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Research Article

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Study of Different Metal Stress on Soybean Plant

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ABSTRACT

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.

In present study the laser spectrofluorometer with computer-controlled data acquisition system is used 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. It has been found that the leaves treated with higher concentration of metals showed, an increase in fluorescence intensity than the lower concentration. The bandwidth decreases in the red band region and increases in the far red band region due to metal stress. In red region bandwidth is minimum for nickel treated plant. In far red region bandwidth is maximum for mercury treated plant. From the various observations it has been cleared that the higher concentration of heavy metals like Aluminium, Nickel and Mercury affect the photosynthetic activity adversely by decreasing the amount of chlorophyll content in the Soybean plant. Mercury proved pronounced stress as compare to Nickel Aluminium and Copper.

Key words: *Metal stress, Fluorescence, Photosynthesis*

INTRODUCTION

LASER-induced fluorescence (LIF) for remote detection of vegetation stress had been initially proposed by Chappelle *et al.*[1]. 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 vigor, 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[1,2]. Saito *et al.*[1] 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 the rate of 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[3].

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This stress indicator has been utilized inactive remote sensing of the plant in fluorescence lidar system by Valentini *et al.*[1]. Subhash and his associates have studied the effect of different stresses on various plants[1,2,3], collecting LIF radiation using optical fiber. Buschmann *et al.*[1] 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.

Heavy metals and photosynthesis

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[1]. In many soils, heavy metals may occur at a toxic level due to natural processes or anthropogenic activities[1]. 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 nitrogenous enzyme which is found in the root nodule of leguminous plants. The aim of the present work is to study the early detection of vegetation characteristics affected by metal, particularly nickel & mercury, and to explore the possibility of using laser as a remote means of measuring vegetation characteristics affected by heavy metal stress.

MATERIAL AND METHOD

Leaves of the Soybean plants used for LICF study were rinsed in distilled water, dried at 80°C for 24 h and wet-ashed in nitric and perchloric acid mixture (3/1 v/v) on an electric thermostatic plate (300°C). Nickel content was determined by atomic absorption spectrophotometry18 (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 show the experimental set-up used in the present study. The LICF spectra for the control and plants harvested after 5 days of nickel chloride (NiCl2×6H2O) 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)10. 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 Parra (2002). The level of total carotenoids in 80% acetone extracted was determined using an extinction coefficient of E4731% = 2500 absorbance units as an average value20. Results are expressed as mg g–1 fresh weight leaf. Soybean plants treated with various metals stress showed variation in the growth performance.

Observations

The Gaussian spectra resulting from the curve-fitting analysis of LICF for the control and the mercury, Aluminium, Copper and Nickel-stressed Soybean 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 metal-treated plants are shown in following Table 1. 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. The ratio F680/F730 for peak height, bandwidth and band area are given in Table2. The peak positions for the LICF spectra of control Soybean plants are located at 680 and 730 nm for red and far-red bands respectively.

When heavy metals accumulate in the plants leaves their photosynthesis activity changes. It is observed in emission spectra as shown in the graph. The blue and red shifting in the emission peaks for red and far-red bands could be correlated with the interaction of heavy metals and the reaction centre assembly of PS II and PS I, which leads to the alteration of the activity of both the photo systems. Apart from the shifting of the bands due to the accumulation of metals in the leaves, it can also be seen from the results that the leaves treated with higher concentration of metals showed an increases in fluorescence intensity than the lower concentration. It is observed that bandwidth in the red band region decreases due to metal stress and it is increases in the far red band region. For control plant bandwidth is minimum in far red region and is maximum in red region. In red region bandwidth is minimum for nickel treated plant. In far red region bandwidth is maximum for mercury treated plant.

