

rate over the Bay of Bengal, Arabian Sea and Indian Ocean is found to be higher during May and June due to the onset of SW monsoon compared to other months. It gradually decreases in the succeeding months, again followed by increase during winter due to winter (NE) monsoon. Low rainfall is found over ocean during March and April due to hot and dry weather. Satellite-derived rain rate is found to be similar to the NCEP/NCAR-derived climate reanalysis data. The MSMR data show the potential in deducing rain rate over the ocean, which will be useful in understanding the complex climatological phenomena over the Indian subcontinent and in the numerical forecast modelling.

1. Quartly, G. D., Srokosz, M. A. and Goymer, T. H., *J. Geophys. Res.*, 1999, **104**, 31489–31516.

2. Gohil, B. S., *First Science Workshop Proceedings of Megha-Tropiques*, ISRO, Bangalore, 19–22 May 1999, pp. 20.1–20.15.
3. Ferraro, R. R., *J. Geophys. Res.*, 1997, **102**, 16715–16735.
4. Mishra, D. R., M Tech thesis, IIT Kanpur (unpublished), 2002, pp. 13–33.
5. Varma, A. K., Gairola, R. M., Mathur, A. K., Gohil, B. S. and Agrawal, K., *Proceedings of PORSEC-2000*, Goa, 2000, pp. 240–243.
6. Hartmann, D. L. and Michelsen, M. L., *J. Atmos. Sci.*, 1989, **46**, 2838–2862.

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## Laser-induced chlorophyll fluorescence spectra of mung plants growing under nickel stress

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**The *in vivo* laser-induced chlorophyll fluorescence (LICF) spectra of mung (*Vigna radiata* Linn.) for the control, and nickel (a heavy metal)-treated plants were recorded in the region 600–800 nm with two characteristic bands lying at 680 nm and 730 nm using 488 nm argon ion laser line for excitation. The chlorophyll fluorescence intensity ratio (FIR) F680/F730, and peak positions were calculated by evaluating curve-fitted parameters using Gaussian spectral function. It was found that the FIR decreases with increasing chlorophyll content in case of 0.1 mM nickel-treated plants. When the nickel concentration was raised to 1.0 mM, the FIR showed increasing trends, although it was much less than the control, with decreasing chlorophyll content. Our study demonstrates the use of LICF spectra in the early detection of heavy metal stress impact on crops, particularly mung.**

LASER-induced fluorescence (LIF) for remote detection of vegetation stress had been initially proposed by Chappelle *et al.*<sup>1</sup>. Later, LIF studies of vegetation were

used to explore the possibility of using laser as a remote means of measuring vegetation characteristics such as plant vigour, as affected by various stress factors such as drought, natural nutrient deficiency, etc. plant type identification and forest biomass estimation. LIF signal can be used to make an inference regarding health and identity of the plants<sup>2,3</sup>. Saito *et al.*<sup>4</sup> have reported fluorescence lidar as a potential new technique for remote terrestrial vegetation monitoring.

The chlorophyll fluorescence spectrum of a green leaf has the maxima near 690 nm and 730 nm. The fluorescence intensity ratio (FIR) of the two maxima red/far-red (F690/F730) is strongly influenced by variation in photosynthetic activity. The intensity of the red and far-red chlorophyll fluorescence is inversely related to the photosynthetic activity. When photosynthesis decreases owing to various stress conditions, the FIR increases. The increase in chlorophyll content in plants results in a decrease in the value of the FIR. The FIR has also been established as an indicator of the *in vivo* chlorophyll content in plants<sup>3</sup>. This stress indicator has been utilized in active remote sensing of the plant in fluorescence lidar system by Valentini *et al.*<sup>5</sup>. Subhash and his associates have studied the effect of different stresses on various plants<sup>6–11</sup>, collecting LIF radiation using optical fibre. Buschmann *et al.*<sup>12</sup> have recorded fluorescence imaging of leaf, which provides ample information about as many as ten thousand pixels over the whole leaf area. This allows detection of local disturbances and the gradients in the fluorescence emission. Single-point or spot-data fluorescence measurements, which are still widely used, usually have the advantage of low cost and possibility of relatively high spectral resolution, but a disadvantage that fluorescence information of one leaf spot seldom represents the whole leaf.

