CHLOROPHYLL ASSESSMENT AND STRESS DETECTION FROM VEGETATION OPTICAL PROPERTIES
Rumiana Kancheva, Denitsa Borisova, Georgi Georgiev

Abstract. Vegetation monitoring is one of the essential applications of remote sensing. Regarding agricultural lands, a primary goal is crop state assessment throughout the growing season. Remote sensing techniques make use of multispectral data to estimate plant biophysical and biochemical characteristics by implementing quantitative relationships between crop growth variables and spectral properties. Chlorophyll is a key biochemical component that is responsible for photosynthesis and is a physiological indicator of plant condition. Changes in plant pigment content can be used to assess the impact of environmental stresses. In this paper, we present results from ground-based spectrometry studies of the spectral behaviour of agricultural species in relation to varying chlorophyll. Multispectral data were analyzed to reveal the performance of different spectral indicators for chlorophyll estimation. Reflectance factors, vegetation indices, red edge shift, transmittance spectra, chromaticity features and fluorescence parameters were related to chlorophyll content and the statistical significance of plant spectral response to chlorophyll variations was examined. High correlations were observed and empirical relationships derived linking plant optical properties and chlorophyll content. These relationships were used for plant stress diagnosis in terms of chlorophyll synthesis inhibition.

Keywords: remote sensing, plant reflectance, vegetation indices, red edge, fluorescence, chlorophyll, stress detection.

Introduction

The increasing ecological concerns at local, regional and global scales are much relevant to vegetation cover. The ever growing pressure on the environment imposes the necessity of efficient means for 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 agricultural species as well as to natural resources degradation. Proper growth and high production of crops are conditioned by a variety of environmental parameters and human-induced impacts. For instance, one third of the world's agricultural plant production is destroyed by pests and diseases. On the other hand, cropland overtreatment with fertilizers and pesticides is a serious source of chemical pollution. Timely crop diagnosis is of particular importance for the management practices and proper production forecasting. Early identification of stress situations allows preventive control measures to be taken and makes it possible to decrease losses. Further development and extension of new methods for early detection of plant stress is therefore of the utmost economic importance. Advanced monitoring and on-time alerting technologies, information extraction, modeling and forecasting techniques are preconditions for successful data application and decision support in environmental studies.

Among different tools used for plant health assessment, spreading role become to play spectroradiometric techniques which are the basis of Earth remote sensing. Plant spectral response to changing conditions lies at the root of vegetation remote sensing. Visible and near infrared measurements have proven abilities in vegetation monitoring. The reason is that this wavelength range reveals significant sensitivity to plant biophysical and biochemical variables. The information is carried by vegetation spectral characteristics which depend on biomass amount, leaf area, chlorophyll content, and etc. These are growth parameters associated with crop phenological development and physiological state, and are closely related to growing conditions. Therefore, the specific spectral behavior of healthy plants and plants subjected to short-term or long-term stress impacts has the potential to be used as a vegetation state indicator. At a time of rising global concern about environmental issues remote sensing techniques acquire increasing importance in vegetation health assessment. Natural and agricultural vegetation is in the focus of numerous investigations, research work and experimental studies as well as a subject of a big number of recent projects and programs related to vegetation resources management and preservation. Spectral data have been successfully used in phenology monitoring, ecosystem forecasting and evaluating year-to-year spatial-temporal variations of vegetation seasonality [1, 2]. Remotely sensed data is implemented to track crop development, assess its dependence on environmental factors and predict crop production at different scales [3-6]. A large amount of work has been published on the derivation of vegetation biophysical parameters from measurements of plant optical properties [7, 8]. Much research is being carried out on vegetation
health issues. Crop stress detection occupies considerable place in monitoring agricultural species during the growing season [9-13]. Various data processing algorithms are applied to provide quantitative crop information.

Chlorophyll content determines vegetation photosynthetic capacity and is a key characteristic of plant physiology. It is an important bioindicator of vegetation performance, especially under unfavorable environmental conditions. Chlorosis when not the result of natural phenological development (maturing), is a basic symptom of vegetation stress. That is why the assessment of chlorophyll and 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 relation of plant chlorophyll with multispectral data in order to use these data in chlorophyll predictive models [14-18]. The goal of our paper is to study plant spectral response to varying chlorophyll, to examine and compare the dependence of various spectral features on chlorophyll content, and to quantitatively describe the existing relations. Multispectral data collected in the visible and near infrared range at leaf and canopy level have been statistically analyzed to derive empirical relationships and reveal the performance of different spectral features for chlorophyll estimation.

