USING MULTISPECTRAL DATA TO ASSESS PLANT CONDITION UNDER CADMIUM-INDUCED STRESS

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ИЗПОЛЗВАНЕ НА МНОГОСПЕКТРАЛНИ ДАННИ ЗА ОЦЕНКА НА СТРЕСОВОТО СЪСТОЯНИЕ НА РАСТЕНИЯ ПРИ КАДМИЕВО ЗАМЪРСЯВАНЕ

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Ключови думи: *кадмий, почвено замърсяване, люцерна, грах, метал-индуциран стрес, спектрални характеристики, вегетационни индекси*

Резюме: *С нарастващата световна загриженост относно опазването на околната среда дистанционните методи придобиват все по-голямо значение за диагностика и оценка на състоянието на растителната покривка. Растителността е особено чувствителен биомаркер за повсеместното замърсяване на почвите и водите в природата. Особено актуален и труден е проблемът* на *замърсяването с тежки метали поради факта, че те не са биоразградими, остават в почвата за продължителен период от време, не се изнасят от коренообитаемия почвен слой и се съхраняват в него дълго след отстраняване на източника. Кадмият е един от най-токсичните метали в тази група. В настоящата работа представяме резултати от експеримент с люцерна и грах, отглеждани в условията на кадмиево замърсяване. Целта е да се установи влиянието на тежкия метал върху биологичния отговор на растенията, както и да се изследва връзката между стресовия фактор и измененията на спектрпалните характеристики на растенията. Анализът на тези стрес-индуцирани изменения цели приложението на спектрални признаци като стрес-индикатори за състоянието на растенията.*

Introduction

Soil contamination is a common thing these days. The issue has received special attention because of the degradation effect of various abiotic pollutants on land resources. The high toxicity, persistent nature, easy take-up by plants, mutagenic effects even at low concentration, and long biological half-life put heavy metals among the most dangerous contaminants [1, 2]. Therefore, particularly great interest has been focused on plant heavy metal-induced stress, its detection. mechanisms of action, consequences, and prevention. Soil is the primary recipient of heavy metals. Contamination is a reason for temporary or permanent decrease of the productive capacity of land. Besides the strong negative impact on natural vegetation and agricultural species which exhibits itself in lowering soil fertility, plant growth depression and yield reduction, heavy metals constitute short-term and long-term healthrisks. The hyper-accumulation and toxicity of heavy metals is now a worldwide problem leading to agriculture losses and hazardous health effects.

Among heavy metals, cadmium (Cd) is a toxic pollutant whose presence in the environment is mainly due to industrial processes and phosphate fertilizers. Cadmium is a non-essential for plant metabolism but when it is present in excessive amount it enters the roots and further translocates to the aerial tissues and exhibits varied degrees of phytotoxicity. Cadmium toxicity has been widely studied in different plant species [3-7]. The studies have demonstrated that Cd is rapidly taken up by plant roots. The high solubility of Cd makes this element an environmental concern especially because it is easily assimilated by plants and disturbs their metabolism. Most plants are sensitive to low Cd concentrations, which inhibit plant growth as a consequence of alterations in the photosynthesis rate and the uptake and distribution of macronutrients and micronutrients. Cadmium causes growth inhibition, metabolic and physiological disorders. Similarly, photosynthesis is sensitive to cadmium. Cadmium tolerance varies with different stages of development in plants.

Modern remote sensing technologies are greatly involved in various ecology-related investigations. Studies are carried out for assessing vegetation condition and response to the impact of different environmental and man-made factors. In agriculture, remotely sensed data are widely employed for crop growth monitoring and health assessment. Numerous papers have the objective of analyzing species reflective and emissive spectra in order to estimate growth variables in relation to stress detection [8-11]. The best results are often obtained where both ground and remote sensing surveys are integrated. Ground-level spectrometric studies are a precondition for the correct interpretation of remotely sensed data and a basis for verification of the information products.

