage	NIR/R	NIR/G	G*NIR/R	(NIR-G)/(NIR+G)	$r_{\lambda=670}/r_{\lambda=700}$
tillering	0.92	0.93	0.84	0.92	-0/93
stem elongation	0.93	0.93	0.87	0.92	-0.94
booting	0.94	0.96	0.88	0.93	-0.94

Table 2. Linear correlation coefficients of spring barley Vis with carotene concentration and the ratio of chlorophyll a+b to carotene concentrations at stem elongation stage

VI	Car	C_{a+b}/Car	VI	Car	C_{a+b}/Car
(NIR-R)/(NIR+R)	-0.91	0.78	(G-R)/(G+R)	-0.89	0.84
NIR/R	-0.90	0.81	G/R	-0.88	0.84
(NIR-G)/(NIR+G)	-0.87	0.70	R/(G+NIR)	0.90	-0.79
NIR/G	-0.89	0.73	$R^2/r_{\lambda=620}*\ r_{\lambda=720}$	0.92	-0.82

In Figure 1 the effects of the chlorophyll content on spring barley spectral reflectance factors at 550 nm, 670 nm, and 700 nm are shown. However, more significantly correlated with plant chlorophyll are different spectral indices. Figure 2 presents the derived empirical relationship between spring barley chlorophyll *a* concentration and the ratio index $r_{\lambda=670)}/r_{\lambda=700}$. It is an illustration of the indices-based approach to chlorophyll retrieval from multispectral data. The chlorophyll content was associated with plant growth and maturing. During maturing and senescence periods the correlation of spectral indices with chlorophyll weakened to insignificant. Besides, chlorophyll exhibited significant variations relevant to growing conditions. In the active vegetative stages decreased chlorophyll was an indicator of plant depression. Stresses inhibited chlorophyll and increased carotenoid pigments.

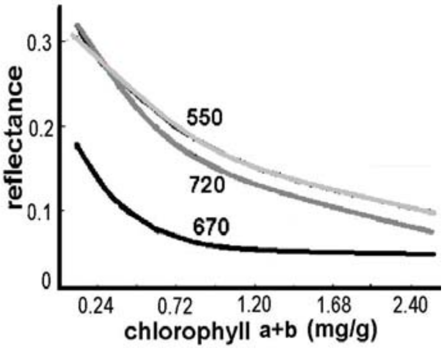


Figure 1 Effects of spring barley chlorophyll concentration on the spectral reflectance factors at 550 nm, 670 nm and 720 nm

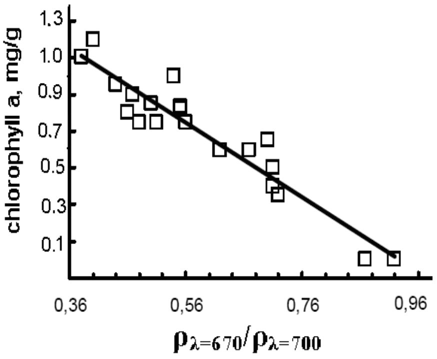


Figure 2 Relationship between spring barley chlorophyll a concentration and the ratio vegetation index $r_{(\lambda=670)}/r_{(l=700)}$

Derivative analysis was another technique used to link plant reflectance to chlorophyll content. Figure 3 shows a typical spectral curve of green vegetation. In Figure 4 the first derivative of the reflectance spectrum is plotted. Chlorophyll variations changed plant reflectance especially around the red band of the chlorophyll absorption.

The spectral response to chlorophyll inhibition was increased reflectance in the red absorption wavelength band and decreased in the red edge interval with shifts towards shorter wavelengths (Figure 5). The red edge shift was examined for deriving quantitative relationships with chlorophyll content. Through regression analysis, linear relationship R^2=0.87) between the red edge wavelength and the total chlorophyll a+b concentration in alfalfa at button stage was revealed. It is plotted in

Figure 6. The sensitivity of the red edge position to chlorophyll content proved the good potential of this spectral feature for quantitative assessment of chlorophyll and reliable stress detection in terms of chlorophyll deprivation.

