STUDY OF LASER-INDUCED FLUORESCENCE SIGNATURES FROM LEAVES OF WHEAT SEEDLINGS GROWING UNDER CADMIUM STRESS

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Summary. Steady state LIF spectra using 488 nm of cw Ar^+ laser and 355 nm of pulsed Nd:YAG laser, fluorescence induction kinetics using 488 nm laser light were studied in wheat seedlings exposed to increasing cadmium (Cd) concentrations (0.01, 0.1, 1.0 and 2.0 mM). In addition, some growth parameters and pigment content were also measured. The LIF spectra of the leaves exhibited two characteristic bands at 685 nm and 730 nm registered at 488 nm excitation wave length and four bands at around 450 nm, 520 nm, 685 nm and 735 nm at 355 nm excitation wave length. The peak parameters of the bands were calculated by Gaussian curve fitting of the LIF spectra. The result showed higher intensity of the red band (685 nm) as compared to the far-red band (730 nm) in the case of 488 nm excited spectra, however, 355 nm excited chlorophyll (Chl) fluorescence spectra exhibited a higher value in the far-red intensity. The plant vitality index (Rfd) of the plants was also calculated for both Chl fluorescence bands using the fluorescence induction kinetics curve. The decrease in plant growth parameters as well as Rfd values at 685 nm and 735 nm in response to increasing Cd concentrations could be considered as symptoms of Cd toxicity. Variations in the Chl FIR and other fluorescence ratios of intensities, bandwidth and band area were also observed. Thus, we demonstrate the use of the LIF spectra for rapid detection of Cd stress in wheat plants.

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Keywords: cadmium stress, chlorophyll content, fluorescence intensity ratio, fluorescence induction kinetics, laser-induced fluorescence, wheat seedlings.

Abbreviations*:* Cd – cadmium; Chl - chlorophyll; Chl FIR – chlorophyll fluorescence intensity ratio; FIR - fluorescence intensity ratio FWHM – full width at half intensity maximum; Fm – maximum fluorescence yield; Fd - fluorescence decay; Fs – steady state fluorescence; ICCD - intensified charge coupled device; LIF - laser induced fluorescence; PMT - photo multiplier tube; Rfd - relative fluorescence decay also called plant vitality index; UV - ultra violet; UV-A - ultra violet-A radiation between 320-400 nm.

INTRODUCTION

The study of LIF spectra in plants under different stress is suitable for the remote sensing of the near distance and has the potential application for further development into a far distance remote sensing system for monitoring the health of terrestrial vegetation crops and forests. This technique can be used for quick determination of plant stress responses even before any visible symptoms have appeared. The fluorescence emission spectrum of higher plants excited by UV light consists of four bands centred at 450 nm (blue), 520 nm (green), 685 nm (red) and 735 nm (far-red). The red and far red bands are due to the fluorescence of chlorophyll while blue and green fluorescence is emitted by cinnamic acid with ferulic acid as a main substance and other plant phenolics covalently bound to the cell walls of the epidermis and mesophyll layer of the leaves (Lichtenthaler and Schweiger, 1998; Buschmann et al., 2000). These fluorescence bands were found to change in relative intensity depending on the species studied (Johnson et al., 2000) and stress conditions (Chappelle et al., 1984; Lichtenthaler et al., 1991; Stober et al., 1994; Lang et al. 1991). Stress can also shift the peak position of the different bands (Subhash and Mohanan 1997; Gopal et al. 2002). Many groups have done extensive research and suggested that Chl FIR as well as FIRs resulting from blue, green, red and far-red bands can give fruitful information about the state of plant health. Moreover, the possible application of FIRs in the remote sensing of vegetation is limited by the lack of information regarding the physiological meaning of FIRs with respect to stresses.

Photosynthetic quantum conversion is affected by exposure to a range of biotic and abiotic stresses. Heavy metals enter into the ecosystem through drainage water, river canal system carrying industrial effluents and air. Plants absorb and accumulate heavy metals in leaves and impede various physiological activities (Bazzaz and Govindjee, 1974; Carlson et al., 1975). Among the heavy metals, Cd is a highly toxic element and its ions can readily be translocated into the leaves of several crop species, such as soybean and wheat (Haghiri, 1973). It has been shown that Cd decreases

CO_2 assimilation (Krupa and Baszynski, 1995), can generate oxidative stress (Schutzendubel et al., 2001) and leads to wilting (Barcelo and Poschenrieder, 1990). The primary mechanisms of Cd toxicity include altered catalytic function of enzymes, damaging of cell membranes and inhibition of root growth (Kastori et al., 1992). Toxic concentrations of heavy metals in the environment affect the physiology of the plants at several levels and the photosynthetic machinery is one of the major targets. Detection of Cd stress in wheat plants which could be useful for precise farming practices in future remote sensing of crops via ground operated or airborn lidar system was the main object of the present study.

