

## Research Article

## Evaluation of the changes of chlorophyll a fluorescence factors in the symbiosis of rosemary plants with mycorrhiza under traffic

Zahra Alinejad<sup>1</sup>, Seyed Ali Abtahi<sup>1\*</sup>, Mojtaba Jafarinia<sup>2</sup>, Jafar Yasrebi<sup>3</sup>

<sup>1</sup>Department of Soil Science, Marvdasht Branch, Islamic Azad University, Marvdasht, Iran

<sup>2</sup>Department of Biology, Marvdasht Branch, Islamic Azad University, Marvdasht, Iran

<sup>3</sup>Department of Soil Science, College of Agriculture, Shiraz University, Shiraz, Iran

(Received: 2023/05/29-Accepted: 2023/12/02)

### Abstract

The phenomenon of pollution emerges as an outcome of progress in industrialization and urban development. A substantial number of heavy metals, which possess the potential to impact both human well-being and the ecological surroundings, are found in traffic dust. Consequently, the discharge of heavy metals from automobiles emerges as a principal contributor to the contamination of metal substances in urban settings, owing to the heightened traffic volume. The presence of heavy metals can adversely influence photosystem reactions, disrupt the normal course of photosynthetic reactions, and ultimately lead to a decline in plant performance. Moreover, the establishment of mutualistic associations between mycorrhizal fungi and plants has the potential to further diminish pollution by inducing alterations and regulation of plant physiological mechanisms. This study was done to evaluate the effects of *Mycorrhiza symbiosis* on chlorophyll a fluorescence measurements in the Rosemary plant grown in high-traffic area of Shiraz city, Fars Province, Iran. The study was performed as a factorial and in the form of a randomized complete block design (RCBD) with three replicas and three factors. The first factor included *Mycorrhiza symbiosis* (control and inoculation); the second factor was traffic (control, 120, 300, 600, 950, 1200, 1800, 2400, 3000, 3600 and 4200 vehicles/h); and the third factor was covering the pot's soil (control and cover). At the end of the experiment, some properties were studied such as by using the OJIP-test, various parameters like Area, F<sub>o</sub>/F<sub>m</sub>, F<sub>v</sub>/F<sub>m</sub>, N, Sm/t (F<sub>m</sub>),  $\psi_o P_o$ ,  $\psi_o E_o$ , and  $\psi_o P_o / (1 - \psi_o P_o)$ . The results showed that inoculation led to increasing of Area, F<sub>v</sub>/F<sub>m</sub>, N,  $\psi_o P_o$ ,  $\psi_o E_o$ , and  $\psi_o P_o / (1 - \psi_o P_o)$  with 57, 1, 7, 2, 25 and 33% by compare to control, respectively. According to the results, it was found that the effects of inoculation, traffic and cover factors were significant at the 1% statistical level on the studied traits. Based on the results of this experiment, it was found that plants inoculated with mycorrhizal fungi had a higher efficiency of the biochemical reaction of electron transfer compared to the control. Rosemary plants were located in the traffic paths of 0 and 120 vehicles/h and had a higher efficiency of the biochemical reaction of electron transfer. Plants that were in the path of 3000, 3600, and 4200 vehicles/h were significantly different in terms of the efficiency of the biochemical reaction of electron transfer with the control treatment (without traffic).

**Keywords:** Chlorophyll a fluorescence, Photosynthesis, Traffic, Pollution

### Introduction

In recent decades, plants have been exposed to high levels of air pollution due to increased urbanization and traffic, as well as dust storms, which have worsened weather conditions. PM<sub>10</sub>, SO<sub>2</sub>, NO<sub>2</sub>, HC, O<sub>3</sub>, and CO are the main air pollutants in big cities (Mohammadi *et al.*, 2016). Heavy metals in street dust are one of the main pollutants in urban environments (Bozdogan Sert *et al.*, 2019). Urban plants are capable of reducing environmental pollution through bioaccumulation of contaminants in their tissues (Hu *et al.*, 2014). One of these plants is rosemary. *Rosmarinus officinalis* L. leaves are used as a decorative plant in gardens and for many medical uses. Also, it is used to flavor foods such

as roast meats. Due to the natural abundance of this plant in the environment and especially its cheapness in Iran, it can be used as an urban plant. Heavy metals are known to interfere with chlorophyll synthesis either through direct inhibition of an enzymatic step or by inducing deficiency of an essential nutrient (Zengin and Munzuroglu, 2005). Pollution, however, exerts detrimental impacts on the efficacy of plants in urban areas, specifically in terms of their growth, biomass, and rate of photosynthesis. For this purpose, some factors can be effective on the efficiency of plants; one of them is bio-fertilizers such as mycorrhizal fungi. Mycorrhizal fungi support plant nutrition by absorbing and translocating mineral nutrients beyond the depletion