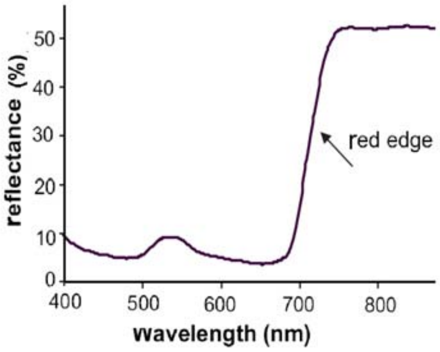


Figure 3. Reflectance of green vegetation in the visible and near infrared spectral range

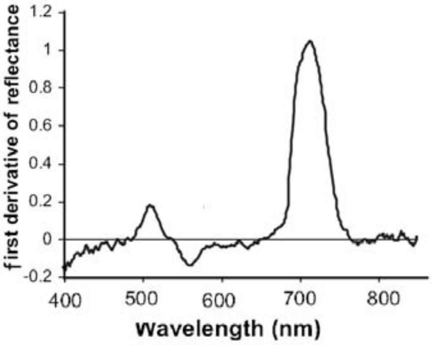


Figure 4. The first derivative of green vegetation reflectance characteristic

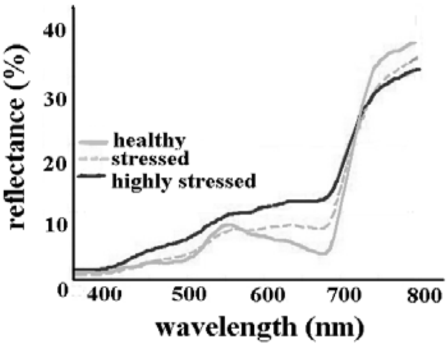


Figure 5. Spectral reflectance signatures of healthy and Cd-stressed alfalfa plants

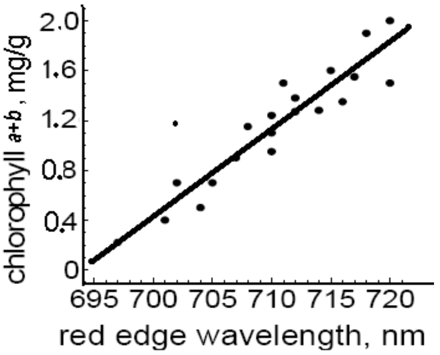


Figure 6. Linear relationship between the total chlorophyll a+b concentration in alfalfa and the red edge position (wavelength)

Vegetation canopy tristimulus values X, Y, Z and chromaticity coefficients x, y, z were calculated from the spectral reflectance curves and examined for their ability to serve as spectral indicators of plant chlorophyll variations. The tristimulus values sum X+Y+Z occurred to be most higly correlated (R^2=0.81) with plant chlorophyll content. Figure 7 presents the obtained regression fit between X+Y+Z and the total C_{a+b} in spring barley.

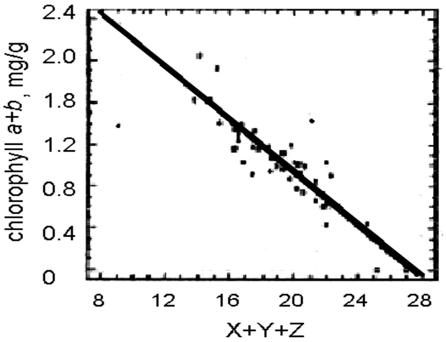


Figure 7. Linear regression between spring barley total chlorophyll *a+b* and the tristimulus values sum X+Y+Z

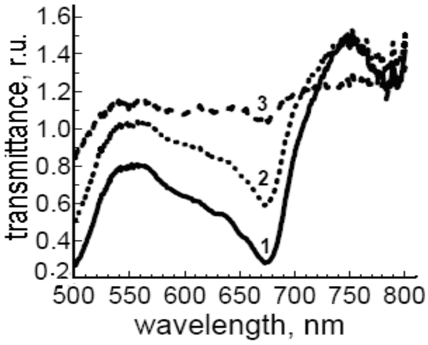


Figure 8. Transmittance of pea plants leaves with different chlorophyll content (decreasing from the control sample 3 to the stressed samples 2 and 1)

In the hydroponic experiment, leaf transmittance was measured and statistically related to plant pigment content in order to determine the presence and strength of correlations and the significance of the spectral response to variations of plant chlorophyll and carotene. Spectral transmittance in the 500-800 nm wavelength range of pea leaves with different chlorophyll content is presented in Figure 8. The spectral feature ND (λ=670 nm) was estimated from plant transmittance spectra. ND is the normalized difference between the spectrograms of the control (3) and Cd-stressed (1 and 2) plants.

