DIFFERENCES ON STRESS RESPONSE OF CERCIUS SILIQUASTRUM PLANTS IN DIFFERENT PLACES IN METALLURGICALL COMPLEX AND IN DIFFERENT PERIODS VIA SCREENING OF PHOTOSYNTHETIC ACTIVITY BY CHLOROPHYLL FLUORESCENCE IMAGING

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Abstract

In Elbasan area plants are repeatedly exposed to a variety of environmental stressors which affect growth, physiological functions and yield. Industrial pollution and other stresses affect the reduction the activity of leaf photosynthetic apparatus. Among the pollutants that are generated from the processing of scrap in Elbasan are sulfur oxides (SOx), nitrogen oxides (NOx), carbon monoxide (CO), fine particles (PM) as well as dioxins and furans The impact of air pollution from metallurgical plants is analyzed in endemic plant Cercius Silicuastrum different distances from the source of pollution. The chlorophyll (Chl) fluorescence signatures of leaves have been widely applied as non-invasive techniques for the *in vivo* analysis of plant stress. The Chl fluorescence provides ample information on the photosynthetic apparatus as first discovered by Kautsky. Various ratios of the Chl fluorescence determined from the induction kinetics can be used as indicators of the stress effect to the photosynthetic apparatus.

Efficiency of photosynthetic apparatus of analyzed endemic plants grown in different environmental stress conditions was evaluated via chlorophyll fluorescence imaging during induction kinetics and the fluorescence ratios which describe the photosynthetic light processes and quantum conversion of light.

Observed differences on imaging of chlorophyll fluorescence signature and photosynthetic pigment metabolism of leaves allowed to characterize effect of environmental factors on photosynthetic performance as well as to estimate the variations among endemic plants in stress conditions.

The chlorophyll fluorescence induction kinetics (Kautsky effect) of pre-darkened leaves (30 min) was measured using the FluorCam 700MF imaging system (Photon Systems Instrument)

Keywords: *Chlorophyll fluorescence, chlorophyll fluorescence imaging, endemic plant, induction kinetics, photosynthetic apparatus.*

Introduction

Acid rain (a product of air pollution) and soil contamination severely affect trees and plants as well. However, two or more pollutants acting together can have a synergistic effect, producing damage at lower concentrations than if they were acting separately (Colls, 2002). Plants grown in the area of the Metallurgical complex of Elbasan are repeatedly exposed to various stressors especially to air pollution gases (sulfide, dioxide etc), highly reactive oxygen species (O_3, OH, H_2O_2) and dusts (Shallari et al., 1998, Sallaku et al., 1999). The contaminants emitted from this complex impact on the environment causing many problems. Smelters, whose emissions contain toxic gases and dusts rich in heavy metals like Pb, Cu, Zn Cr, Ni and Cd, caused particularly these effects. The soils around metallurgical complex are contaminated by heavy metals such as cadmium, nickel, chromium, lead and copper as consequence of the industrial activity (Shallari et al., 1998, Sallaku et al., 1999)

These stressors can affect the photosynthetic performance of leaves either directly or indirectly modifying their optical and fluorescence properties. The chlorophyll (*Chl*) fluorescence signatures of plants as a rapid, nondestructive diagnostic method allow to investigate the photosynthetic light processes and quantum conversion in plants (Lichtenthaler and Miehe, 1997; Govindjee. 2004; Krause and Weis, 1991; Lichtentaler and

Babani, 2004; Schreiber, 1986).

The objective of the presented work is the evaluation of damage by Metallurgical complex on spontaneous plants (*Cercius Silicuastrum*) grown in sites with different level of air pollution characterizing the photosynthetic performance by chlorophyll fluorescence imaging and performing.

Material and Methods

Plant material

Endemic-spontaneous plant *Cercius Siliquastrum* grown in different steel plant pollution conditions were analyzed. Study areas were chosen for *Cercius Siliquastrum* plants in two locations in Albania: Site 1 –200m area from source of pollution ferrocrom), Site 2 - in a distance of 4km from source of pollution), in two different periods.

Chlorophyll fluorescence imaging of induction kinetics

Chlorophyll (*Chl*) fluorescence induction kinetics was measured using the FluorCam 700MF kinetics imaging system constructed by Photon Systems Instrument (Photon Systems Instruments, 2012). *Chl* fluorescence images and induction kinetics were measured on pre-darkened leaves (30 min) using the FluorCam quenching protocol. The images of the measured Chl fluorescence intensity were obtained on false colour, whereby black is the lowest (zero) and red the highest fluorescence intensity. The fluorescence emission is induced by two sets of 325 super-bright orange light emitting diodes (LED's) (wavelength 605nm) that provide excitation flashes or a continuous actinic irradiance controlled by defined protocol. Fluorescence images are captured by a CCD camera at 12-bit resolution in 512x512 pixels of CCD chip. The size of an analyzed object is up to 13×13 cm.

Chlorophyll fluorescence images of parameters during induction kinetics as F_0 and F_0' (minimum fluorescence in the dark and in the light-adapted states), Fm and Fm' (maximum fluorescence in the dark and in the light-adapted states), Fp (initial fluorescence increase caused by the actinic light exposure) and Fs, (steady-state fluorescence in actinic light exposure) were recorded during induction kinetics.

Images of various Chl fluorescence ratios obtained by pixel to pixel arithmetic operations performed by FluorCam software were: maximum quantum yields of Photosystem II Fv/Fm and Fm/Fo, effective quantum yields of Photosystem II Fv'/Fm' and Fm'/Fo', fluorescence decline ratio in steady-state which assess plant vitality Rfd=(Fp-Fs)/Fs where Fv=Fm-Fo and Fv'=Fm'-Fo'.