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In the present communication the authors studied laser-induced chlorophyll fluorescence (LICF) spectra of mung plants treated with nickel, using a technique which enjoys the benefit of both the existing techniques of high spectral resolution and fluorescence imaging from a large area of the leaf. Heavy metals, one of the major pollutants in the environment, are spilled in the soil through several agencies. Plants often take up these metals which eventually exert toxic effects on them<sup>13–15</sup>. In many soils, heavy metals may occur at a toxic level due to natural processes or anthropogenic activities<sup>16,17</sup>. Sometimes these heavy metals act as micronutrients at very low concentrations, which stimulate the growth of the plants by increasing the chlorophyll content and the photosynthetic activity of the plants. Nickel, one of the heavy metals, stimulates many enzymatic activities at very low concentration, and also acts in substitution of molybdenum in nitrogenase enzyme which is found in the root nodule of mung. The aim of the present work is to study the early detection of vegetation characteristics affected by metal, particularly nickel, and to explore the possibility of using laser as a remote means of measuring vegetation characteristics affected by heavy metal stress.

Healthy and uniform-sized mung bean (*Vigna radiata* Linn.) seeds were surface sterilized in 4% sodium hypochlorite solution for 20 min and washed thoroughly with sterilized double distilled water, and then germinated in acid-washed sterilized sand for 6 days under controlled environmental conditions. Ten seedlings of uniform size were selected and carefully transferred into each pot containing 100 ml, 0.2-strength, modified Rorison medium with nickel as  $\text{NiCl}_2$  (0.1 and 1.0 mM). Seedlings treated with and without (control) nickel were placed in a growth chamber at  $27 \pm 1^\circ\text{C}$  under the 12 h photoperiod ( $75 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) for a day. The basic composition of nutrient solution was as follows; in mM: 0.4  $\text{Ca}(\text{NO}_3)_2$ , 0.2  $\text{MgSO}_4$ , 0.2  $\text{KH}_2\text{PO}_4$ ; in  $\mu\text{M}$ : 0.1  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.2  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 9.2  $\text{H}_3\text{BO}_3$ , 1.8  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 0.2  $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$  and 10 Fe EDTA. Plants harvested after 5 days of heavy metal treatment were used for estimation of chlorophyll and carotenoids, and a similar set of experiments was also performed for the control sample. Healthy leaves of the seedlings were used for LICF study. The experiment was repeated five times to determine each parameter.

Leaves of the plants used for LICF study were rinsed in distilled water, dried at  $80^\circ\text{C}$  for 24 h and wet-ashed in nitric and perchloric acid mixture (3/1 v/v) on an electric thermostatic plate ( $300^\circ\text{C}$ ). Nickel content was determined by atomic absorption spectrophotometry<sup>18</sup> (Perkin-Elmer 2380).

The laser spectrofluorometer used in the present study is a computer-controlled data acquisition system, which provides fluorescence information. A cw argon ion laser (Spectra Physics, USA model 2016) operating at 488 nm was used for exposing the full intact leaf with the help of

the beam expander. The fluorescence light was collected with the help of a convex lens on the slit of computer-controlled 0.5 M monochromator (Acton Research Corporation, USA) having a resolution of 0.03 nm and reciprocal linear dispersion of 1.1 nm/mm, with R928 PMT detector. The PMT signals were sent to the computer and the data have been collected and analysed using Grams-32 (Galactic) software. Laser light intensity was measured with the help of power meter (Spectra Physics, USA model 407A-2). Figure 1 shows the experimental set-up used in the present study.

The LICF spectra for the control and plants harvested after 5 days of nickel chloride ( $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ ) treatment, excited by 488 nm of argon ion laser (power 30.0 mW) have been recorded in the region 600–800 nm with two peaks lying nearly at 680 and 730 nm, which are due to chlorophyll from PS II and PS I respectively. The curve fitting has been done in the region between 650 and 800 nm using the Grams-32 software with the Curve-fit.AB program. This curve-fit is based on the original algorithm of nonlinear peak fitting as described by Marquardt and also known as the Levenberg–Marquardt method. It fits the Gaussian, Lorentzian, mixed Gaussian–Lorentzian, log normal, Pearson-VII, and Voigt line shape. We have chosen Gaussian spectral function for the curve fitting, since it provides a reasonably matching fit of the spectral data with good  $F$ -statistics, standard errors for peak amplitude, peak centre, and bandwidth or full width at half intensity maximum (FWHM)<sup>10</sup>.