MATERIALS AND METHODS

Plant material and growth conditions

Healthy and uniform in size wheat seeds (*Triticum aestivum* v- RR.21) were disinfected and soaked in sterilized double distilled water over the double layers of filter paper for two days. Besides, 25 germinating seedlings of the same size were selected and transferred carefully into Petri plates, each containing an equal amount of 0.2 strength modified Rorison medium for control plants and different concentrations of Cd (0.01, 0.1, 1.0 and 2.0 mM) applied as $CdCl_2.2H_2O$ for treated plants. The composition of the nutrient solution was as follows (in mM): 0.4 $Ca(NO_3)_2$, 0.2 $MgSO_4$, 0.2 KH_2PO_4; (in mM): 0.1 $CuSO_4.5H_2O$, 0.2 $ZnSO_4.7H_2O$, 9.2 H_3BO_3, 1.8 $MnCl_2.4H_2O$, 0.2 $NaMoO_4.2H_2O$ and 10 FeEDTA. The pH of the nutrient solution was adjusted to 6.5. The plants were grown in a growth chamber at a photon flux density of 175 μmol m^{-2} s^{-1}, at room temperature under a12/12 h dark/light photoperiod. After five days intact leaves were harvested and used for LIF study and determination of Chl *a, b* and carotenoids.

Experimental setup for blue laser light excitation studies

The laser spectrofluorimeter used in the present study was a computer controlled Acton 0.5 m monochromator with a data acquisition system. A cw Ar^+ laser (Spectra Physics, USA Model 2016) operating at 488 nm (2 mW) was used for exposing the intact leaves with the help of the beam expander. The laser beam fell on the intact leaves by reflecting the beam using two front surface polished mirrors and passing through a beam expander. The mirror and the beam expander were aligned to obtain about 2.0 cm^2 expanded laser beam at the surface of the leaves. The fluorescence light was collected using a convex lens on the slit of the monochromator having resolution 0.03 nm and reciprocal linear dispersion of 1.1 nm mm^{-1}, with R928 PMT detector. The steady-state spectra were recorded in the spectral region 650 – 800 nm. The PMT signals were sent to the computer and the data collected were analysed

using Grams-32 (Galactic) software. The laser light intensity was measured by a power meter (Spectra-Physic). The experimental set-up used to study fluorescence parameters is presented in Fig. 1. Each spectrum presented in the paper with 488 nm excitation light is an average of fifteen different spectra.

Experimental setup for UV-A laser light excitation studies

Third harmonics (355 nm) of Nd:YAG laser (Spectra Physics - Quanta Ray), operating at 10 Hz with a pulse width of 10 ns and pulse energy of 2.5 mJ was used for the excitation of plant leaves. The fluorescence radiation was collected using a convex lens on the slit of a computer-controlled Spex 0.32 m monochromator with 600 grs/mm grating having resolution of 0.12 nm and linear reciprocal dispersion 5.28 nm mm^{-1}, fitted and Spex TE cooled ICCD detector. The steady state LIF spectra were recorded in the region 418-766 nm. The ICCD signals were collected on the computer using SpectraMax software and the data were analysed using Grams-32 (Galactic) software. The detector took "snap shots" and SpectraMax were used to combine them into a single spectrum or present them separately. Multiple spectra were recorded for control and Cd-treated leaves with 20 accumulations in three replicates. The multiple spectra were merged into a single data file for each measurement.

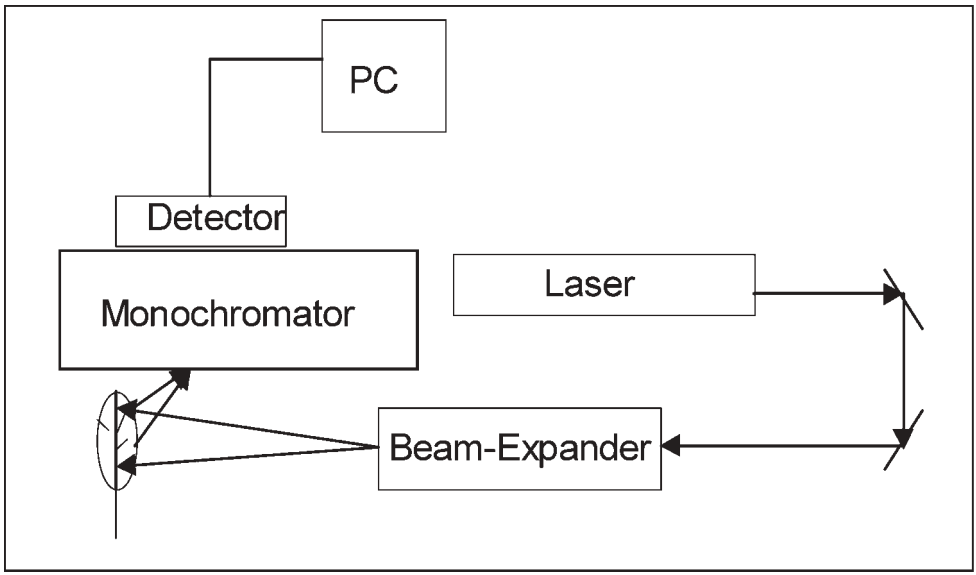

Fig. 1. Experimental scheme for investigation of LIF spectra. The set-up consists of a laser as an excitation source, a beam expander, a computer-controlled monocromator and a detector system (details are given in Materials and Methods).