\*Corresponding Author, Email: seyedaliabtahi@yahoo.com

zones of the plant rhizosphere and inducing changes in secondary metabolism, leading to improved nutraceutical compounds. In addition, it interferes with the phytohormone balance of host plants, thereby influencing plant development and inducing tolerance to soil and environmental stresses (Rouphael *et al.*, 2015). The amount of carbon fixation by mycorrhizal symbiosis is much higher than the fungus needs, so mycorrhizal fungi increase the rate of photosynthesis in their symbiotic plant (Reid *et al.*, 1983; Lendenmann *et al.*, 2011). Mycorrhiza can increase the rate of net photosynthesis in the host plant by increasing the phosphorus concentration (Choi *et al.*, 2005). Also, in research under water stress conditions, the rate of net photosynthesis in inoculated *Helianthus* plants was about twice that of the control (Morte *et al.*, 2000). Hence, the aim of this study was the evaluation of Shiraz traffic's effect on chlorophyll fluorescence measurements of the *Rosemary* plant under mycorrhiza symbiosis conditions.

### Material and methods

Shiraz, the center of Fars Province, located in the southwestern part of Iran, is one of the seven most crowded cities in the country, with more than 1500000 populations. This city covers an area of 1268 sq. km<sup>2</sup> with an altitude of 1540 meters above the mean sea level and is located at latitude 29.37 N and longitude 52.32 E.

This study was performed as a factorial and in the form of a randomized complete block design (RCBD) with three replicas and three factors. The first factor included *Mycorrhiza symbiosis* (control and inoculation); the second factor was traffic (control, 120, 300, 600, 950, 1200, 1800, 2400, 3000, 3600 and 4200 vehicles/h); and the third factor was covering the pot's soil (control and cover). Initially, root cuttings with a length of 15-20 cm and a diameter of 0.5 cm were prepared from the mother stocks of rosemary, all of which grew in the same conditions for rooting in greenhouse. Pots with a diameter of 22 cm were filled with soil, leaf litter and sand in a ratio of 1-1-1. The analysis of the physical and chemical properties of soil is shown in Table 1. In each pot, 3 rosemary rooted cuttings were planted. 30 g of inoculum for each seedling (10 cm transplant) were spread in each pot around the roots of the seedlings and covered with soil. The prepared pots were moved to selected locations. The pots remained in the selected locations for 9 months. Heavy metals present in street dust serve as a primary source of contamination for urban surroundings, resulting from the heavy volume of traffic, the wearing down of tires, and the utilization of vehicle components. Certain contaminants permeate the plant tissue by means of the stomatal pores, while other atmospheric pollutants may eventually infiltrate the soil, subsequently becoming accessible to the roots and

entering the aerial organs through deposition. Half of the experimental pots were covered with a plastic covering over the soil, with the exception being the placement of three rosemary cuttings.

**The amount of traffic:** The traffic volume at the selected points was calculated using the following formula:

$AADT = \text{annual average daily traffic volume of motor vehicles}$

The total volume of traffic passing through a given section or point of a road in a year is divided by 365 days for the selected points.

$ADT = \text{average daily traffic volume of motor vehicles}$

It is the average number of motor vehicles that pass a given point in 24 hours on both sides of the road and is usually calculated for a year. Table 1 shows the annual average volume of daily traffic in selected points.

**Chl *a* fluorescence measurement:** Chl *a* fluorescence was measured at room temperature with the Plant Efficiency Analyzer (HandyPEA, Hansatech Instruments Ltd., King's Lynn, UK). The fluorescence-activating light was provided by an array of three light-emitting diodes, which were focused onto the leaf surface to provide homogenous illumination. All measurements were performed on the upper surface of the fully expanded leaves by using the leaf clips. All samples were dark-adapted for 30 min prior the measurements. The leaves exhibit the Chl *a* fluorescence rise during the first second of illumination after the dark adaptation period. The fluorescence signals were detected by a high-performance PIN photodiode detector, received by the sensor head during recording, and digitized in the control unit using a fast Analogue/digital converter. Every measurement record 118 data points (Handy PEA manual user's guide). These data were analyzed and conducted using the software Biolyzer 4HP (The fluorescence analyzing program by Bioenergetics Laboratory, University of Geneva, Switzerland). In the present study, among the various parameters that were calculated according to OJIP test (Table 2), our focus was on the parameters that were significantly affected by traffic.