Materials and Methods

This paper takes as its focus the study of species spectral response to Cd pollution. The goal is to use remote sensing techniques as a tool to detect plant stress and quantify the stress impact from spectral indicators. Ground-based experiments were performed, using alfalfa (Medicago sativa) and pea (Pisum sativum) grown under Cd contamination. Alfalfa plants were grown in green-house in soil-pots contaminated with Cd in concentrations 5, 10, 20, 30, and 40 mg per kg soil. Two soils were used with pHs of 5.5 and 7.5. Each trial was replicated 5 times. The pea treatments were grown in 4 replications over alluvial-meadow soil under Cd contamination of 0, 10, 15, 20, and 30 mg/kg. The heavy metal was introduces through $CdCl_2.2.5H_2O$. The reflectance signatures of the treatments were measured in multiple bands within the visible and near infrared wavelength range from 400 to 820 nm with a 10 nm step. The spectral measurements were carried out at canopy level and weekly intervals during plant development. Plant performance was characterized by a set of growth variables: canopy fraction cover, stem length, above-ground biomass and pigment content.

The datasets were statistically analyzed to describe the variations in plant performance as a function of the stress-impact The multispectral data acquired at different phenological stages were linked to plant parameters and Cd concentration. The differences of the spectral reflectance characterstics between the treatments were assessed through various vegetation indices. The sensitivity of the spectral response to the contamination level and plant variables was evaluated using statistical analysis tools. Spectral features were examined for the ability to detect and quantify stress-induced changes in plants. Plant phenology was taken into consideration. Along with the influence of the soil type and the hydroponic medium, the phenological stage was studied as a factor of plant spectral variability. Analysis of variance was conducted to determine the statistical significance of the differences within replications and between treatments. The analysis of variance allowed also to reveal the individual and interactive effects of the applied factors using F-tests. Correlation analysis was performed in order to determine the presence and strength of the correlation between plant parameters and spectral response as well as to reveal the the significance of their stress-induced variations depending on the level of Cd conramination. Through regression analysis, empirical relationships were derived describing plant spectral and physiological response to the stress factor and quantifying cadmium stress impact.

Results and Discussion

We report some results from a study designed to make use of plant spectral response in assessing the performance of species grown under different conditions and subjected to different levels of Cd contamination. Multispectral data obtained from ground-based spectroradiometric measurements are analyzed in terms of the ability to serve as plant stress estimators.Cd toxicity manifested itself by inhibition of plant growth and synthesis of photosynthetic pigments. Multispectral reflectance was measured and examined for the suitability to detect differences in plant condition. Statistical analysis was performed and empirical relationships were established between Cd concentration, plant growth variables and spectral response Various spectral properties proved to be indicative of plant performance and predictive estimators of the degree of the Cd-induced stress.

Various vegetation indices have been developed from the performed narrowband multispectral measurements. Combinations of spectral bands are a common tool for analyzing vegetation reflectance data and characterization of plant performance. In Table 1 some of the examined in this study vegetation indices (VIs) are presented. Many of them exploit vegetation reflectance and absorption features in the green (G), red (R) and near infrared (NIR) bands as well as in the red edge region.

Cd causes disturbances in plant vital processes with symptoms of depressed growth, root system damages, chlorosis, etc. As a consequence, there was a pronounced plant spectral response to the varying Cd concentration (Fugure 1).