Experimental setup for fluorescence induction kinetics (Kautsky effect)

The fluorescence induction kinetics of pre-darkened leaves was recorded on an Acton 0.5 m monochromator with the intensity vs time scan mode of the RD CARD software exposed with 488 nm of Ar^+ laser (82 μmol m^{-2} s^{-1} PFD). The plants were dark adapted for 20 min and were placed in front of the entrance slit of the monochromator to record the kinetics at 685 nm and 735 nm for 5 min.

Measurements of LIF spectra of intact leaves and their curve-fitting

Intact leaves were dark adapted for 20 min and stacked in a black wood cardboard. The geometry was set up in such a way that the fluorescence was excited at an angle of 60° and sensed at an angle of 30° to the leaf surface. Fluorescence radiation emitted by the leaf was collected at the entrance slit of a monochromator using a convex lens and fluorescence induction kinetics were measured for 5 min using 488 nm of Ar+ laser followed by steady-state fluorescence spectra using 488 nm and 355 nm laser lights. The background signals of the spectra were also recorded for each sample and subtracted from the respective data file. The curve fitting of the recorded spectra was done using Grams-32 software with the Curve-fit.AB program. The peak parameters, such as exact peak position, intensity, bandwidth and band area were determined. The Curve-fit is based on the original algorithm of nonlinear peak fitting as described by Marquardt and also known as the Levenberg-Marquardt method. Gaussian spectral function for the curve fitting provides the reasonable matching of the spectral data with good F-statistics, standard errors for peak amplitude, peak centre and bandwidth or FWHM (Subhash and Mohanan, 1997). The intensities of the fluorescence bands were corrected in light of response curves of the used grating and detector and normalised fluorescence ratios were calculated.

Determination of pigments

Chlorophyll and carotenoids were extracted in 80% acetone. Concentrations of total Chl, Chl *a* and Chl *b* were determined using a spectrophotometer (model 108, Systronic, India) according to the method of Arnon (1949), however, the true values for Chl *a, b* and total Chl were calculated by applying the correction equation given by Porra (2002). The level of total carotenoids was determined using the extinction co-efficient of E_{473} = 2500 absorbance units as an average value (Goodwin, 1954).

RESULTS AND DISCUSSION

Pigment content and pigment ratio

An inhibition of the growth of wheat seedlings was observed as a result of exposure to Cd for 5 days. Chl *a, b* and total Chl content decreased with increasing Cd concentration while carotenoids content was minimum at 0.01 mM Cd and it increased with increasing Cd concentration (Table 1). Our results showed also that Cd inhibited shoot length. The fresh weight of the leaves was slightly stimulated at 0.01 mM Cd while higher Cd concentrations inhibited strongly the fresh weight of the seedlings. Larsson et al. (1998) and Baryla et al. (2001) found that even 5 mM Cd reduced growth, Chl content, photochemical quantum yield and led to stomatal closure in *Brassica napus*. The Chl *a* /*b* ratio increased by 8.1% at 0.01 mM Cd followed by a decrease with increasing Cd concentration (Fig. 2B). A rapid decrease in the total Chl/carotenoids ratio with increasing Cd concentration was also observed. Babani and Lichtenthaler (1996) reported that total Chl/carotenoids ratio increased with greening of barley seedlings. Bazzaz et al. (1974) mentioned that cadmium caused degradation in Chl and carotenoids, inhibited their biosynthesis, and induced oxidative stress by disturbing the chloroplast. Carotenoids are known as antioxidants that reduce oxidative stress by acting as scavengers of reactive oxygen species (Stratton and Liebler, 1997). Therefore, the increasing content of carotenoids in Cd-treated wheat seedlings could reflect the overall level of antioxidant defence against stress. Han et al. (2005) reported an increase in carotenoids content in some lower plants under oxidative stress.

Table 1. Content of photosynthetic pigments and growth parameters measured in control and Cd-treated wheat seedlings.