Materials and Methods

The study comprised field, green-house and laboratory experiments. Agricultural species were cultivated under different growing conditions and controlled combinations of factors in order to provide a wide range of chlorophyll variation. Spring barley and alfalfa were grown on two soil types which differed by their organic content and acidity: neutral chernozem soil and acid grey forest soil. Different nitrogen supply rates and fertilizer compounds were applied effecting plant development. Peas was grown on slightly acid alluvial soil. The greenhouse trials comprised heavy metal (Ni or Cd) contamination with varying concentration. The soils exhibited different behaviour to heavy metals whose mobility and uptake by plants increased with increasing soil acidity. A second set of experiments included spring barley and peas grown hydroponically in different media (water and algae supernatant) and subjected to Cd contamination. The variety of growing conditions and their interactive effects ensured a wide range of plant performance and physiological status thus causing considerable changes in plant pigment content.

Spectral reflectance measurements were conducted at canopy level over plants grown in soil. A multichannel spectrometer recording in the wavelength band 400-820 nm with a 10 nm step and 12° field-of-view was used. The measurements were carried out at different phenological stages of plant development. Data processing included the calculation of spectral transforms which as a common technique for multispectral data analysis [3, 8, 9, 12-15]. These spectral transforms are called vegetation indices (VIs) and represent various combinations of the measured reflectance factors \( r(\lambda) \) at two or more wavelengths \( \lambda \), usually in the form of simple \( r(\lambda) / r(\lambda_i) \) or other ratios such as, for instance, \([ r(\lambda) - r(\lambda_i) ] / [ r(\lambda) + r(\lambda_i) ]\), differences \( r(\lambda) - r(\lambda_i) \), weighted sums \( a r(\lambda) + b r(\lambda_i) \), and normalized differences \([ r(\lambda) - r(\lambda_i) ] / [ r(\lambda) + r(\lambda_i) ]\). Wavelengths used for calculating various VIs depend usually on the particular goal of the research but as a whole they exploit the specifics of vegetation reflectance, transmittance and absorbance of incident light (Fig. 1a).

Pigments play dominant role in determining leaf reflectance in the visible to near infrared spectral region. To develop a technique for non-destructive estimation of pigment content, it is essential to find spectral bands where reflectance is maximally sensitive to a pigment of interest and minimally sensitive to other pigments and canopy variables (vegetation fraction, biomass, leaf area index, etc.). In our study which aimed at examining the potential of plant optical properties for chlorophyll estimation, wavelengths were selected in vegetation strong absorption bands (Fig. 1b): blue (450 nm), and red (670 nm) and high reflectance bands: green (550 nm) and near infrared (800 nm). Wavelengths located along the transition region between the red absorption and near infrared reflectance (690-800 nm) were used as well. In this interval of steep reflectance increase, the point of maximum slope on the spectral reflectance curve is the so called red edge. By derivative analysis, the red edge position (wavelength) was determined and related to plant chlorophyll content. From the measured spectral reflectance, vegetation canopy tristimulus values X, Y, Z and colour coordinates \( x, y, z \) (relative X, Y, Z stimulus) were calculated for D65 light source according to the CIE 1964 methods. The efficiency of each single colorimetric variable, their ratios and sums was examined in respect to the 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 500-800 nm spectral range were gathered from detached leaves of hydroponically grown plants. These data were used to detect plant stress associated with chlorophyll decrease. Fluorescence emission spectra from detached plant leaves were also acquired and used to discriminate between healthy and depressed plants. Red and far red fluorescence was excited by a blue light source at 470 nm. Chlorophyll fluorescence, being a measure of the efficiency of photosynthesis, was used as a spectral indicator of vegetation health and vitality in terms of chlorophyll content.