Table 1. Vegetation indices

1	$(R_{800}-R_{670})/(R_{800}+R_{670})$	12	$[(R_{800}-R_{670})/(R_{800}+R_{670})+0.5]^{0.5}$
2	$(R_{550}-R_{670})/(R_{550}+R_{670})$	13	R_{800}/R_{670}
3	R_{710}/R_{670}	14	$(R_{800}-R_{550})/(R_{800}+R_{550})$
4	$(R_{800}-R_{670})/R_{800}$	15	R_{800}/R_{550}
5	$(R_{550}-R_{670})/R_{550}$	16	$(R_{550}-R_{670})/(R_{550}+R_{670})$
6	$(R_{720}-R_{700})/R_{720}$	17	R_{550}/R_{670}
7	$(R_{720}-R_{670})/R_{720}$	18	$R_{800}(R_{550}-R_{670})/(R_{550}+R_{670})$
8	$R_{670}/(R_{550}+R_{800})$	19	$R_{670}//(R_{550}-R_{670})$
9	$R_{550}/(R_{650}+R_{670})$	20	$R_{550}/(R_{670}+R_{800})$
10	$R_{670}/(R_{800}+R_{670}+R_{550})$	21	$R^2_{670}/R_{620}R_{720}$
11	$R_{800}/(R_{670}+R_{680}+R_{690}+R_{700}+R_{710}+R_{720})$	22	$(R^2_{550}+R^2_{800})^{0.5}$

As measures of spread of VIs values of a treatment (species, growing medium, Cd concentration and growth stage) the mean, average deviation, variance, standard deviation and coefficient of variation were estimated. Then the statistical significance of the differences of VIs values between treatments with different Cd level was evaluatef using t-test statistics. In Table 2 the derived t-test values for VI_5 of the alfalfa grey soil trials at button stage are presented. Dashes point out that the t-value is not statistically meaningful (at 0.05 probability). In other words, VI_5 cannot distinguish between the respective two Cd concentrations, as it is the case of the adjacent concentrations 0 and 5 mg/kg, 5 and 10 mg/kg, and 10 and 20 mg/kg. Spectrally distinguishable, however, are all the rest possible pairs. This means that VI_5 possesses class separability for these pairs and quantitative assessment of the stress factor is possible using (G-R)/G vegetation index.

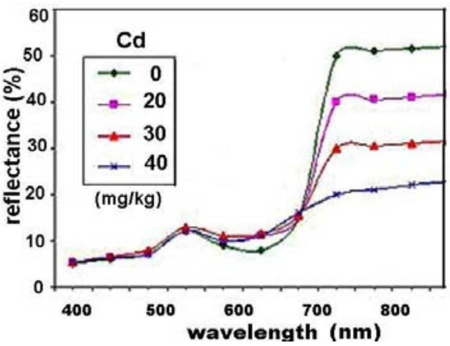


Fig. 1. Spectral reflectance signatures of alfalfa treatments on grey forest soil

Table 2. Student's t-test values for (G-R)/G vegetation index (VI_5) of alfalfa treatments on Cd contaminated (mg/kg) grey forest soil

Cd	5	10	20	30	40
0	--	2.8	7.0	8.4	10.8
5		---	3.8	5.7	8.6
10			---	2.6	5.6
20				3.0	6.7
30					3.9

This result is graphically depicted in Figure 2 by the box and whiskers diagram of VI_5 values.