Cd concentration(mM)	Chl *a* (mg g^{-1} FW)	Chl *b* (mg g^{-1} FW)	Total Chl (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)	Shoot length (cm)	Fresh weight (mg/shoot)
0.00	0.76 ± 0.01	0.44 ± 0.02	1.20 ± 0.04	0.18 ± 0.01	10.4 ± 0.6	163 ± 1
0.01	0.71 ± 0.03	0.38 ± 0.01	1.09 ± 0.03	0.17 ± 0.02	9.8 ± 0.6	167 ± 3
(-6.6)	(-13.6)	(-9.2)	(-5.6)	(-5.8)	(2.5)	
0.10	0.64 ± 0.02	0.43 ± 0.02	1.07 ± 0.04	0.25 ± 0.01	6.9 ± 0.6	124 ± 2
(-15.8)	(-2.3)	(-10.8)	(38.9)	(-33.7)	(-23.9)	
1.00	0.47 ± 0.01	0.38 ± 0.03	0.85 ± 0.02	0.27 ± 0.02	6.4 ± 0.2	112 ± 3
(-38.2)	(-13.6)	(-29.2)	(50.0)	(-38.5)	(-31.3)	
2.00	0.39 ± 0.02	0.30 ± 0.01	0.69 ± 0.02	0.29 ± 0.01	4.2 ± 0.1	48 ± 1
(-48.7)	(-32.8)	(-42.5)	(61.1)	(-59.6)	(-70.6)	

Mean (n=3) ± SD of triplicate.
*Values in parentheses represent the percent increase or decrease compared to the control.

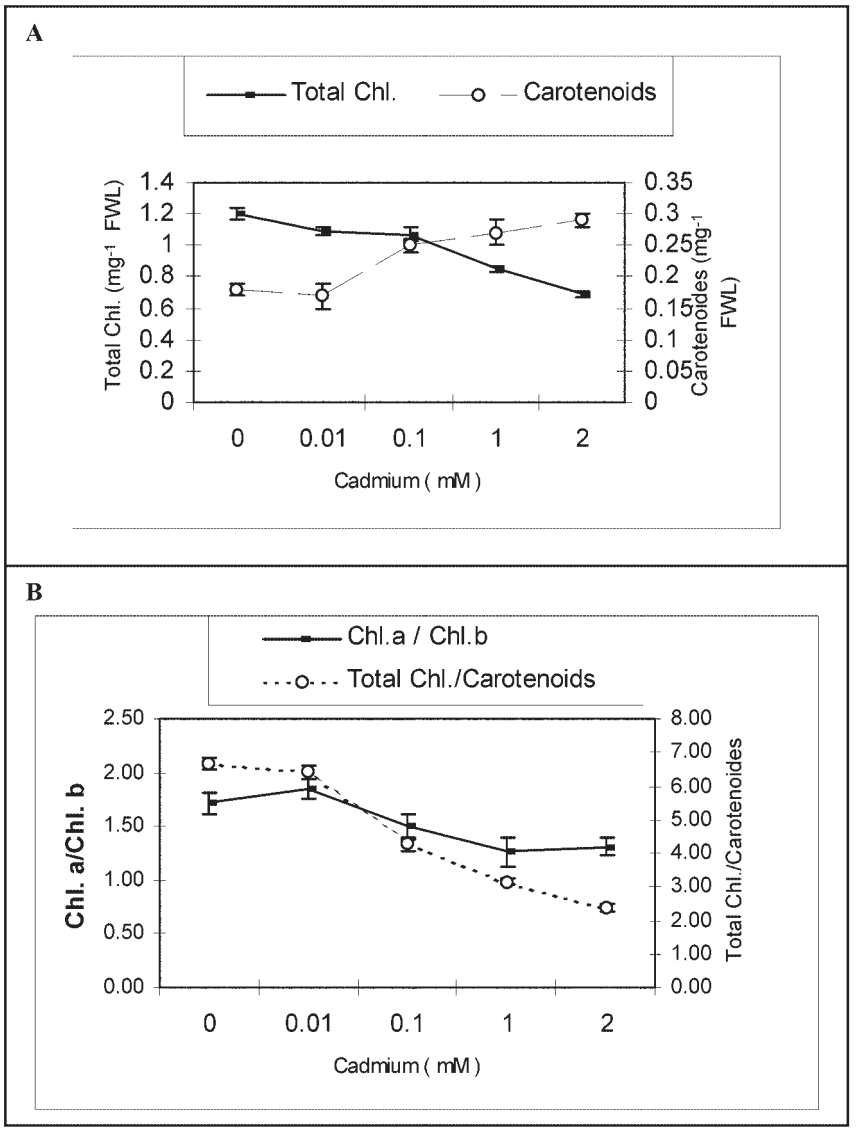

Fig. 2. Variations in (A) total chlorophyll and carotenoids content, (B) Chl *a*/Chl *b* and total Chl/carotenoids ratios in Cd-treated wheat plants. Points represent the mean value (n=3) and bars represent the standard error (SE).