Along with spectral measurements, the content of chlorophyll a, chlorophyll b, total chlorophyll a+b and carotene content were measured, and the ratios of chlorophylls (C$_a$/C$_b$), and of the total chlorophyll to carotenoids (C$_{ab}$/Car) were determined. Empirical approach was used to test the performance of spectral data for detection and estimation of pigment changes. Correlation analysis was carried out to reveal the strength of the relationships between various spectral variables and plant physiological state defined by chlorophyll and carotenoid content. In order to quantitatively relate spectral features to chlorophyll and produce statistical models that can be applied for chlorophyll predictions, regression analysis was performed. Empirical relationships were established between plant chlorophyll concentration and various spectral response features (spectral factors, vegetation indices, red edge wavelength, colorimetric characteristics, transmittance and fluorescence parameters).

**Results and Discussion**

As far as the main 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 results presented herein illustrate some of the relevant findings. Our experimental design produced treatments with a wide range of chlorophyll content that resulted in clearly different spectral signatures. This can be seen in Figure 2a where spectral reflectance characteristics of spring barley with different total chlorophyll are shown. In general, visible reflectance (400-700 nm) increased in response to chlorophyll degradation. The reflectance amplitude at 550 nm and near 700 nm were found to be particularly sensitive to changes in chlorophyll content that occurred with foliar senescence during plant maturing or as a result of stress effects. This is illustrated by Figure 2b where canopy reflectance values at 550, 670 and 700 nm are plotted against the total chlorophyll. The reflectance around 680 nm was more sensitive to low chlorophyll content and less sensitive to moderate and high chlorophyll values. This is because reflectance of 670 nm dropped rapidly at initial chlorophyll increase up to 0.7 mg/g and then saturated quickly at higher chlorophyll concentrations.

The results of the correlation analysis of chlorophylls and carotenoids with the reflectance factors at different wavelengths in the spectral range 400-750 nm are presented in Figure 3. Reflectance at 550 nm was closely correlated to chlorophyll content for a wide range of plant greenness. There was a broad region from 550 to 620 nm and a narrow zone around 700 to 730 nm that showed highly significant (with confidence level P<0.005) correlation coefficients and, consequently, considerable sensitivity to pigments variations.

However, a single measure of reflectance at a certain wavelength is unsuitable for chlorophyll estimations because spectral reflectance is sensitive
to the impact of other factors as well, such as sensor characteristics, measurement geometry, variable irradiance, soil background reflectance properties, and etc. Measures that are less sensitive to these factors are various spectral transforms known as vegetation indices (VIs). They minimize the combined effects of the underlying soil reflectance and the canopy nonphotosynthetic materials. We developed and examined a great number of indices for their correlation with the major photosynthetic pigments of higher plants: chlorophylls and carotenoids. The formulas of some of these vegetation indices are given in Table 1. Most of the indices exploited characteristic wavelengths of vegetation reflectance spectrum in the green, red and red-edge to near infrared spectral bands where the highest correlations with chlorophyll had been observed.

Many VIs were significantly correlated with the concentration of chlorophyll a ($C_a$), chlorophyll b ($C_b$), total chlorophyll a+b ($C_{ab}$), carotene (Car) and chlorophyll to carotenoid ratio ($C_{ab}$/Car). Some of the highest correlation results for spring barley and peas are given in Table 2 and Table 3 respectively. Vegetation indices proved to be more feasible in chlorophyll content estimation than single reflectance factors. The strong correlation between VIs and plant pigments existed for all active vegetative stages before maturing.

Through regression analysis quantitative statistical relationships were established between plant pigment composition and various spectral indices. An example is presented in Figure 4 where the derived empirical dependences of spring barley VI$^{17}$ and VI$^1$ on chlorophyll a content are plotted. The regression function between the simple ratio VI$^{17}$ and chlorophyll a ($C_a=1.88-2.04 \text{ VI}^{17}$) was a better prediction model ($R^2=0.88$) because it better described chlorophyll content in the whole range of...
implementing vegetation indices for stress detection

stress bioindicator, in Figure 5b the possibility of chlorophyll-to-carotenoid ratio can be used as a response to the effect of the stress factor. While ab result of plant depression, C