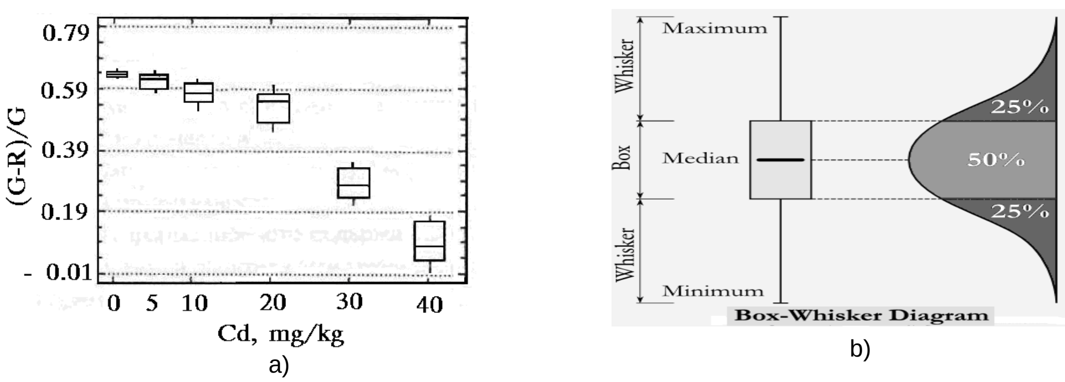
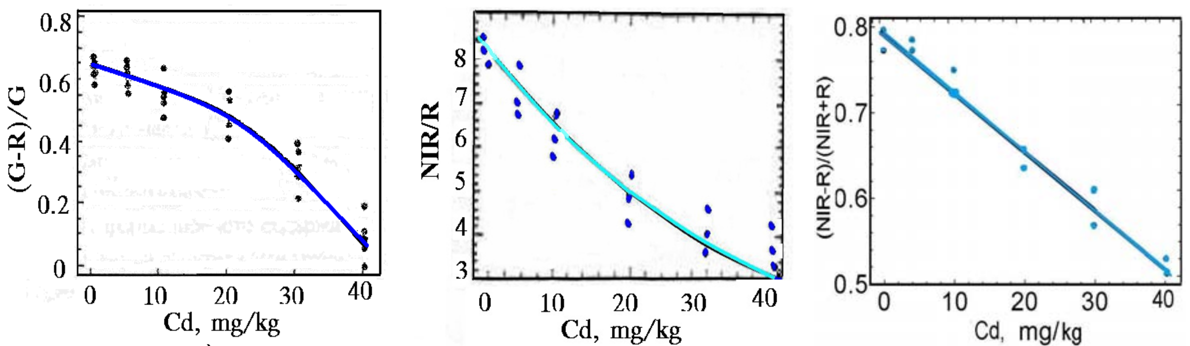


Fig. 2. Box and whiskers diagram of (G-R)/G index (VI_5) of alfalfa treatments on grey soil with Cd contamination (a); illustrative explanation of the concept of box and whiskers diagram (b)

While the t-test compares the actual difference between the means of two groups, the box and whiskers diagram displays the spread of each group and provides a visual representation of the values range (whiskers), median (indicated with a line in the group box), the highest 25 percent (quartile) and lowest 25 percent of each sample. A "box" of the plot shows the middle or "most typical" 50% of the values. Two groups on the diagram in Figure 2a appear most different in the cases where there is no or relatively little overlap between the boxes. Then it means that the impact of the respective Cd concentrations is non-chance, but systematic. The growth of the control plants was the same at the two pHs studied but plants grown in the presence of the heavy metal showed different behavior at each pHs being most depressed in the case of the acid grey forest soil.

For the most sensitive VIs, regression analysis was carried out to model the statistical relationships between Cd contamination level and plant spectral response. Many of the examined VIs of alfalfa plots revealed high correlation with the degree of Cd contamination. This correlation became stronger from the initial (real leaf) towards the later (button and flowering) phenological stages.The derived empirical relationships for two of the VIs are shown in Figure 3. It should be noted that vegetation indices (VI_6, VI_7, VI_9, VI_{11}) located in the spectral region of the red shift (670-720 nm) were as a rule most highly correlated with the Cd level.



a) b) c)

Fig. 3. Empirical relationships between Cd concentration in the grey forest soil and alfalfa vegetation indices VI_5 (a), VI_{13} (b) and VI_1 (c) at button stage

Cd contamination caused worsening of the performance of pea trials as well. The heavy metal-induced stress manifested itself in plant growth depression and resulted in reduced biophysical parameters. Cadmium inhibited chlorophyll synthesis and accelerated carotenoid cumulation. Stress-induced changes of growth variables effected in turn plant reflectance. The correlation coefficients of VIs of the pea treatments with growth variables and Cd concentration are given in Table 3.