Results

Image Fluorescence parameters

Image fluorescence parameters measured during induction kinetics (Tab 1, Tab 2) represent the mean values of six different leaves for the *Cercius Siliquastrum*. Whereby, the values of each parameter of every leaf calculated by FluoCam software, indicate the mean of the fluorescence signals of all pixels over the leaf area. The mean values of image fluorescence parameters demonstrate the differences between leaves grown in different conditions. The observed rising of the values of standard deviations from optimal growth conditions to pollution conditions can be illustrated the increase of the variability through the leaves as the effect to steel plant pollution exposure.

Chlorophyll flurescence decline ratios images – Rfd images

Images of the fluorescence decline ratio Rfd of a green leaf grown in optimal conditions at Site 2 in 4km area - control show almost no irregularities and a uniform distribution of the values of this ratios over the leaf area (Fig. 1, Fig 2).

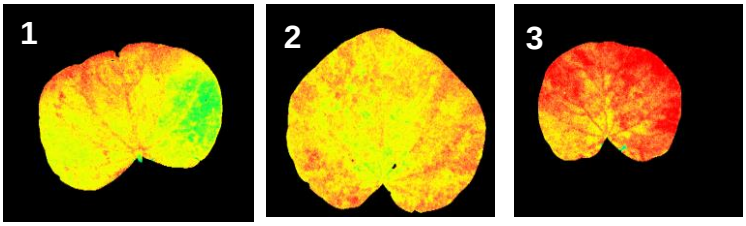

Figure 1 The fluorescence decline ratio image Rfd of a *Cercius Siliquastrum* leaf grown in plan steel air pollution conditions conditions (Site 1- 200m) and (Site 2- 4km) on June.

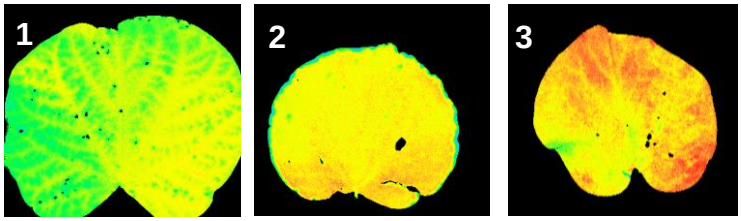

Figure 3 Image fluorescence ratios of *Cercius Siliquastrum* leaves grown in area of plan steel air pollution conditions (Site 1- 200m) and (Site 2- 4km) on October (mean of six leaves).

The values of Rfd ratios as plant vitality indicator demonstrated that these green leaves can be characterized by a high photosynthetic activity on June and October (Tab. 2).

The distribution of the Rfd indices over the leaf area display an increase of the gradients and local irregularities of the leaves grown in plan steel air pollution growth conditions, Site 1 200m area, in comparison of leaves in 4km distance from source of pollution ferrocrom especially in the leaves grown in Site 1, where the level of pollution is higher related to the nearer distance from the metallurgical complex.

Very low Rfd signal identified at local areas on leaves grown in Site 1 and 2 on October show the high reduction of photosynthetic performance as damage of the photosynthetic apparatus and photosynthetic electron transport.

Table 1 Induced fluorescence image parameters of leaves of *Cercius Siliquastrum* leaves grown in area of plan steel air pollution conditions (Site 1- 200m) and (Site 2- 4km) on June and October (mean of six leaves).

Cercius Siliquastrum		**Site 1** (200m)	**Site 4** (4km)	**Site 1** (200m)	**Site 4** (4km)
Image Fluorescence parameters		**June**		**October**	
Fo	mean	143.1	147.1	111.1	114.4
	std	6.94	2.29	13.44	4.12
Fm	mean	469.2	492.1	305.3	415.2
	std	29.27	42.10	36.23	18.09

Table 2 Image fluorescence ratios of *Cercius Siliquastrum* leaves grown in area of plan steel air pollution conditions (Site 1- 200m) and (Site 3- 4km) on June and October (mean of six leaves).

Cercius Siliquastrum		**Site 1** (200m)	**Site 4** (4km)	**Site 1** (200m)	**Site 4** (4km)
Image Fluorescence ratios		**June**		**October**	
Fm/Fo	mean	3.19	3.31	2.77	3.66
	std	0.03	0.08	0.24	0.20
Fv/Fm	mean	0.68	0.69	0.63	0.73
	std	0.01	0.008	0.02	0.01
Rfd	mean	**1.78**	**2.14**	**1.09**	**1.75**
	std	0.14	0.04	0.19	0.09

Conlcusions

- Fluorescence images measured during induction kinetics, values of the fluorescence parameters and distribution of the fluorescence signal and the Rfd values over the leaf area in the leaves of spontaneous plant *Cercius Siliquastrum* grown in distance 4 km, Site 2of source of pollution exhibited a high photosynthetic activity compare to the other areas as is demonstrated by the values of fluorescence ratios which evaluate the plant vitality and maximum quantum yield of photosynthetic apparatus: Rfd=2.14, 1.75 respectively on June and October.
- Activity of photosynthetic apparatus of leaves of *Cercius Siliquastrum* grown in plant steel air pollution conditions in Site 1 area in a distance of 200m from source of pollution ferrocromwas generally lower than activity of plants in other areass is expressed by a non uniform distribution and increase of irregularities of the fluorescence signal at Fm and Rfd ratios over the leaf area as well as by the values of the fluorescence ratios: Rfd=1.78, 1.09 respectively on June and October.

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