Data analyzed by SAS software and mean comparison performed by Duncan test at 5% statistically level (Littell *et al.*, 1988).

### Results and discussion

According to the analysis of variances, it was found that the effect of inoculation had a significant effect on area, N, Sm/t (Fm), and  $\psi_{O_{E_0}}$  at the 1% statistically level (Table 3). Another factor, traffic, had a significant effect on Fo/Fm, Fv/Fm,  $\psi_{O_{P_0}}$  at 5%, Sm/t (Fm),  $\psi_{O_{E_0}}$ , and  $\psi_{O_{P_0}} / (1 - \psi_{O_{P_0}})$  at 1% statistically level (Table 3). In relation to cover treatment, this factor had a significant effect on N and Sm/t (Fm) at the 5% and 1% statistical

Table 1. Results of the soil's analysis of pots

Parameters	Unit	results
(EC)	dS/m	1.79
(pH)	-	7.51
(Ca)	meq/l	9
(Mg)	meq/l	3.5
(Na)	meq/l	4.0
(SAR)	-	1.6
(K)	mg/kg	200
(P)	mg/kg	15
(Clay)	%	30
(Silt)	%	30
(Sand)	%	40
(Ca/Mg)	-	2.57
(Fe)	mg/kg	2.57
(Zn)	mg/kg	0.41
(Cu)	mg/kg	0.22
(Mn)	mg/kg	1.78
(Cd)	mg/kg	0.005
(Pb)	mg/kg	0.037

Table 2. Summary of the JIP-test formulae using data extracted from the OJIP chlorophyll a fluorescence transient  
Extracted and technical fluorescence parameters

$F_0 = F_{50\mu s}$ , fluorescence intensity at 50  $\mu s$   
 $F_J$  = fluorescence intensity at the J-step (at 2ms)  
 $F_I$  = fluorescence intensity at the I-step (at 30 ms)  
 $F_M$  = maximal fluorescence intensity  
 $T_{FM}$  = time to reach  $F_m$  (ms)  
 $VJ$  = relative variable at the J-step =  $(F_{2ms} - F_0) / (F_M - F_0)$   
 $Area$  = area between fluorescence curve and  $F_M$   
 $F_v/F_0$  = activity of the water-splitting complex  
 $dV/dt_0 = M_0 = 4 \cdot (F_{300} - F_0) / (F_M - F_0)$   
 $Sm = Area / (F_M - F_0)$   
 $N$  = turnover number of QA  
**Quantum efficiencies or flux ratios or yields**  
 $\phi_{Po} = TR_o / ABS = [1 - (F_0 / F_M)] = F_v / F_M$   
 $\phi_{Eo} = ET_o / ABS = [1 - (F_0 / F_M)] \cdot \psi_o$   
 $\psi_o = ET_o / TR_o = (1 - VJ)$   
**Specific fluxes or specific activities**  
 $ABS/RC = Mo \cdot (1/VJ) \cdot (1 - \phi_{Po})$   
 $TR_o / RC = Mo \cdot (1 / VJ)$   
 $ET_o / RC = Mo \cdot (1 / VJ) \cdot \psi_o$   
 $DI_o / RC = (ABS / RC) - (TR_o / RC)$   
**Phenomenological fluxes or phenomenological activities**  
 $ABS / CS = ABS/CSChl = Chl/CS$  or  $ABS/CS_o = F_o$  or  $ABS/CSM = FM$   
 $TR_o / CS = \phi_{Po} \cdot (ABS / CS)$   
 $ET_o / CS = \phi_{Po} \cdot \psi_o \cdot (ABS / CS)$   
 $DI_o / CS = (ABS/CS) - (TR_o / CS)$   
**Density of reaction centres**  
 $RC / CS = \phi_{Po} \cdot (VJ / Mo) \cdot ABS/CS$   
**Performance indexes**  
 $PIABS = (RC/ABS) \cdot [\phi_{Po} / (1 - \phi_{Po})] \cdot [\psi_o / (1 - \psi_o)]$   
 $PICS = (RC/CS) \cdot [\phi_{Po} / (1 - \phi_{Po})] \cdot [\psi_o / (1 - \psi_o)]$   
**Driving forces**  
 $DFABS = \log (PIABS)$   
 $DFCS = \log (PICS) = \log (PIABS) + \log (ABS/CS)$