Table 3. Linear correlation between the ratio of the total chlorophyll to carotenoids (Ca+b/car) in pea leaves for 14-day and 20-day old plants and ND (λ=670 nm)

Plant age	14 days	20 days
Correlation	0.91	0.94

$$ND(\lambda_i) = \frac{t_{\lambda_i}^{contr} - t_{\lambda_i}^{pollut}}{t_{\lambda_i}^{contr} + t_{\lambda_i}^{pollut}}$$

The variable ND (λ=670 nm) was relared to the pigment ratio chlorophyll *a*+*b*/carotenoids. This ratio is indicative of vegetation health condition. It decreases in case of plant depression and chlorophyll inhibition. In Table 3 the coefficients of correlation between the ratio of total chlorophyll to carotenoids in pea leaves for 14 and 20-day old plants and the transmittance feature ND (λ=670 nm) are given. The correlation is higher for the older plants because of the bigger and more distinct differences in the pigment ratio. Higher levels of carotene, on the other hand, are plant protective reaction to improve resistance to stresses. Figure 9 shows ND (λ=670 nm) values for leaves with different chlorophyll and carotenoid concentrations. In Figure 10 the obtained statistical dependence of this spectral feature on the pigment ratio (in percentage to the control non-stressed plants) is plotted. Indicators of the adequacy and accuracy of the fitted model are the high coefficient of determination (R^2=0.93) and the narrow confidence limits. The ratio of C *a*+*b* to carotenoids is an indicator of the greenness of plants. Lower values of the ratio are indicators of senescence, stress, and damage to the plant and the photosynthetic apparatus, which is expressed by faster breakdown of chlorophylls than carotenoids.

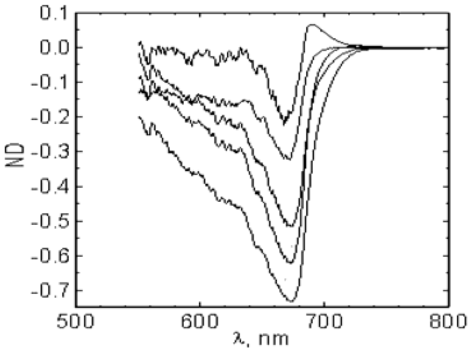


Figure 9. Transmittance $ND_{(\lambda=670\ nm)}$ values of pea leaves with different chlorophyll and carotenoid concentration

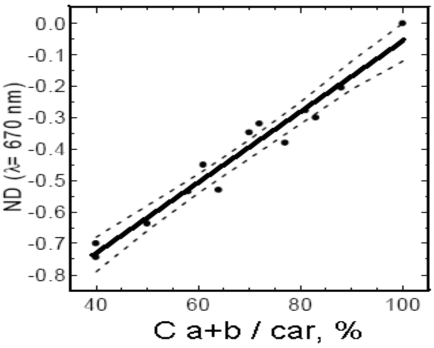
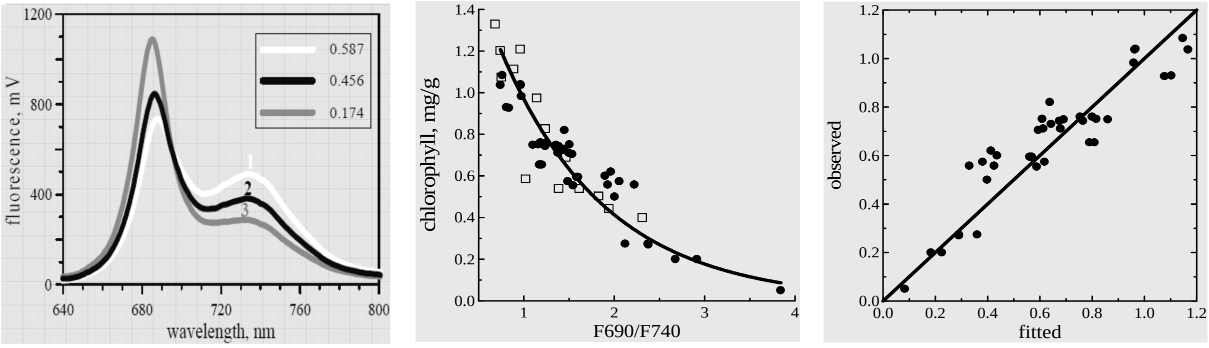