20 plant spectral behaviour represented by VI contamination rates on peas carotenoid content and view of the simultaneous impact of Cd carotenoid pigments. Figure 5a shows a graphical plant depression. Unfavourable growing conditions decreased chlorophyll content was an indicator of growing conditions. In the active vegetative stages exhibited significant range of variations relevant to plant growth and maturing but chlorophyll content are more suitable for chlorophyll predictions. wavelength, such as 550 nm, 700 nm, and 720 nm absorption in 670 nm region. Therefore, VI low chlorophyll content is sufficient to saturate chlorophyll estimator. The reason is that relatively exceeded 0.6 mg/g and wasn’t considered a reliable values. It saturated when chlorophyll content increase becoming insensitive at higher chlorophyll relationship and responded to initial chlorophyll difference index VI variations. On the contrary, the normalized difference index VIi showed a non-linear relationship and responded to initial chlorophyll increase becoming insensitive at higher chlorophyll values. It saturated when chlorophyll content 0.6 mg/g and wasn’t considered a reliable chlorophyll estimator. The reason is that relatively low chlorophyll content is sufficient to saturate absorption in 670 nm region. Therefore, VI wavelength, such as 550 nm, 700 nm, and 720 nm are more suitable for chlorophyll predictions.

Chlorophyll variations were associated with plant growth and maturing but chlorophyll content exhibited significant range of variations relevant to growing conditions. In the active vegetative stages decreased chlorophyll content was an indicator of plant depression. Unfavourable growing conditions inhibited chlorophyll biosynthesis and increased carotenoid pigments. Figure 5a shows a graphical view of the simultaneous impact of Cd contamination rates on peas carotenoid content and plant spectral behaviour represented by VI30. As a result of plant depression, Cab/Car ratio decreased in response to the effect of the stress factor. While chlorophyll-to-carotenoid ratio can be used as a stress bioindicator, in Figure 5b the possibility of implementing vegetation indices for stress detection via C_ab/Car estimation from spectral models is illustrated. During maturing (senescence) periods the correlation of spectral indices with chlorophyll weakened to insignificant.

Table 1 Vegetation indices

| 1 | (r800×r670)/(r800+r670) | 8 | r670/(r800+r550) | 15 | r550×r800/r670 |
|---|----------------.|---|-----------------|---|----------------|
| 2 | (r550×r670)/(r550+r670) | 9 | r550/(r550+r670) | 16 | (r800×r550)/(r900+r550) |
| 3 | (r800×r670)/r800 | 10 | r670/(r550+r800) | 17 | r670/r700 |
| 4 | (r550×r670)/r550 | 11 | r800/(r670+r680+r590+r700+r710+r720) | 18 | r550/r670 |
| 5 | r800×(r650+r670)/(r670×r720) | 12 | r700×(r670×r720) | 19 | r550×r670/r450 |
| 6 | (r800×r730)/(r670×r730) | 13 | r800/r670 | 20 | r670/(r550×r670) |
| 7 | (r720×r670)/r720 | 14 | r800/r550 | 21 | r800/r730 |

Table 2 Linear correlation coefficients between spring barley spectral indices and chlorophyll a+b concentration at different phenological stages

<table>
<thead>
<tr>
<th>Growth stage/VI</th>
<th>1</th>
<th>2</th>
<th>6</th>
<th>8</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>19</th>
<th>20</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>3rd leaf</td>
<td>0.8</td>
<td>0.82</td>
<td>0.9</td>
<td>-0.81</td>
<td>0.92</td>
<td>0.93</td>
<td>0.84</td>
<td>0.92</td>
<td>-0.93</td>
<td>0.85</td>
<td>-0.83</td>
<td>0.9</td>
</tr>
<tr>
<td>tillering</td>
<td>0.82</td>
<td>0.85</td>
<td>0.95</td>
<td>-0.85</td>
<td>0.93</td>
<td>0.93</td>
<td>0.87</td>
<td>0.92</td>
<td>-0.94</td>
<td>0.84</td>
<td>-0.85</td>
<td>0.93</td>
</tr>
<tr>
<td>stem elongation</td>
<td>0.75</td>
<td>0/81</td>
<td>0.81</td>
<td>-0.78</td>
<td>0.94</td>
<td>0.96</td>
<td>0.88</td>
<td>0.93</td>
<td>-0.94</td>
<td>0.74</td>
<td>-0.8</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Table 3 Linear correlation coefficients of peas spectral indices with carotenoid concentration and chlorophyll a+b to carotenoid ratio