Chlorophyll and carotenoids were extracted in 80% acetone from the leaf samples used for taking the spectra. Concentration of chlorophyll  $a$  and  $b$  and total chlorophyll was determined colorimetrically (Spectro Colorimeter, 108, Systronic, India) using the formulae of Arnon<sup>19</sup>. The level of total carotenoids in 80% acetone extracted was determined using an extinction coefficient of  $E_{473} 1\% = 2500$  absorbance units as an average value<sup>20</sup>. Results are expressed as  $\text{mg g}^{-1}$  fresh weight leaf.

Mung plants treated with various concentrations of nickel chloride showed variation in the growth performance. At very low concentration of nickel (0.1 mM or below) the plant showed better growth than the control, as the photosynthetic pigments, i.e. chlorophyll  $a$ ,  $b$  and carotenoids (Table 1) content (leaf) of plants was increased

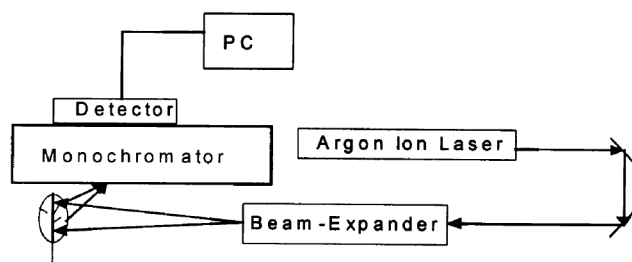


Figure 1. Experimental arrangement to obtain LICF spectra.

**Table 1.** Effect of nickel on photosynthetic pigments of mung plants

Plant treatment with nickel (mM)	Chlorophyll <i>a</i> (mg g <sup>-1</sup> fresh weight leaf)	Chlorophyll <i>b</i> (mg g <sup>-1</sup> fresh weight leaf)	Total chlorophyll (mg g <sup>-1</sup> fresh weight leaf)	Carotenoids (mg g <sup>-1</sup> fresh weight leaf)
0.0 (control)	1.26	0.78	2.04	0.54
0.1	1.43 (13.5)	0.89 (14.1)	2.32 (13.7)	0.56 (3.7)
1.0	1.14 (- 9.5)	0.61 (- 21.8)	1.75 (- 14.2)	0.60 (11.1)

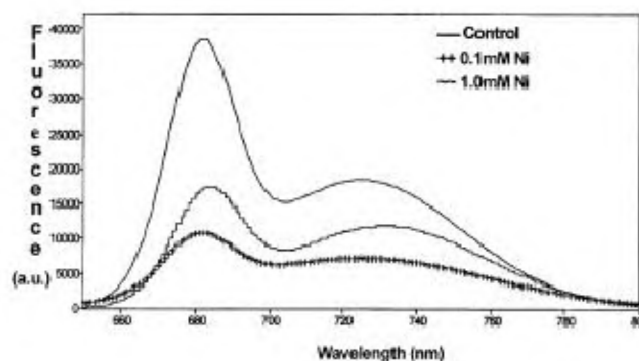
Values given in parentheses are per cent increase or decrease over the control.

**Table 2.** Nickel accumulation in leaves of mung plants after 5 days of exposure to increasing concentration of nickel

Plant treatment with nickel (mM)	Nickel content in leaves (mg g <sup>-1</sup> dw)
0.0 (control)	0.0
0.1	2.4 ± 0.1
1.0	28.6 ± 0.3

by 13.5, 14.1 and 3.7% respectively. Nickel also stimulated the plant height at this concentration (data not shown). Nickel-induced enhancement in chlorophyll content may be due to its stimulatory role in the chlorophyll biosynthetic process. Nickel can also increase various enzymatic activities like those of nitrogenase, enzymes in the Calvin cycle and urease<sup>21</sup>, at very low concentration, and thus enhances the general growth of the plants. Plants with 1.0 mM nickel exhibited inhibition at all parameters studied and further rise in metal concentration (2 mM or above) caused a poor growth of the plant (data not shown). The accumulation of nickel in the leaves of 0.1 mM nickel-treated plants was 2.4 mg g<sup>-1</sup> dw. It was further increased up to 28.6 mg g<sup>-1</sup> dw in 1.0 mM nickel-treated plants (Table 2). As expected, nickel accumulation was more in the root (37%) than in the shoot (26%) (data not shown) and 29% of absorbed nickel was translocated to the leaves.