$ABS$ , absorption energy flux;  $CS$ , excited energy cross-section of leaf sample;  $DI$ , dissipation energy flux at the level of the antenna chlorophyll;  $ET$ , flux of electron from  $Q_A^-$  into the electron transport chain;  $\phi_{Do}$ , quantum yield of dissipation;  $\phi_{Eo}$ , probability that an absorbed photon will move an electron into electron transport further than  $Q_A^-$ ;  $\phi_{Po}$ , maximum quantum yield of primary photochemistry;  $PIABS$ , performance index;  $\psi_o$ , efficiency by which a trapped excitation, having triggered the reduction of  $QA$  to  $Q_A^-$ , can move an electron further than  $Q_A^-$  into the electron transport chain;  $RC$ , reaction center of PSII;  $RC/CS$ , fraction of active reaction centers per excited cross-section of leaf;  $TR$ , excitation energy flux trapped by a  $RC$  and utilized for the reduction of  $QA$  to  $Q_A^-$

**Table 3. Analysis of variances (mean squares) for experiment factors**

Source of variation	d.f	mean squares							
		Area	Fo/Fm	Fv/Fm	N	Sm/t(Fm)	$\psi_o Po$	$\psi_o Eo$	$\psi_o Po/(1 - \psi_o Po)$
Replication	2	11598975	0.00001	1.05	17.114	0.00003	0.0003	0.0008	0.031
Inoculation (A)	1	113727667**	0.001	0.001	212.954**	0.003**	0.001	0.033**	0.215
Traffic (B)	10	22271940*	0.001*	0.001*	14.968	0.004**	0.001*	0.013**	0.266**
(A) × (B)	10	48713138**	0.001	0.001	85.235**	0.001**	0.001	0.002*	0.096
Cover (C)	1	5968140	0.0003	0.0002	33.502*	0.002**	0.00001	0.0002	0.039
(A) × (C)	1	33409607	0.0002	0.0001	17.107	0.0001	0.00002	0.001	0.128
(B) × (C)	10	29628496**	0.001**	0.001**	11.719	0.005**	0.001**	0.006**	0.408**
(A) × (B) × (C)	10	28827130	0.001**	0.001**	10.908	0.0001	0.001**	0.013**	0.298**
Error	86	9497485	0.00037	0.0004	8.507	0.0001	0.0003	0.001	0.062
C.V		12.07	9.036	2.3	10.23	9.27	2.62	8.32	6.19

\*and\*\* show significant effects at 5 and 1% statistically levels. Area between fluorescence curve and F = FM (The upper level of the fluorescence intensity), Fo/Fm: Minimal fluorescence (all PSII RCs are assumed to be open)/Maximal fluorescence, when all PSII RCs are closed, Fv/Fm: Maximal variable fluorescence/Maximal fluorescence, when all PSII RCs are closed, N: The number of turnovers, Sm/t (Fm): Normalized total area above the OJIP curve,  $\psi_o Po$ : Maximum quantum yield for primary photochemistry,  $\psi_o Eo$ : Efficiency/probability for electron transport (ET),  $\psi_o Po/(1 - \psi_o Po)$ : Quantum efficiencies or flux ratios or yields.

levels, respectively. The responses of all traits were significant to the interaction of treatments except area, N and Sm/t (Fm). The results obtained from the study of chlorophyll *a* fluorescence parameter in rosemary, show that the placement of these plants in the traffic lane of vehicles, especially busy lanes, disrupts the function of the photosynthetic system, which is in line with the results of (Zengin and Munzuroglu, 2005) in soybean and (Poorakbar and Ebrahimzade, 2012) in beans. Based on the results of this experiment, it was found that inoculation of rosemary plants significantly affected and increased the fluorescence kinetics of chlorophyll *a*. The results showed that inoculation led to increasing of Area, Fv/Fm, N,  $\psi_o Po$ ,  $\psi_o Eo$ , and  $\psi_o Po/(1 - \psi_o Po)$  with 57, 1, 7, 2, 25 and 33% by compare to control, respectively. In relation to traffic, there were found that traffic led to significant changes on area, Fv/Fm, N,  $\psi_o Po$ ,  $\psi_o Eo$ ,  $\psi_o Po/(1 - \psi_o Po)$  by comparing to control. Figure 1 shows the relationship between traffic and the studied fluorescence parameters. Soil cover treatment led to an increase of N with 4% and this treatment led to the increase of Sm/t (Fm) of 13%. Another parameter shows no significant response to pot cover compared to the control (Table 4). The Area parameter indicates the amount of electron acceptors, including QA, QB and plastoquinone, which in this study has decreased with the increase in traffic in the path of rosemary plants. Any factor that affects these acceptors will affect the amount of Area (Dewez *et al.*, 2007).