<table>
<thead>
<tr>
<th>VI</th>
<th>1</th>
<th>2</th>
<th>5</th>
<th>8</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>16</th>
<th>18</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Car</td>
<td>-0.91</td>
<td>-0.89</td>
<td>-0.89</td>
<td>0.90</td>
<td>0.92</td>
<td>-0.92</td>
<td>-0.89</td>
<td>-0.87</td>
<td>-0.88</td>
<td>0.89</td>
</tr>
<tr>
<td>C_ab/Car</td>
<td>0.78</td>
<td>0.84</td>
<td>0.81</td>
<td>-0.79</td>
<td>-0.82</td>
<td>0.81</td>
<td>0.73</td>
<td>0.70</td>
<td>0.84</td>
<td>-0.84</td>
</tr>
</tbody>
</table>

variants. As a contrast, normalized difference index VIi showed a non-linear relationship and responded to initial chlorophyll increase becoming insensitive at higher chlorophyll values. It exceeded 0.6 mg/g and wasn’t considered a reliable chlorophyll estimator. The reason is that relatively low chlorophyll content is sufficient to saturate absorption in 670 nm region. Therefore, VI wavelength, such as 550 nm, 700 nm, and 720 nm are more suitable for chlorophyll predictions. Chlorophyll variations were associated with plant growth and maturing but chlorophyll content exhibited significant range of variations relevant to growing conditions. In the active vegetative stages decreased chlorophyll content was an indicator of plant depression. Unfavourable growing conditions inhibited chlorophyll biosynthesis and increased carotenoid pigments. Figure 5a shows a graphical view of the simultaneous impact of Cd contamination rates on peas carotenoid content and plant spectral behaviour represented by VI30. As a result of plant depression, C_ab/Car ratio decreased in response to the effect of the stress factor. While chlorophyll-to-carotenoid ratio can be used as a stress bioindicator, in Figure 5b the possibility of implementing vegetation indices for stress detection via C_ab/Car estimation from spectral models is illustrated. During maturing (senescence) periods the correlation of spectral indices with chlorophyll weakened to insignificant.

Very characteristic spectral feature of vegetation is the so-called red edge (Figure 6a). It refers to the region of rapid change in reflectance in the near infrared range of the electromagnetic spectrum (see also Figure 1a). Chlorophyll contained in vegetation absorbs most of the light in the visible part of the spectrum but becomes almost transparent at wavelengths greater than 700 nm. The red edge position is the point of maximum slope in vegetation reflectance spectra that occurs in the 680-750 nm region. This phenomenon is caused by the strong chlorophyll absorption in the red spectrum and canopy scattering in the near infrared. As chlorophyll concentration increases, the absorbency in the red region also increases, resulting in lower reflectance. In addition, the absorption area increases in width. This causes the red edge to move towards longer wavelengths and its slope to become less steep. The red-edge inflection point is termed red edge and marks the boundary between the processes of chlorophyll absorption in red wavelengths and within-leaf scattering in near infrared wavelengths.
The highest peak of the first derivative reflectance shows the position of the red edge inflection point and determines the red edge wavelength $\lambda_{re}$. The traditional red-edge position extraction technique, i.e. finding the wavelength location of the first derivative $dr/d\lambda$ maximum, is shown in Fig. 6a. In our experimental data analysis, the position of the red edge ($\lambda_{re}$) was statistically linked to plant chlorophyll a, chlorophyll b and chlorophyll a+b. The red edge wavelengths were found to be very closely correlated ($R^2 > 0.82$) to plant chlorophyll pigments. The empirical relationships derived for spring barley chlorophyll a and chlorophyll a+b concentrations are plotted in Fig. 6b.