The Gaussian spectra resulting from the curve-fitting analysis of LICF for the control and the nickel-stressed mung plants are presented in Figure 2. The curve-fitting parameters such as peak centre, peak height, bandwidth (FWHM) and the area under each Gaussian curve for both the control and nickel-treated plants are shown in Table 3. The ratio F680/F730 for peak height, bandwidth and band area are given in Table 4. The peak positions for the LICF spectra of control mung plants are located at 681.5 and 725.5 nm for red and far-red bands respectively. In plants treated with 0.1 mM nickel, the leaves accumulated 2.4 mg g<sup>-1</sup> dw of nickel, which has caused a blue shifting of both the red and far-red bands by 1.0 nm. Further, leaves from 1.0 mM nickel-exposed plant accumulated about 12 times more nickel content than

**Figure 2.** Curve-fitted LICF spectra of mung.

0.1 mM nickel-treated sample. In contrast to the blue shifting, the higher accumulation of nickel (28.6 mg g<sup>-1</sup> dw) resulted in a major red shifting by 6.0 nm at the far-red band (Figure 2 and Table 3). The blue and red shifting in the emission peaks for red and far-red bands could be correlated with the interaction of nickel and the reaction centre assembly of PS II and PS I, which leads to the alteration of the activity of both the photosystems. Subhash and Mohanan<sup>10</sup> observed a notable blue shifting about 9 nm in the far-red band in the case of nitrogen-deficient *Salvia splendens* plant, whereas in the case of phosphorous-deficient plant, the blue shifting in this region was only 1.5 nm.

Apart from the shifting of the bands due to the accumulation of nickel in the leaves, 0.1 mM nickel-treated leaves showed a decrease of 77% in the peak height (intensity) for red band and 61% for the far-red band compared to the control. The leaves treated with 1.0 mM of nickel exhibited only 56% decrease in the peak height for red band and 35% for far-red band. Further, it can also be seen from the results that the leaves treated with higher concentration of nickel (1.0 mM) showed an increase in fluorescence intensity than the lower concentration (0.1 mM) nickel-treated sample. Lowering of the peak height due to 0.1 mM nickel could be correlated with the increase in the photosynthetic pigments (Table 1) and photochemical electron transport involving both reaction centres PS I and PS II (data not shown). But, at higher concentration of 1.0 mM nickel, the smaller

**Table 3.** Results of curve fitting on LICF spectra of mung plants excited by 488 nm argon ion laser

Plant treatment with nickel (mM)	Red band				Far-red band			
	Peak position (nm)	Peak height (au $\times 10^2$ )	Bandwidth (nm)	Band (au $\times 10^4$ )	Peak position (nm)	Peak height (au $\times 10^2$ )	Bandwidth (nm)	Band (au $\times 10^4$ )
0.0 (control)	681.5	330	19.5	68	725.5	185	65.5	128
0.1	680.5	76 (- 77)	22.0 (12.8)	18 (- 73)	724.5	72 (- 61.1)	80.5 (22.9)	60 (- 53.1)
1.0	681.8	145 (- 56.1)	24.0 (23.1)	37 (- 45.6)	731.5	121 (- 34.6)	62.5 (- 4.6)	80 (- 37.5)

Values in parentheses are per cent increase or decrease over the control.

decrease in the intensity in the red and far-red bands compared to the lower concentration (0.1 mM) nickel-treated plants, could be explained on the basis of nickel-induced alteration in the thylakoid membranes, and hence a retardation in general growth of the plants was expected.

Recently, the shape of the chlorophyll fluorescence spectra at room temperature, i.e. the ratio F690/F735 of the two chlorophyll fluorescence maxima of the leaves are being widely used to differentiate between healthy and stressed plants. In the present study, leaves from the plant treated with 0.1 mM nickel showed decrease in the ratio (F680/F730) of peak height, bandwidth (FWHM) and band area by 41, 43 and 10% respectively compared to the control. However, leaves from 1.0 mM nickel-treated plants showed decrease in the ratio of peak height and band area by 32 and 11% respectively, but an increase in the bandwidth ratio by 27%. Several workers studied the FIR F680/F730 and established this ratio as the *in vivo* indicator of chlorophyll content of the leaves. The F680/F730 ratio is chlorophyll-dependent and decreases with increasing chlorophyll content. Therefore it can be used to monitor changes in the chlorophyll content during leaf development<sup>22-24</sup>, autumnal chlorophyll breakdown<sup>25,26</sup>, the course of a year<sup>27</sup>, and also as a result of natural and anthropogenic stress or damage events<sup>28,29</sup>. It is clear from Table 4 that the F680/F730 ratio for the intensity is maximum for the control leaf, which decreases after the nickel treatment at 0.1 mM concentration. Since nickel stimulates the general growth of the plant at 0.1 mM concentration and increases the chlorophyll content (Table 1) as well as photosynthetic activity, thus the F680/F730 ratio appears to be minimum. Leaf from plants treated with 1.0 mM nickel showed an increase in the F680/F730 ratio in comparison to the 0.1 mM nickel-treated plants. Results clearly demonstrated that nickel inhibited the growth of the plant at 1.0 mM concentration and decreased the chlorophyll content as well as photochemical processes, and therefore this increase in the F680/F730 ratio was obvious. The chlorophyll content in 1.0 mM nickel-treated plants was less than that in the control; but F680/F730 ratio in the control was found to be more. Comparatively, the smaller F680/F730 ratio in leaves of 1.0 mM nickel-treated plants compared to the control