Table 3. Correlation between vegetation indices of pea treatments, Cd concentration in the soil and plant bioparameters

VI	Height	Biomass	Cover	Caroten.	$Chl_{(a+b)}$/Carot	Cd
1	0.88	0.86	0.97	-0.91	0.78	-0.97
8	-0.86	-0.86	-0.97	0.9	-0.79	0.97
13	0.93	0.92	0.99	-0.92	0.81	-0.97
14	0.75	0.75	0.03	-0.87	0.7	-0.96
15	0.77	0.76	0.94	-0.89	0.73	-0.97
16	0.96	0.96	0.07	-0.89	0.84	-0.93
17	0.97	0.98	0.96	-0.88	0.84	-0.91
18	0.89	0.9	0.98	-0.89	0.81	-0.95
19	-0.9	-0.9	-0.96	0.89	-9.84	0.94
20	-0.71	-0.71	-0.91	0.85	-0.67	0.95
21	-0.9	-0.89	-0.98	0.92	-0.82	0.97
22	0.5	0.5	0.75	-0.64	0.52	-0.77

A consequence of the varying degree of plant depression were significant variations of canopy spectral response. Vegetation indices with reliable variations between the treatments were subjected to correlation and regression analysis in order to establish their relationship with the Cd contamination level. Fugure 4 and Figure 5 illustrate the statistically meaningful changes of plant spectral behaviour due to Cd-induced stress in pea plants. Cd effect on plant spectral behaviour became more pronounced with time as the same was observed for alfalfa plots. The strong correlation between plant biophysical and spectral response to the stress factor makes the use of spectral models a reliable approach for the assessment of plant performance.

Chlorophyll as a marker indicating plant growth status. Chlorosis is one of the primary symptoms of heavy metal toxicity.

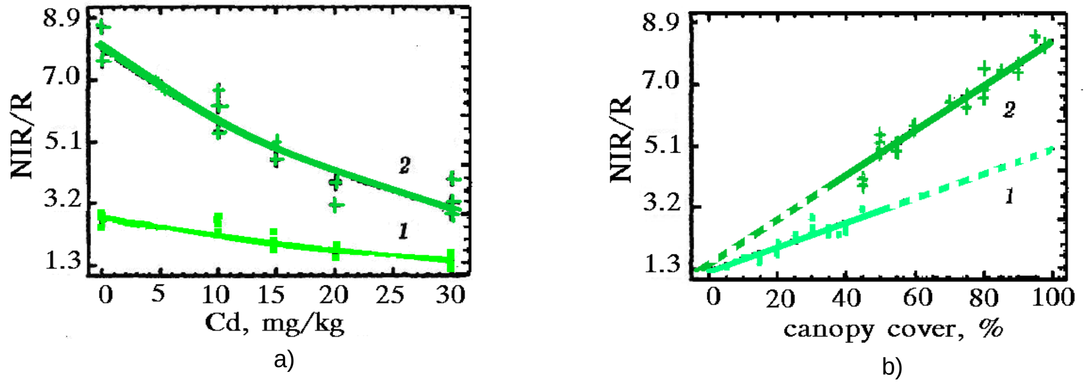


Fig. 4. Dependence of pea spectral index NIR/R (VI_{13}) on Cd concentration in the soil (a) and on the fractional canopy cover (b) at an initial (1) and later (2) growth stages