Measured chlorophyll was positively and linearly related to the red edge wavelengths $\lambda_{re}$. For chlorophyll a+b concentrations the fitted regression equation was $C_{ab} = -64.3 + 0.092 \lambda_{re}$ with coefficient of determination $R^2 = 0.85$. The correlation for chlorophyll a was even stronger ($R^2 = 0.9$). The obtained results indicate that $\lambda_{re}$ is a good estimator of chlorophyll content, even at canopy level. The red edge is a spectral measure that is less sensitive to the effects of variable canopy biophysical parameters, soil background and environmental conditions and therefore a more accurate chlorophyll estimator than...
combinations of near infrared and visible spectral bands. Chlorophyll variations significantly changed plant reflectance curves especially around chlorophyll absorption bands. We developed a spectral index $S=S_2/S_1$ (Figure 7a) that exploited two areas of vegetation reflectance spectrum referring to the red absorption depth ($S_1$) and the red edge region of abrupt reflectance increase ($S_2$). The examination of the performance of this index revealed very high correlation with plant chlorophyll content. The derived for alfalfa nonlinear regression function that described the relationship between $S$ and total chlorophyll $C_{ab}$ is presented in Figure 7b. The quadratic model for chlorophyll $a+b$ estimation $C_{ab} = 0.136S + 0.081S^2$ yielded a coefficient of determination $R^2=0.88$. Besides the strong relation to chlorophyll content, this index showed low sensitivity to soil background reflectance and this raised its predictive accuracy.

![Fig. 7 Spectral index S (a) and its relationship with chlorophyll a+b concentrations in alfalfa (b)](image)

As a whole, plant spectral response to chlorophyll decrease was increased visible reflectance. The goal of multispectral data processing is to quantitatively associate this trend with chlorophyll variations. From measured spectral reflectance characteristics, canopy tristimulus values $X$, $Y$, $Z$ and chromaticity coordinates $x$, $y$, $z$ were calculated, and examined for the ability to serve as spectral estimators of plant chlorophyll. Among single chromaticity features and their combinations the tristimulus values sum $X+Y+Z$ occurred to be most highly correlated to chlorophyll content ($R^2=0.87$ for $C_a$ and $R^2=0.85$ for $C_{ab}$ of alfalfa). Figure 8 presents the obtained regression fit between $X+Y+Z$ and total chlorophyll $C_{ab}$ in alfalfa treatments. The negative correlation indicated that reflected visible radiation (400-700 nm) decreased with higher chlorophyll content. These reflectance changes were statistically linked to chlorophyll variations by a simple linear model.

Light transmittance is an optical property which, along with reflectance and absorbance, governs energy interception and conservation in vegetation canopies. Green vegetation transmission (see Fig. 1a) is typically high in the near infrared range and low in the red range because green plants absorb visible radiation for photosynthesis and transmit near infrared, which they do not use. As far as transmittance also depends on pigment constituents, we measured and analyzed plant transmittance in...
order to determine the strength of correlations and the sensitivity of this optical feature to pigment composition changes. Transmittance spectra of detached leaves of hydroponically cultivated peas were recorded for wavelengths from 500 to 800 nm. Plant pigment content varied considerably as a result of Cd-induced stress. Figure 9 shows transmittance of peas with different chlorophyll content. Chlorophyll decreased from control treatments to stressed plants. As a spectral variable we used the normalized difference between the spectrograms of control and polluted plants at 670 nm: \[ \text{ND}\lambda=670 = \frac{(t_{\text{contr}}-t_{\text{pollut}})}{(t_{\text{contr}}+t_{\text{pollut}})} \] and statistically related it to the ratio of total chlorophyll to carotenoids. In Figure 10 the obtained dependence of \( \text{ND}\lambda=670 \) values on \( \text{C}_{\text{ab}}/\text{Car} \) ratio (in percentage to non-stressed control plants) is presented (\( R^2=0.88 \)). This ratio is very indicative of vegetation health. Lower values of the ratio are indicators of senescence, stress, and damages to the plant and the photosynthetic apparatus, which are expressed by faster breakdown of chlorophylls than carotenoids. Spectral data resembled the stress impact and clearly discriminated between plant condition. The strength of the correlation was higher for 20-day old plants in comparison to 14-day old plants due to the more pronounced stress effect and distinct differences in the pigment ratio.