**Table 4.** The F680/F730 ratio for the peak height, band area and bandwidth for the control and the nickel-treated mung plants

Plant treatment with nickel (mM)	[F680/F730] ratio		
	Peak height	Band area	Bandwidth
0.0 (control)	1.78	0.53	0.30
0.1	1.05 (- 41.01)	0.30 (- 43.40)	0.27 (- 10.00)
1.0	1.21 (- 32.0)	0.47 (- 11.3)	0.38 (26.7)

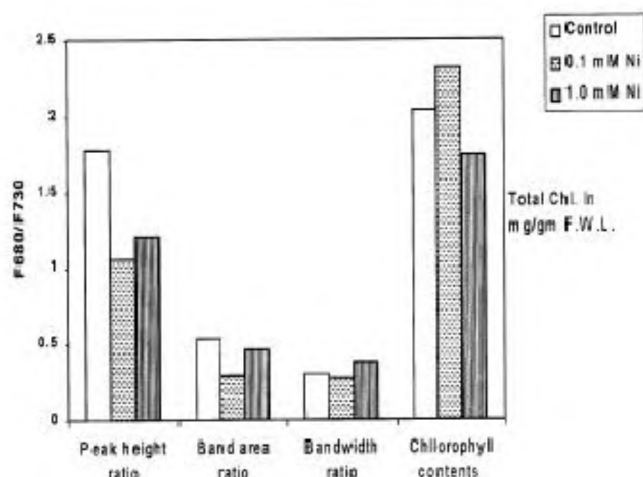
Values in parentheses are per cent increase or decrease over the control.

could be correlated with the interaction of nickel and the photosystems, thereby reducing the physical and chemical properties (absorption and photosynthesis). Furthermore, nickel may also interact with the CO<sub>2</sub>-fixation process, which is supposed to be a sink for electron released from the photosystems. It can also be argued that 1.0 mM concentration of nickel inhibited the photosynthetic activity and the transportation of electrons through PS II and PS I; therefore the F680/F730 ratio was found to be less in the case of 1.0 mM nickel-treated plants than in the control.

Figure 3 shows the mean chlorophyll content and F680/F730 ratio from curve-fitted peak amplitudes, Gaussian curve area and the bandwidth (FWHM). The F680/F730 ratio of peak height and band area exhibited similar trends and represents a correlation with the decrease and increase in the chlorophyll content of the respective sample. At the same time, bandwidth ratio has slight dissimilarity with the peak height and band area ratio, as the bandwidth ratio of the control sample was even less than that of the 1.0 mM nickel-treated sample.

It is also noteworthy to mention that the present study demonstrates the use of nickel as a trace element for crops besides its exclusion from the list of trace element as at the lower concentration (0.1 mM) it has stimulated growth and other metabolic processes.

This study explores the possibility of detection of vegetation stress affected by heavy metal using laser. Our



**Figure 3.** Variation of F680/F730 ratio from curve-fitted peak height, Gaussian curve area, and bandwidth with chlorophyll content for control and nickel-treated mung plants.

study shows that the FIR can be used to monitor metal stress on the vegetation. It was found that the lower concentration of nickel decreases the FIR, but the higher concentration of nickel not only increases the FIR compared to the lower concentration treated sample, but also changes the fluorescence peak positions, bandwidth and band area significantly. The blue and red shifts of red and far-red bands, increase and decrease in the bandwidth (FWHM) together with the FIR obtained from the curve-fitted parameters which seem to be great potential for the determination of heavy metal stress. We can also use the F680/F730 ratio to determine whether the stress has a positive or negative impact on the growth and the development of crop plants and forests.