LIF spectra from intact leaves of the seedlings

The curve-fitted steady state level LIF spectra excited by 488 nm (blue) and 355 nm (UV-A) laser light are presented in Fig. 3 and Fig. 4, respectively. The sum of squared deviation (c^2) and correlation coefficient (R^2) of the fitted LIF spectra are presented

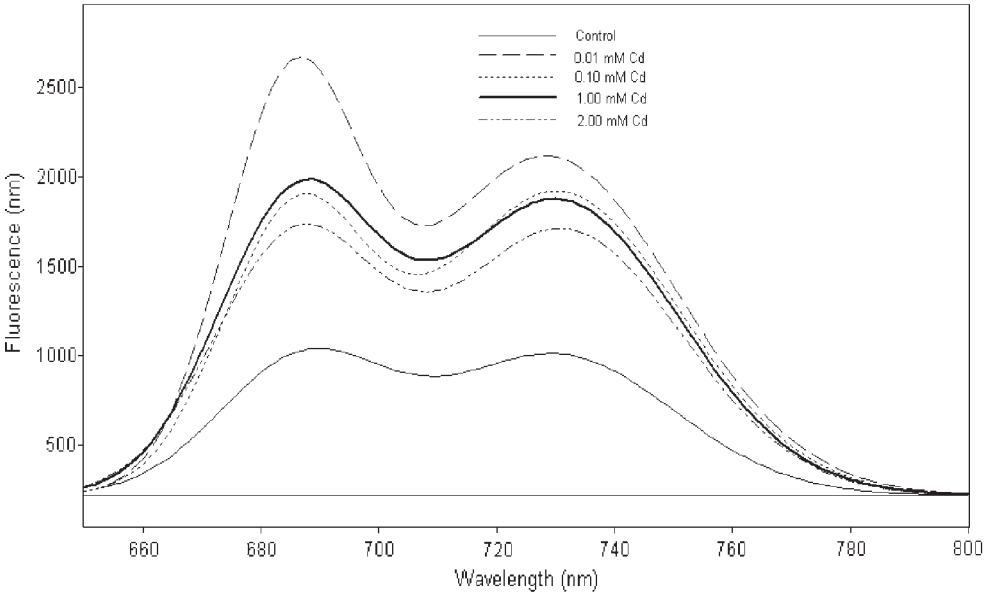

Fig. 3. Gaussian curve fitted steady state LIF spectra of control and Cd treated wheat plants excited by 488 nm of Ar^+ laser. Each spectrum in the figure is the average of fifteen spectra.

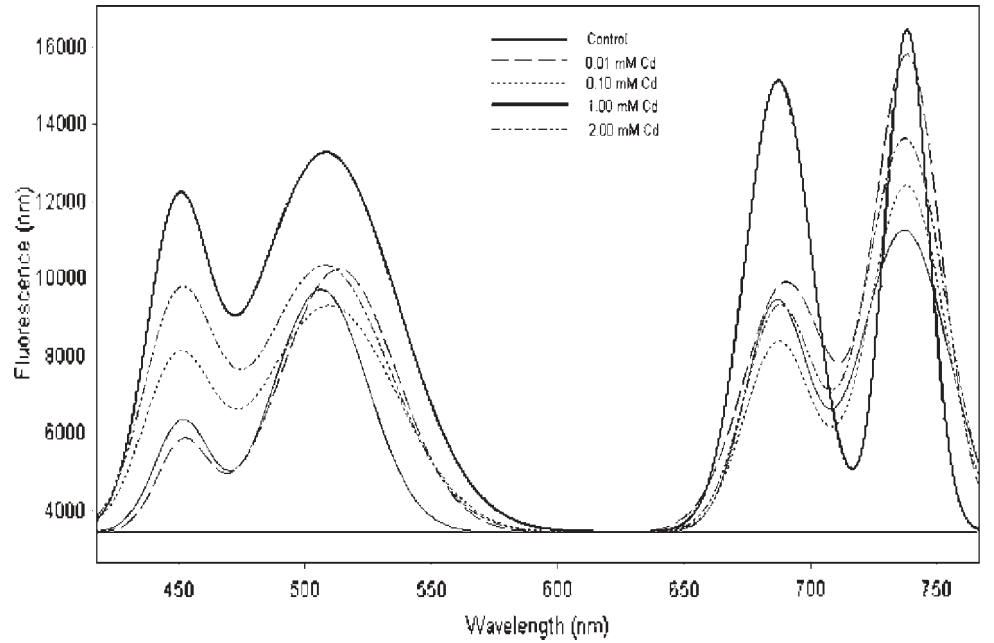

Fig. 4. Gaussian curve fitted steady state LIF spectra of control and Cd treated wheat plants excited by 355 nm of Nd:YAG laser. The fluorescence signal was collected using an ICCD detector system by choosing seven wavelength regions with twenty accumulations for each region with three replicates. The multiple spectra were merged into a single data file within the region 418-766 nm.

Table 2. Curve - fitting parameters reduced chi² (χ^2) and correlation (R^2) of the fitted LIF spectra of control and Cd-treated wheat seedlings at 488 nm and 355 nm excitation wave lengths. These parameters show the quality of fitting of the measured LIF spectra.

Cd concentration (mM)	488 nm excitation		355 nm excitation	
	Reduced Chi² (χ^2)	Correlation (R^2)	Reduced Chi² (χ^2)	Correlation (R^2)
0.00	4.1	0.991	5.92	0.972
0.01	3.6	0.992	5.27	0.977
0.10	3.7	0.991	1.36	0.973
1.00	3.0	0.989	1.71	0.978
2.00	4.5	0.989	2.32	0.976

Table 3. Peak positions of the fluorescence bands of the curve fitted spectra of control and Cd-treated wheat seedlings. The measured LIF spectra were averaged and curve-fitted using GRAMS-32 software and peak positions of the existing bands were determined.