One more technique used in our research for linking plant spectral response to chlorophyll variations was the analysis of fluorescence emission. Fluorescence occupies a special place in the study of vegetation photosynthesis system and stress-induced chlorophyll deprivation [18]. Chlorophyll fluorescence is a measure of the efficiency of photosynthesis and can be used, therefore, as an indicator of vegetation vitality. Fluorescence spectra in the wavelength band 640-800 nm excited by a blue light source (470 nm) were taken from detached leaves of 14-day old spring barley plants grown hydroponically in two different media and subjected to Cd-contamination. The objective was to evaluate the sensitivity of fluorescence parameters to chlorophyll content and the applicability of fluorescence measurements for detection and assessment of plant stress. The examination of fluorescence emission revealed high correlation of leaf chlorophyll with red F690 and far red F740 fluorescence intensities. Fluorescence ratio F690/F740 was regressed to leaf chlorophyll a and an exponential relationship was derived (●). The empirically fitted model is presented in Figure 10a. Besides the strong correlation (\( R^2=0.83 \)), the adequacy of the model was validated by an independent dataset (□) from treatments repetitions and thus the consistence of the established relationship under the given experimental conditions was confirmed. Figure 10b shows the correspondence between measured and estimated chlorophyll a values. Fluorescence was very responsive to chlorophyll depression already at early stages of plant development, before visual colour or morphological signs had been observed. The predicted (modelled) chlorophyll concentrations were in good agreement with the experimental (measured) data. Compared to reflectance, chlorophyll fluorescence was a more sensitive estimator of plant chlorophyll able to detect slight changes at early plant growth.

![Fig. 9 Transmittance spectra (a) of peas leaves with different chlorophyll content decreasing from control samples (1) to stressed plants (2 and 3); dependence of ND\(\lambda=670\) values on the ratio of total chlorophyll a+b to carotenoids relatively to control plants (b)](image-url)
Conclusions

From the obtained experimental results it can be concluded that damage to plants caused by stress impacts can be assessed from changes of plant optical properties through analyzing the spectral response to chlorophyll deprivation. This is possible as far as reduced foliar chlorophyll concentration is symptomatic of stress and, on the other hand, is a factor which determines plant spectral behavior. Chlorophylls exhibit absorption peaks and reflectance maxima which are used in spectral data analysis. Transmittance and fluorescence spectra also depend on plant chlorophyll content. The sensitivity of different spectral features to chlorophyll variations and the observed high correlations indicate good potential for quantitative assessment of pigment content and reliable stress detection from multispectral data acquired at leaf and canopy level. Most of the examined spectral variables proved effective in describing changes in chlorophyll content. Remote sensing techniques that estimate pigments in higher plants are a prominent tool for determining vegetation physiological state. This study can serve as a basis for nondestructive estimation of chlorophyll and carotenoid contents in plants needed to assess the physiological status of vegetation and to detect the early stages of stress. Such spectrally-based approach can also be applied to assist interpreting remotely sensed image data.

References


ОЦЕНКА НА ХЛЮРОФИЛНОТО СЪДЪРЖАНИЕ И УСТАНОВЯВАНЕ НА СТРЕС ПО ОПТИЧНИТЕ СВОЙСТВА НА РАСТИТЕЛНОСТ

Румяна Кънчева, Деница Борисова, Георги Георгиев

Резюме. Растителният мониторинг е едно от основните приложения на дистанционните изследвания. По отношение на земеделските земи основна цел е да се оценят състоянието на културите по време на вегетацияния процес. Методите за дистанционни изследвания използват многоспектрални данни за определение на растителни биофизични и биохимични характеристики чрез установяване на количествени връзки между спектралните показатели и спектралните свойства на културите. Като физиологичен показател съдържанието на хлорофил е важен биопараметър за оценка на развитието и състоянието на растенията. В настоящата работа представяме някои резултати от използване на спектрални данни за анализ на различни земеделски съдържания над различни земеделски култури. Данните са използвани за оценка на способността и точността на различни спектрални показатели за съдържанието на хлорофил. Коefициентите на отражение, вегетационни индекси, червената граница на хлорофилното поглъщане, спекти на пропускане, флуоресцентно излъчване, цветови характеристики на растенията са статистически съюзени с хлорофилното съдържание, за да бъде изследвана значимостта на измененията на спектралните характеристики като функция от пигментните изменения. Получената висока корелация е позволила извеждането на достоверни количествени съотношения между хлорофил и различни спектрални признаки. Тези зависимости са използвани за оценка на състоянието на растението и установяването на стрес от гледна точка на хлорофилното съдържание.

Ключови думи: растителност, хлорофил, стрес, спектрални характеристики, вегетационни индекси.

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