- Chappelle, E. W., Wood, F. M., McMurtrey, J. E. and Newcomb, W. W., *Appl. Opt.*, 1984, **23**, 134–138.
- Chappelle, E. W., Wood, F. M., Newcomb, W. W. and McMurtrey, J. E., *ibid*, 1985, **24**, 74–80.
- Lichtenthaler, H. K. and Rinderle, U., *CRC Crit. Rev. Anal. Chem. (Suppl. 1)*, 1988, **19**, 29–85.
- Saito, Y., Kanoh, M., Hataka, K., Kawahara, T. D. and Nomura, A., *Appl. Opt.*, 1998, **37**, 431–437.
- Valentini, R. *et al.*, *Remote Sensing Environ.*, 1994, **47**, 29–35.
- Subhash, N., Mazzinghi, P., Agati, G., Fusi, F. and Lercari, B., *Photochem. Photobiol.*, 1995, **62**, 711–718.

- Subhash, N., Agati, G., Fusi, F. and Mazzinghi, P., in *Proceedings of Laser '93 Conference*, Nevada, USA, 1993, pp. 113–117.
- Subhash, N., *LAMP*, ICTP, Italy, 1995, **4**, 1–24.
- Subhash, N. and Mohanan, C. N., in *Proceedings of the International Geoscience and Remote Sensing Symposium*, Firenze, Italy, 1995, vol. III, pp. 2323–2325.
- Subhash, N. and Mohanan, C. N., *Remote Sensing Environ.*, 1997, **60**, 347–356.
- Subhash, N., Wenzel, O. and Lichtenthaler, H. K., *ibid*, 1999, **69**, 215–223.
- Buschmann, C., Langsdorf, G. and Lichtenthaler, H. K., *Photosynthetica*, 2000, **38**, 483–491.
- Foy, C. D., Chaney, R. L. and White, M. C., *Annu. Rev. Plant Physiol.*, 1978, **29**, 511–566.
- Woolhouse, H. W., *Encyclopedia of Plant Physiology* (eds Lange, O. L., Osmond, C. K. and Ziegler, H.), Springer-Verlag, New York, 1983, vol. 12C, pp. 245–300.
- Mukherji, C. and Mukherjee, S., *Indian J. Plant Physiol.*, 1990, **33**, 190–196.
- Nriagu, J. O. and Pacyna, J. M., *Nature*, 1988, **333**, 134–139.
- Nriagu, J. O. and Farmer, J. G. (eds), *International Conference*, Edinburgh, 1991, vol. 1, pp. 1–5.
- Gabbrielli, R., Mattrioni, C. and Vergnano, O., *J. Plant Nutr.*, 1991, **14**, 1067–1080.
- Arnon, D. I., *Plant Physiol.*, 1949, **24**, 1–15.
- Goodwin, T. W., *Carotenoids* (eds Paech, K. and Tracey, M. W.), Springer-Verlag, 1954, vol. III, pp. 272–311.
- Klucas, R. V., Hanus, F. J. and Russell, S. A., *Proc. Natl. Acad. Sci. USA*, 1983, **90**, 2253–2257.
- Buschmann, C., in *Photosynthesis* (ed. Akoyunoglou, G.), Balaban International Science Services, Philadelphia, 1981, vol. 5, pp. 417–426.
- D'Ambrosio, N., Szabo, K. and Lichtenthaler, H. K., *Radiat. Environ. Biophys.*, 1992, **31**, 51–62.
- Tuba, Z., Lichtenthaler, H. K., Csintalan, Z. and Pocs, T., *J. Plant Physiol.*, 1993, **142**, 103–108.
- Lichtenthaler, H. K., *ibid*, 1987, **131**, 101–110.
- Hak, R., Lichtenthaler, H. K. and Rinderle, U., *Radiat. Environ. Biophys.*, 1990, **29**, 329–336.
- Rinderle, U., Schindler, C. and Lichtenthaler, H. K., in *Proceedings of the 5th International Colloquium on Physical Measurements and Spectral Signatures in the Remote Sensing*, Courchevel, ESA Publication Division, Noordwijk, 1991, pp. 731–734.
- Lichtenthaler, H. K., Buschmann, C., Rinderle, U. and Schmuck, G., *Radiat. Environ. Biophys.*, 1986, **25**, 297–308.
- Rinderle, U. and Lichtenthaler, H. K., in *Application of Chlorophyll Fluorescence* (ed. Lichtenthaler, H. K.), Kluwer Academic Publisher, Dordrecht, 1998, pp. 189–196.

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