The Fv/Fm index indicates the initial efficiency of photosystem II (Kocheva *et al.*, 2004). The results of this study showed that the placement of rosemary plants in the paths of 3000, 3600 and 4200 cars per hour reduced the activity of the water decomposing complex (Fv/Fo). The  $\phi Po$  parameter is proportional to Fv/Fm and TR / ABS and expresses the maximum efficiency of PSII. According to Zhang *et al.* (2010), the decrease in  $\phi Po$  is under stress due to optical inhibition, but if the excited energy surplus cannot be dispersed and redistributed, PSII will be more exposed to light

damage. According to the interaction of treatments, the highest mean of area was obtained by inoculation×0 cars/h ×covered with 31740 value, and the lowest mean was observed by non-Inoculation ×4200 cars/h ×non-covered with 17160 value (Table 4). According to the findings, the inoculation× 0 cars/h ×non-covered treatment exhibited the highest mean value of Fo/Fm at 0.24, whereas the non-Inoculation ×4200 cars/h ×non-covered treatment displayed the lowest mean value at 0.18. In continuation, the 0 cars/h × covered treatment yielded the highest mean value of Fv/Fm with a value of 0.82, while the lowest mean value was observed in the 4200 cars/h × non-covered treatment and the inoculation or non-inoculation × 1800-3600 cars/h treatment, both with a value of 0.19. The Fv/Fm ratio is frequently employed as an indicator of inhibition or other forms of damage to the PSII complex (Rohacek, 2002). According to the interaction of inoculation × traffic × covered, the highest mean of N was obtained by inoculation×0 cars/h × covered with 37.1 value, and the lowest mean was observed by non-Inoculation ×4200 cars/h ×non-covered with 23.4 value. In relation to Sm/t (Fm), the highest mean of Sm/t (Fm) was obtained by inoculation×0 cars/h ×covered and inoculation treatment ×4200 cars/h ×non-covered with 0.4 value, and the lowest mean was observed by non-Inoculation×4200 cars/h ×non-covered with 0.39 value (Table 4).

According to interaction of 3 factors, the highest mean of  $\psi_o Po$  was obtained by inoculation×0 cars/h × covered with a 0.82 value, and the lowest mean was observed by non-Inoculation ×4200 cars/h × non-covered with 0.76 value. Reduction of  $\phi Po$  under stress can be due to the inactivation of reaction centers, which increases energy loss in the form of heat and fluorescence, and ultimately reduces electron transfer from P680 to photosystem I (Hermans *et al.*, 2003). Decreasing  $\phi Po$  leads to decreasing  $\phi Eo$  and  $\psi_o$  (Zhang *et al.*, 2010). The decline in the highest attainable quantum efficiency of Photosystem II ( $\phi Po$ ) and the reduction in  $\psi_o$  suggest that rosemary plants without