Figure 10. Dependence of $ND_{(\lambda=670\ nm)}$ values of pea leaves on the total chlorophyll *a*+*b* to carotenoid ratio relatively to the control plants

One more technique used in this study to relate plant spectral response to chlorophyll content was the analysis of plant fluorescence emission. Chlorophyll fluorescence is a measure of the efficiency of photosynthesis and can be used, therefore, as an indicator of vegetation vitality. Fluorescence spectra excited by a blue light source (470 nm) were taken from leaves of 14-day old spring barley plants grown hydroponically in two media and subjected to Cd-contamination. Each measurement record consisted of 10 consecutive spectra, automatically averaged. The fluorescence was obtained from the upper side of the leaf samples after 3 min predarkening. The objective was to evaluate the sensitivity of fluorescence parameters to chlorophyll content in order to detect and assess plant depression from fluorescence measurements. Visible light illumination excited chlorophyll fluorescence in the red and far red spectral band (675-740 nm) with peaks at about 685-690 nm and 735-740 nm. Fluorescence spectra obtained from barley leaves with different chlorophyll concentration are shown in Figure 11a.

The analysis of the fluorescence emission revealed high correlation of the red and far red fluorescence intensities with leaf chlorophyll. Fluorescence appeared to be very responsive to chlorophyll decrease at early stage of plant development before visual color or morphological signs of stress had been observed. Changes of fluorescence maxima intensities (termed as F690 and F740) as well as of the minimum emission values F710 were observed. Plants with less chlorophyll content exhibited increased red fluorescence F690 and decreased far-red intensity F740 along with decreased F710 values. With decreased chlorophyll, the red F690 peak manifested shifts towards shorter wavelengths while the position of the far-red maximum F740 remained consistent. Small wavelength shifts were observed also in the F710 emission minimum but with the opposite to F690 behaviour, i.e. to longer wavelengths. Thus, chlorophyll variations were readily traceable through fluorescent measurements.



a) b) c)

Figure 11. Red and far-red fluorescence of spring barley leaves with different chlorophyll *a* concentration (a); relationship between chlorophyll in barley leaves and fluorescence F690/F740 ratio (●), independent data (□) (b); correspondence between the observed (measured) and fitted chlorophyll values (c)

In order to reveal the most reliable spectral indicator, correlation analysis was run over fluorescence and chlorophyll data sets. In Table 4 the results of the linear correlation are given (about 50 measurements). As commented above, the chlorophyll correlation with F710 and F740 intensities was positive while the correlation with the F690 emission and the wavelength bandwidths $\Delta\lambda_1=\lambda_{F710}-\lambda_{F690}$ and $\Delta\lambda\ =\lambda_{F740}-\lambda_{F690}$ were negative. Highest correlation with chlorophyll revealed the intensity ratio of the red to far-red fluorescence F690/F740.

Table 4. Coefficients of linear correlation between chlorophyll concentration and fluorescence parameters

Fluorescence parameter	F690	F710	F740	$\lambda_{F710}-\lambda_{F690}$	$\lambda_{F740}-\lambda_{F690}$	F690/F740
Correlation coefficient	- 0.69	0.49	0.68	- 0.81	- 0.86	- 0.91

Fluorescence response F690/F740 was exponentially regressed to leaf chlorophyll. The obtained empirical dependence is presented in Figure 11b. The results were confirmed by repeated experiments proving the consistence of the established relationships under the given experiment conditions. The adequacy of the fitted model was tested and confirmed by an independent dataset from treatments repetitions. Figure 11c shows the good correspondence between the observed (measured) and fitted chlorophyll values. The predicted (modeled) chlorophyll concentrations are in good agreement with the experimental data. Compared to reflectance, chlorophyll fluorescence was a more accurate predictor of plant chlorophyll able to detect slight changes at early plant growth.

Conclusions

The presented results obtained in the study of the relationships between chlorophyll content and plant spectral features come to prove the capabilities of different spectrally-based techniques to quantitatively estimate chlorophyll and detect slight chlorophyll variations from plant spectral response. Ground-based experiments and precise measurements under controlled conditions serve as a basis for remote sensing data verification and validation of retrieval algorithms.

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