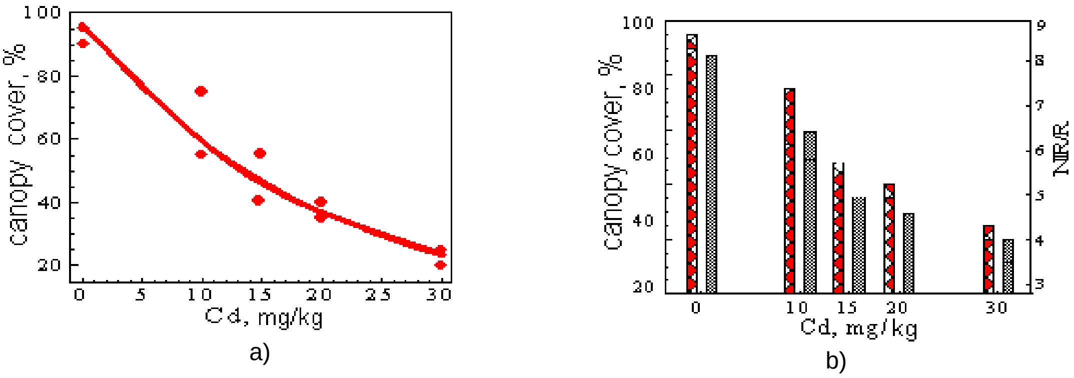


Fig. 5. Dependence of pea fractional cover on Cd concentration in the soil (a); simultanious effect of Cd contamination on NIR/R index (VI_{13}) and canopy fraction (b)

Plant chlorophyll depression when occurring at the active growth stages is a serious bioindicator of stress. Stresses inhibite chlorophyll and increase the concentrations of carotenoid pigments. We found variation in pigment content to be pollution load dependent. On the other hand, these variations led to pronounced changes of plant reflectance features. One of the most consistent spectral indicators for chlorophyll evaluation proved to be the red edge wavelength estimated through derivative analysis. The highest peak of the first derivative of reflectance shows the position of the inflection point and determines the red edge wavelength. The red edge position is the point of maximum slope in vegetation reflectance spectra that occurs in the 680-750 nm region. For all Cd-treatments the red edge wavelength was found to be very closely correlated (r >0.92) with plant chlorophylls. Red-edge spectral shifls effectively detected plant stress in terms of chlorophyll depression as it can be seen in Figure 6a. Figure 6b presents the obtained empirical dependence of the red edge wavelength of alfalfa on Cd concentration in the soil.

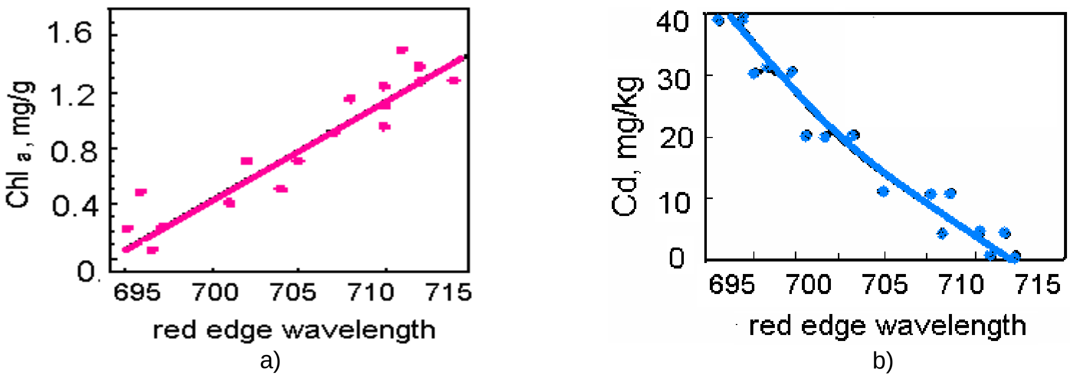


Fig. 6. Empirical relationships of the red edge position on alfalfa chlorophyll-a at button growth stage (a) and on Cd concentration in the grey forest soil (b)

Conclusions

The obtained experimental findings clearly suggeste that growing conditions cause significant variations of plant spectral response. Damage to plants caused by Cd contamination can be assessed from variations of plant reflectance properties. Various spectral indices appear to be highly correlated with Cd contamination and allow to distinguish between healthy and depressed vegetation as well as to discriminate between the stress levels. Therefore we conclude that spectral indices can be successfully used as sustainable vegetation stress indicators. Multispectral data proves applicable for detection of stress symptoms and reliable diagnosis of species.One more general conclusion that can be drawn from this study is the importance of performing multitemporal remote sensing measurements during plant growing season. Vegetation assessment and stress detection should be performed at different stages of plant development since phenology is a significant factor of plant spectral response and because of the time-dependent stress impact. Soil type also should be considered as an environmental factor of plant performance.

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