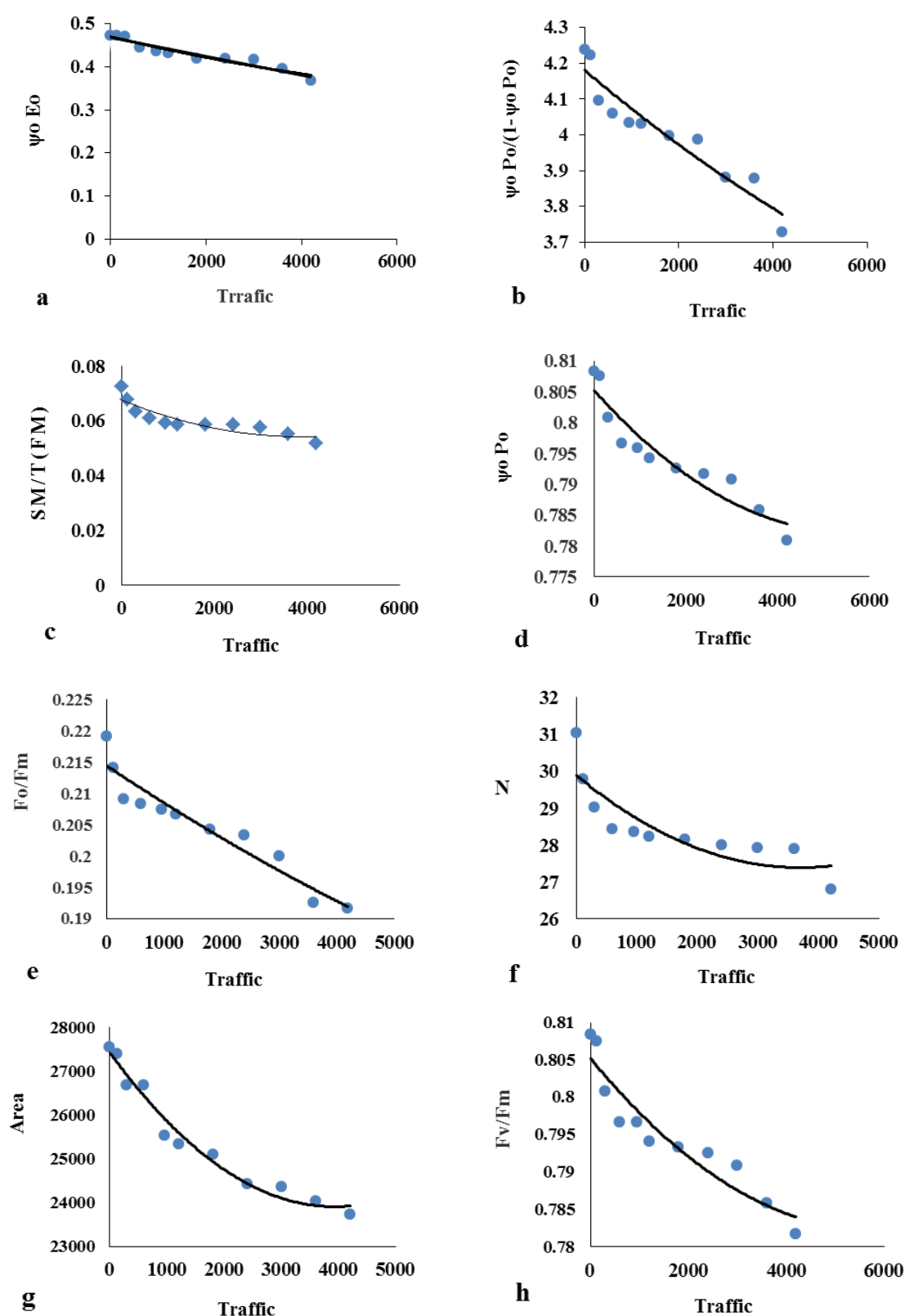


Figure 1. The relationship between traffic and studied fluorescence parameters. a)  $\psi_o E_o$ : Efficiency/probability for electron transport (ET), b)  $\psi_o P_o / (1 - \psi_o P_o)$ : Quantum efficiencies or flux ratios or yieble, c)  $SM/t(F_m)$ : Normalized total area above the OJIP curve, d)  $N$ : The number of turnovers, e)  $F_o/F_m$ : Minimal fluorescence (all PSII RCs are assumed to be open)/Maximal fluorescence, when all PSII RCs are closed, f)  $\psi_o P_o$ : Maximum quantum yield for primary photochemistry g) Area: area between fluorescence curve and  $F_m$ , h)  $F_v/F_m$ : Maximal variable fluorescence/Maximal fluorescence, when all PSII RCs are closed

**Table 4. Mean comparisons for studied trait in response to treatment's interaction effects (Duncan test at 5% statistically level)**