Table.1. Result of curve fitting of LICF Spectra of Soybean plant excited by 405 nm diode laser

Plant Treatment	Red Band				Far Red Band			
	Peak position in nm	Peak height a.u. x 10^2	Band width in nm	Band area a.u. x 10^4	Peak position in nm	Peak height a.u. x 10^2	Band width in nm	Band area a.u. x 10^4
Control	680	1427	31	55959	730	844	35	37122
Mercury stress	680	14727	24	435507	730	5028	42.41	267327
Aluminum stress	680	9114	24	272976	730	3317	42.10	175037
Copper stress	680	6028	25.2	190423	730	2042	38.49	98515
Nickel stress	680	7791	23.5	229724	730	2592	41.36	134385

Recently, the shape of the chlorophyll fluorescence spectra at room temperature, i.e. the ratio F680/F730 of the two chlorophyll fluorescence maxima of the leaves are being widely used to differentiate between healthy and stressed plants. 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, autumnal chlorophyll breakdown the course of a year and also as a result of natural and anthropogenic stress or damage events. In the present work the ratio F680/F730 for peak height is maximum for nickel treated plant and lowest for aluminium treated plant. It indicates the chlorophyll content is more in aluminium treated plant and is less in nickel treated plant. Nickel affects adversely the growth of Soybean plant compared to aluminium.

Comparatively, the larger F680/F730 ratio in leaves of metal treated plants compared to the control could be correlated with the interaction of metal and the photo systems, thereby reducing the physical and chemical properties (absorption and photosynthesis). Furthermore, metals may also interact with the CO_2-fixation process, which is supposed to be a sink for electron released from the photo systems.

The F680/F730 ratio of peak height and band area exhibited similar trends and represents a correlation with the decrease 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 more compared to stress plants.

Table.2. The F680/F730 ratio for the peak height, band area and bandwidth for the control and the metal-treated Soybean plants

Plant treatment	F680/F730 ratio		
	Peak height	Band width	Band area
control	1.6907	0.88571	1.5074
Mercury stress	2.9289	0.56590	1.6291
Aluminum stress	2.7476	0.57007	1.5595
Copper stress	2.9520	0.65471	1.9329
Nickel stress	3.0057	0.56818	1.7094

Fig. 1: Experimental arrangement to obtain LICF spectra

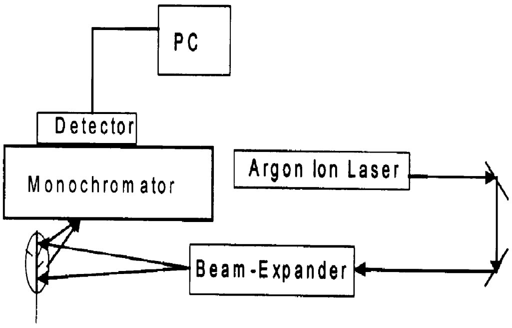

Fig. 2: Curve fitted LICF spectra of Soybean leaf under control and stress conditions