Cd concentration (mM)	488 nm excitation			355 nm excitation		
	Red band Peak (nm)	Far-red band Peak (nm)	blue band Peak (nm)	Green band Peak (nm)	Red band Peak (nm)	Far –red Peak (nm)
0.00	685.9	728.5	453.4	511.8	686.5	737.7
0.01	685.0	728.5	452.0	513.7	690.2	738.3
0.10	685.8	730.2	449.7	510.0	687.2	737.8
1.00	685.6	730.2	449.5	508.6	687.2	738.0
2.00	685.5	731.2	450.2	508.1	687.6	737.3

in Table 2. The peak positions of the LIF spectra of control leaves at 488 nm excitation were centred at 685.9 nm and 728.5 nm while 355 nm excited spectra consisted of four bands at around 453.4 nm, 511.8 nm, 686.5 nm and 737.7 nm (Table 3). It is evident from the results that red and far-red bands showed minimal shifting in the peak position while blue and green bands showed quite significant shifting upon Cd treatment. The shifting of the blue and green bands could be due to the interaction of Cd with various components of the cell walls, such as cinnamic acid, ferulic acid, quercitin, berberin, etc. (Lang et al., 1991).

The LIF spectra of the Cd-treated wheat seedlings showed increased values of fluorescence intensity of the red and far red bands as compared to the spectra of control seedlings in the case of 488 nm excited spectra. The increase in the intensity of the spectra could be correlated with the decrease in chlorophyll content and reduction of photosynthetic activity as observed by Rinderle and Lichtenthaler (1988) in DCMU treated *Phaseolus vulgaris* plants. The lower intensities obtained for 0.1 mM, 1.0 mM and 2.0 mM Cd-treated leaves compared to of 0.01 mM Cd-treated plants were probably due to the interaction of Cd with the reaction centres of the two photosystems. The higher concentrations of Cd (0.1, 1.0 and 2.0 mM) may induce alter-

ations in the thylakoid membranes and inhibit photochemical electron transport involving both reaction centers PS I and PS II. Hence, reduction in the intensity as well as in the general growth of the plants is obvious. At 355 nm excitation wave length, the radiation is absorbed by flavonol and cinnamic acid in the epidermal and mesophyll layers which fluoresce blue (450 nm) and green (520 nm) light. At 488 nm excitation, fluorescence comes directly from the outer mesophyll layers because of the strong absorption of the blue light by chlorophyll and carotenoids. Thus, a higher chlorophyll fluorescence yield was obtained. The difference in the intensity distribution pattern of the red and far-red bands of the spectra with blue and UV-A laser light excitation could be attributed to a change in the light penetration through the leaf on different energy transfer processes among pigments (Bornman and Vogelmann, 1988; Cen and Bornman, 1993). The intensity of the blue band decreased by 20.5% whereas the intensity of the green bands increased by 8.6% in 0.01 mM Cd-treated seedlings. The fluorescence intensity of the blue band increased with increasing Cd concentration. The intensity of the green band decreased after 0.1 mM Cd treatment while the intensity increased after 1.0 mM and 2.0 mM Cd treatment. Treatment with Cd may cause alterations in the amount of blue-green fluorescing substances in the cell walls resulting in an increase or decrease in blue green fluorescence intensity of the bands. The increase of blue-green fluorescence emission in Cd-treated *Phaseolus vulgaris* plants has been interpreted to be a result of the synthesis of secondary metabolites, such as ferulic and cumaric acids (Valcke et al., 1999). The accumulation of many metabolites in response to various stresses has been illustrated by Zang et al. (2000).

The Chl FIR has been established as a stress indicator and it increases with de-

Table 4. Normalized FIRs of spectra of control and Cd-treated wheat seedlings. The measured LIF spectra were averaged and processed through Gaussian curve fitting. The peak heights of the bands were determined and normalized with respect to the response curve of the used grating and detector system and FIRs were calculated.