			Area	Fo/Fm	Fv/Fm	N	Sm/t(Fm)	ψo Po	ψo Eo	ψo Po/ (1-ψo Po)
Inoculation	Control	Covered	29910 <sup>a-c</sup>	0.24 <sup>a</sup>	0.82a <sup>c</sup>	37.11 <sup>a</sup>	0.6567 <sup>a</sup>	0.82 <sup>a</sup>	0.5333 <sup>a</sup>	4.559 <sup>a</sup>
		Non-covered	28760 <sup>a-e</sup>	0.24 <sup>a</sup>	0.81 <sup>a</sup>	34.05 <sup>abc</sup>	0.6267 <sup>ab</sup>	0.8133 <sup>bc</sup>	0.5133 <sup>ab</sup>	4.359 <sup>ab</sup>
	120	Covered	29910 <sup>a-c</sup>	0.23 <sup>b</sup>	0.81 <sup>ab</sup>	33.38 <sup>a-d</sup>	0.62 <sup>abc</sup>	0.81 <sup>cd</sup>	0.4933 <sup>a-d</sup>	4.336 <sup>ab</sup>
		Non-covered	25890 <sup>a-h</sup>	0.23 <sup>b</sup>	0.81 <sup>ab</sup>	32.57 <sup>a-f</sup>	0.6167 <sup>abc</sup>	0.81 <sup>cd</sup>	0.49 <sup>a-e</sup>	4.237 <sup>abc</sup>
	300	Covered	29480 <sup>a-d</sup>	0.23 <sup>b</sup>	0.81 <sup>ab</sup>	31.37 <sup>a-f</sup>	0.6 <sup>a-d</sup>	0.81 <sup>cd</sup>	0.4867 <sup>a-f</sup>	4.237 <sup>abc</sup>
		Non-covered	26730 <sup>a-h</sup>	0.23 <sup>b</sup>	0.81 <sup>c-e</sup>	30.22 <sup>a-g</sup>	0.57 <sup>a-f</sup>	0.81 <sup>cd</sup>	0.46 <sup>b-h</sup>	4.225 <sup>abc</sup>
	600	Covered	28430 <sup>a-e</sup>	0.21 <sup>c-d</sup>	0.81 <sup>e</sup>	29.5 <sup>a-g</sup>	0.57 <sup>a-f</sup>	0.8067 <sup>de</sup>	0.46 <sup>b-h</sup>	4.214 <sup>abc</sup>
		Non-covered	24690 <sup>c-h</sup>	0.23 <sup>b</sup>	0.81 <sup>a-c</sup>	28.22 <sup>b-h</sup>	0.5667 <sup>b-f</sup>	0.8067 <sup>de</sup>	0.4533 <sup>b-i</sup>	4.203 <sup>abc</sup>
	950	Covered	25120 <sup>b-h</sup>	0.2 <sup>e-h</sup>	0.8 <sup>ab</sup>	27.84 <sup>b-h</sup>	0.5633 <sup>b-f</sup>	0.8033 <sup>ef</sup>	0.45 <sup>c-i</sup>	4.203 <sup>abc</sup>
		Non-covered	23470 <sup>d-h</sup>	0.23 <sup>b-c</sup>	0.8 <sup>ab</sup>	27.65 <sup>b-j</sup>	0.5567 <sup>b-f</sup>	0.8033 <sup>ef</sup>	0.43 <sup>c-j</sup>	4.155 <sup>abc</sup>
	1200	Covered	25380 <sup>d-h</sup>	0.2 <sup>e-h</sup>	0.8 <sup>ab</sup>	26.79 <sup>b-j</sup>	0.54 <sup>b-g</sup>	0.8033 <sup>ef</sup>	0.4267 <sup>f-j</sup>	4.155 <sup>abc</sup>
		Non-covered	23480 <sup>d-h</sup>	0.22 <sup>b-j</sup>	0.8 <sup>a-e</sup>	26.64 <sup>b-j</sup>	0.54 <sup>b-g</sup>	0.8033 <sup>ef</sup>	0.42 <sup>g-k</sup>	4.091 <sup>a-d</sup>
	1800	Covered	23350 <sup>d-h</sup>	0.2 <sup>e-h</sup>	0.8 <sup>b-e</sup>	26.64 <sup>c-j</sup>	0.53 <sup>c-g</sup>	0.8033 <sup>ef</sup>	0.42 <sup>g-k</sup>	4.067 <sup>a-d</sup>
		Non-covered	22610 <sup>c-i</sup>	0.19 <sup>b-j</sup>	0.79 <sup>c-e</sup>	26.64 <sup>c-j</sup>	0.5167 <sup>d-g</sup>	0.7967 <sup>gh</sup>	0.4167 <sup>g-k</sup>	3.986 <sup>b-e</sup>
	2400	Covered	23620 <sup>d-h</sup>	0.19 <sup>f-h</sup>	0.78 <sup>a-c</sup>	26.5 <sup>c-j</sup>	0.5167 <sup>d-g</sup>	0.79 <sup>i</sup>	0.4167 <sup>g-k</sup>	3.945 <sup>b-e</sup>