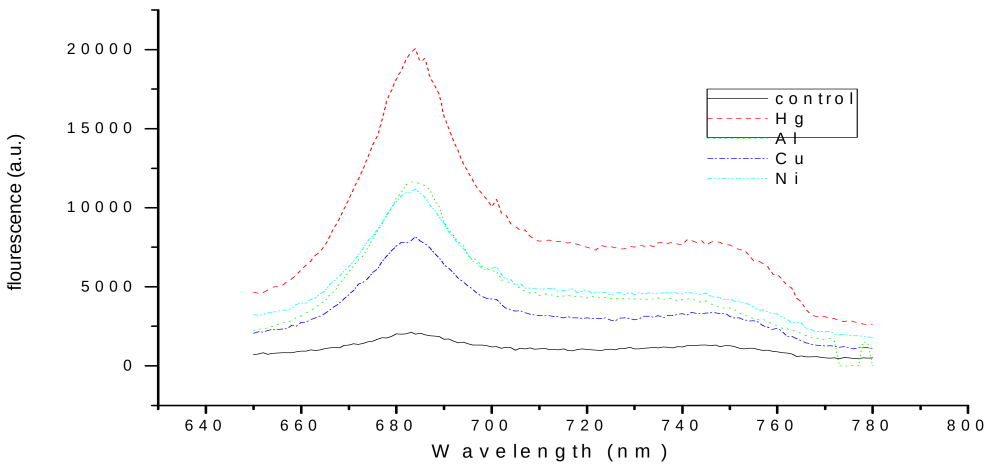

Fig. 2 a: Curve fitted LICF spectra of Soybean leaf under control conditions

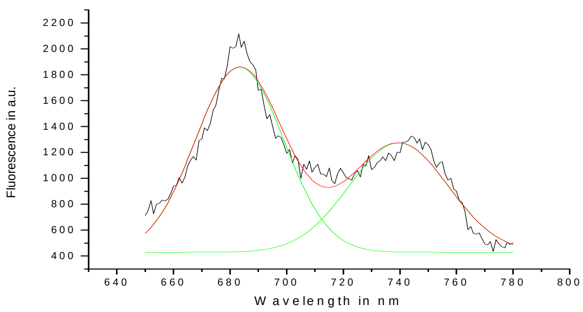

Fig. 2 b: Curve fitted LICF spectra of Soybean leaf under mercury stress

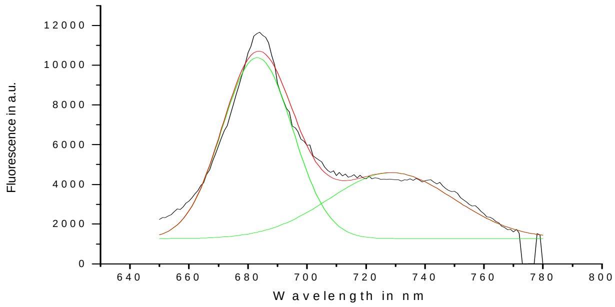

Fig. 2 c: Curve fitted LICF spectra of Soybean leaf under Aluminum stress

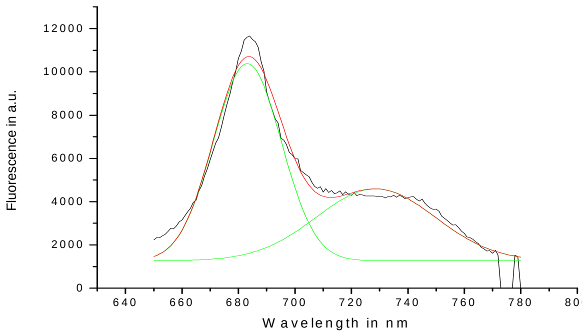

Fig. 2 d: Curve fitted LICF spectra of Soybean leaf under copper stress

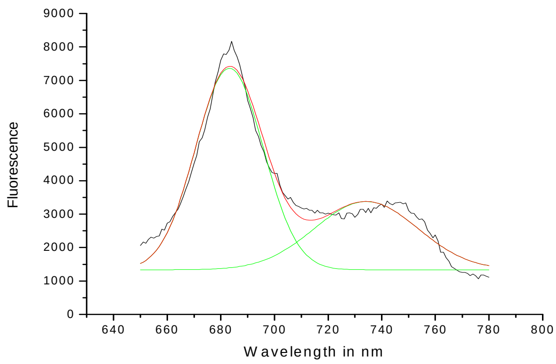

Fig. 2 e: Curve fitted LICF spectra of Soyabean leaf under nickel stress

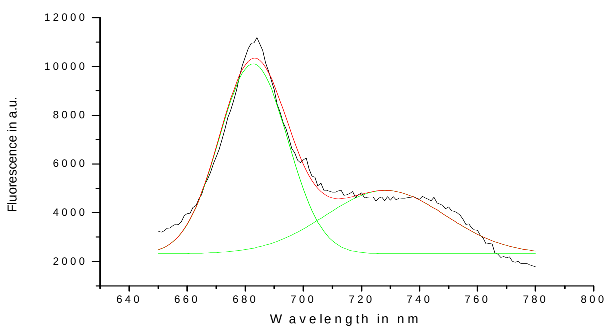

Table : 3 Effect of heavy metal stress on chlorophyll content of Soybean plant

S. No.	Metal Stress	Chlorophyll a	Chlorophyll b	Total Chlorophyll
1	Controlled plant	34.488676	18.380584	52.869256
2	Copper (Cu)	34.180301	16.669652	50.849954
3	Aluminum (Al)	27.850763	9.9201193	37.770882
4	Nickel (Ni)	24.954886	7.830877	32.785764
5	Mercury (Hg)	24.265267	8.401796	32.667063

RESULT

This study explores the possibility of detection of vegetation stress affected by heavy metal using laser. This study shows that the FIR can be used to monitor metal stress on the vegetation. It was found that metal concentration increases the FIR and 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.

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