Cd concentration (mM)	Chl FIR with 488 nm	Other FIR with 355 nm excitation					
	F685/ F730	F450/ F520	F450/ F685	F450/ F735	F520/ F685	F520/ F735	F685/ F735
0.00	0.83	2.16	8.29	5.75	3.83	2.66	0.69
0.01	0.98	1.58	5.95	2.91	3.77	1.84	0.49
	(18.1)	(-26.9)	(-28.2)	(-49.4)	(-1.6)	(-30.8)	(-29.0)
0.10	0.83	3.46	14.92	7.46	4.31	2.15	0.50
	(0.0)	(60.2)	(80.0)	(29.7)	(12.5)	(-19.2)	(-27.7)
1.00	0.82	3.74	11.28	9.36	3.02	2.50	0.83
	(-1.2)	(73.1)	(36.1)	(62.8)	(-21.2)	(-6.0)	(20.3)
2.00	0.78	4.01	17.16	8.99	4.28	2.23	0.52
	(-6.0)	(85.6)	(107.0)	(56.3)	(11.7)	(-16.2)	(-24.6)

* Values in parentheses represent the percent increase or decrease compared to the control.

creasing chlorophyll content and photosynthetic activity of the leaves. Table 4 represents the normalised Chl FIR as well as other FIRs resulting from both the 488 nm and 355 nm excited LIF spectra. The Chl FIR resulting from 488 nm excitation was 0.83 for the control sample and it increased by about 18 % in response to 0.01 mM Cd treatment while for 0.1 mM and 1.0 mM Cd-treated plants the Chl FIR was simi-

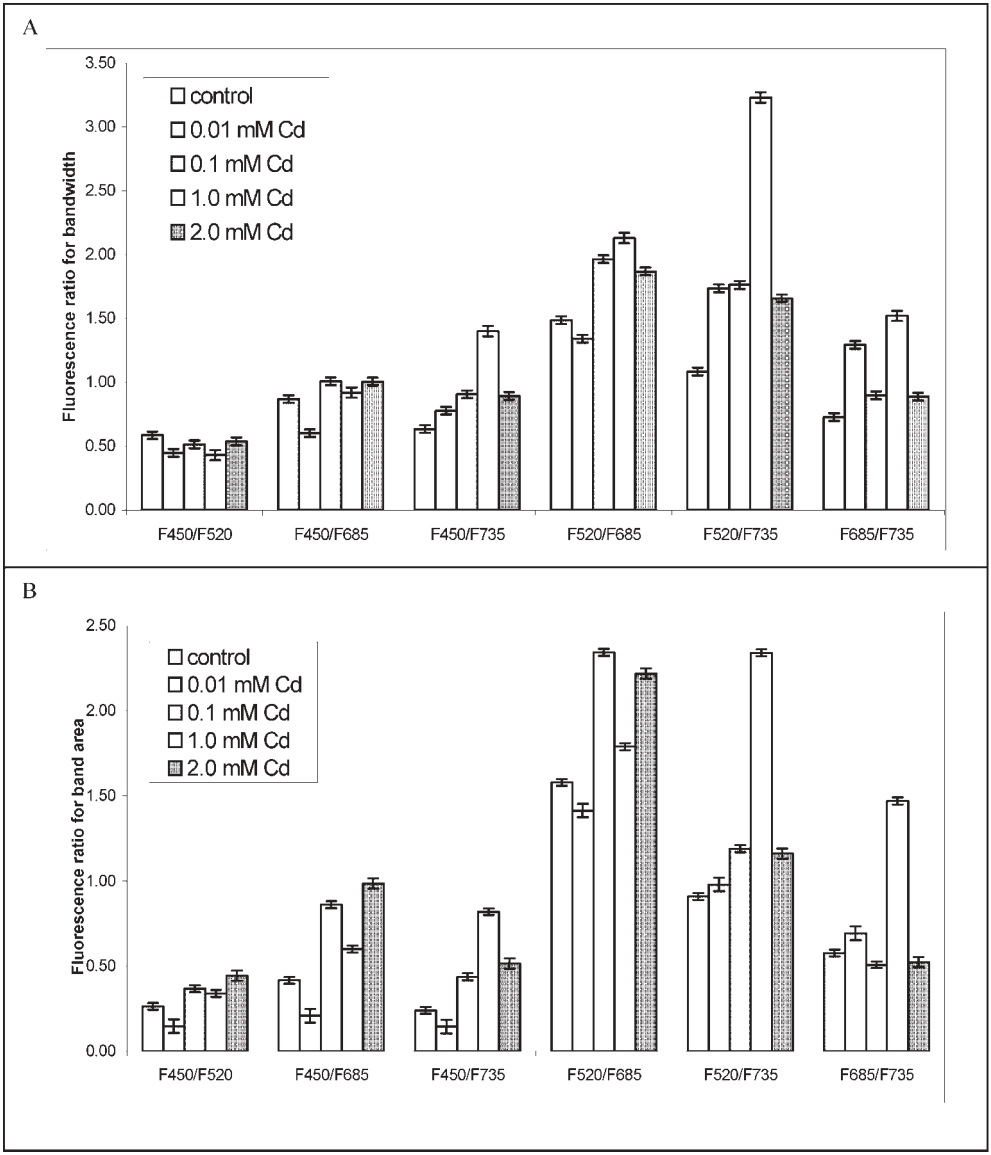

Fig. 5. Variations in the fluorescence ratios for the (A) bandwidth and (B) band area of 355 nm excited LIF spectra**.** Columns represent the mean value (n=3) and bars represent the standard error (SE).

lar to the control plants. In the case of 355 nm excited spectra, the Chl FIR decreased in 0.01 and 0.1 mM Cd-treated seedlings and increased by about 20% after treatment with 1.0 mM Cd. An increase in Chl FIR by 28% was found during the inhibition of photosynthetic electron transport by the herbicide diuron in *Phaseolus vulgaris* plants (Rinderle and Lichtenthaler, 1988). Our results showed that treatment with 2.0 mM Cd decreased the Chl FIR by about 6 % and 25 % as compared to controls at 488 nm and 355 nm excitation wave lengths, respectively. These results clearly demonstrated that higher concentrations of Cd inhibited severely the growth of the seedlings and decreased chlorophyll content, shoot length and fresh weight. The decrease in both Chl FIR and chlorophyll content could be correlated with the interaction of Cd with PSII reaction centre. The change in Chl FIR could also be due to the redox state of Q_A as Cd affects the electron transport /Calvin cycle, spill over changes, state changes and non photochemical quenching. The continuous increase in the FIR (F450/F520) values in 0.1 mM, 1.0 mM and 2.0 mM Cd-treated wheat seedlings may be due to the increase in the amount of blue fluorescing substances in the epidermis layer (Lang et al., 1992). The FIRs 450/F520, F450/F685, F450/F735, F520/F735 and F685/F735 decreased significantly after treatment with 0.01 mM Cd. The significantly higher values of the F450/F685 and F450/F735 ratios at higher concentrations of Cd could be an indication of a higher content of secondary metabolites, such as ferulic acid or cumaric acid which are involved in the biosynthesis of lignin, a component of cell walls (Valcke et al., 1999). The increases in synthesis accompanied by a stimulation of some enzymes of the Shikimate pathway have been observed during stress (Krause

Table 5. Rfd values for control and Cd-treated wheat seedlings. These values were calculated from the fluorescence induction kinetics curve, recorded up to five minutes at both red (685 nm) and far-red (735nm) of Chl fluorescence bands, using the method of Lichtenthaler.

Cd concentration (mM)	Rfd (685 nm)	Rfd (735 nm)
0.00	2.11	1.63
0.01	1.90	1.03
	(-10.0)	(-36.8)
0.10	1.39	0.96
	(-34.1)	(-41.1)
1.00	0.92	0.77
	(-70.1)	(-52.8)
2.00	0.63	0.60
	(-70.1)	(-63.2)

Each value is the mean of triplicate with SD less than 5%.

and Weis, 1992). The fluorescence ratios for the bandwidth and band area are presented in Fig. 5. The F450/F735 and F520/F735 ratios for the bandwidth together with the F520/F735 ratio for the band area showed similar patterns with increasing Cd concentration. On the other hand, the highest concentration of Cd applied (2.0 mM) was found to decrease the above ratios.

Fluorescence induction kinetics

The fluorescence induction kinetics measured on pre-darkened leaves consists of a fast fluorescence rise to a maximum intensity level (F_m) followed by a slow fluorescence decay (F_d) and a steady-state level (F_s). The fluorescence decrease ratio (Rfd = F_d/F_s) is a measure of photosynthetic capacity of a leaf (Lichtenthaler et al., 1986; Strasser, 1986) and the full potential capacity of a leaf may depend on the water status and the degree of stomata opening. Rfd values are known as a plant vitality index which declines steadily with increasing stress (Lichtenthaler, 1988). The Rfd values decline steadily with increasing Cd stress at both wavelengths and they were always higher for the Cd-treated seedlings in the 685 nm region than those measured in the 735 nm region (Table 5). Lichtenthaler (1988) has observed a similar decrease in the Rfd value at 690 nm and 730 nm during water stress induced by abscission of maple leaves (*Acer platanoides*) or spruce needles (*Picea omorika*). A decrease in the Rfd values at different Cd concentrations could be related to a decrease in the photosynthetic pigments as well as a damage caused to the photosynthetic apparatus. This result suggests also a reduced rate of CO_2 fixation caused by Cd which could be due to stomata closure and interaction of Cd with Calvin cycle enzymes (Weigel, 1985).

CONCLUSION

The spectral behaviour of the measured fluorescence bands depended mainly on the absorption properties of the leaves. The results presented here indicate that Chl FIR together with the other FIRs can give a lot of information regarding the physiological status of a leaf. The fluorescence signature of 488 nm and 355 nm laser light, plant vitality index calculated from fluorescence induction kinetics and the pigment data analysis revealed that even 0.01 mM Cd was harmful to the growth of the wheat seedlings. The lidar remote sensing of fluorescence signature of terrestrial vegetation can be useful for evaluation of plant health. However, the plant vitality index, calculated from fluorescence induction kinetics, seems to provide more accurate information regarding the state of plant health. On the other hand, it is not suited for remote sensing because induction kinetics requires longer measurement time and plants must be dark-adapted. The measured LIF could be processed by a curve-fit-

ting procedure using a linear combination of Gaussian spectral function to assist observation of the shift in the band peaks position and determination of the FIRs.

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