		Non-covered	22070 <sup>f-i</sup>	0.19 <sup>b-j</sup>	0.77 <sup>c-e</sup>	26.04 <sup>d-j</sup>	0.5167 <sup>d-g</sup>	0.78 <sup>j</sup>	0.4133 <sup>g-k</sup>	3.876 <sup>b-f</sup>
	3000	Covered	21690 <sup>g-i</sup>	0.19 <sup>b-j</sup>	0.77 <sup>c-e</sup>	25.14 <sup>f-j</sup>	0.5167 <sup>d-g</sup>	0.77 <sup>k</sup>	0.4133 <sup>g-k</sup>	3.752 <sup>c-g</sup>
		Non-covered	21690 <sup>g-i</sup>	0.19 <sup>b-j</sup>	0.77 <sup>ab</sup>	25.14 <sup>f-j</sup>	0.4933 <sup>fgh</sup>	0.77 <sup>k</sup>	0.4 <sup>h-k</sup>	3.752 <sup>c-g</sup>
	3600	Covered	20810 <sup>hi</sup>	0.19 <sup>b-j</sup>	0.77 <sup>c-e</sup>	25.14 <sup>hi-j</sup>	0.4933 <sup>fgh</sup>	0.77 <sup>k</sup>	0.3767 <sup>jk</sup>	3.742 <sup>c-g</sup>
		Non-covered	21690 <sup>g-i</sup>	0.19 <sup>j</sup>	0.77 <sup>ab</sup>	24.66 <sup>hi-j</sup>	0.46 <sup>ghi</sup>	0.77 <sup>k</sup>	0.36 <sup>kl</sup>	3.578 <sup>efg</sup>
	4200	Covered	21690 <sup>hi</sup>	0.19 <sup>j-i</sup>	0.77 <sup>a-c</sup>	23.71 <sup>j</sup>	0.46 <sup>ghi</sup>	0.76 <sup>l</sup>	0.36 <sup>kl</sup>	3.508 <sup>efg</sup>
		Non-covered	17160 <sup>g-i</sup>	0.18 <sup>j</sup>	0.74 <sup>de</sup>	23.71 <sup>j</sup>	0.4 <sup>i</sup>	0.7567 <sup>l</sup>	0.32 <sup>l</sup>	3.508 <sup>efg</sup>
Non-Inoculation	Control	Covered	31740 <sup>ab</sup>	0.22 <sup>b-c</sup>	0.82 <sup>a-c</sup>	34.1 <sup>ab</sup>	0.62 <sup>abc</sup>	0.8167 <sup>ab</sup>	0.5 <sup>abc</sup>	4.516 <sup>a</sup>
		Non-covered	31000 <sup>a</sup>	0.24 <sup>a</sup>	0.81 <sup>a-c</sup>	33.1 <sup>ab</sup>	0.62 <sup>abc</sup>	0.8133 <sup>bc</sup>	0.5 <sup>abc</sup>	4.336 <sup>ab</sup>
	120	Covered	31050 <sup>a-e</sup>	0.22 <sup>b-c</sup>	0.81 <sup>a-c</sup>	32.88 <sup>abc</sup>	0.62 <sup>abc</sup>	0.81 <sup>cd</sup>	0.5 <sup>abc</sup>	4.234 <sup>abc</sup>
		Non-covered	28430 <sup>ab</sup>	0.23 <sup>b</sup>	0.8 <sup>a-c</sup>	32.2 <sup>a-e</sup>	0.59 <sup>a-e</sup>	0.81 <sup>cd</sup>	0.4767 <sup>a-g</sup>	4.225 <sup>abc</sup>
	300	Covered	31240 <sup>a-e</sup>	0.2 <sup>d-f</sup>	0.8 <sup>a-c</sup>	32.2 <sup>b-g</sup>	0.58 <sup>a-f</sup>	0.81 <sup>cd</sup>	0.4733 <sup>a-g</sup>	4.225 <sup>abc</sup>
		Non-covered	28620 <sup>ab</sup>	0.21 <sup>c-e</sup>	0.8 <sup>a-c</sup>	31.8 <sup>b-h</sup>	0.58 <sup>a-f</sup>	0.8067 <sup>de</sup>	0.46 <sup>b-h</sup>	4.225 <sup>abc</sup>
	600	Covered	28740 <sup>a-e</sup>	0.2 <sup>e-h</sup>	0.8 <sup>a-c</sup>	31.72 <sup>b-i</sup>	0.5767 <sup>a-f</sup>	0.8067 <sup>de</sup>	0.46 <sup>b-h</sup>	4.217 <sup>abc</sup>
		Non-covered	27960 <sup>a-f</sup>	0.2 <sup>e-g</sup>	0.8 <sup>a-c</sup>	30.28 <sup>c-j</sup>	0.57 <sup>a-f</sup>	0.8033 <sup>ef</sup>	0.46 <sup>b-h</sup>	4.155 <sup>abc</sup>
	950	Covered	28040 <sup>a-f</sup>	0.2 <sup>e-h</sup>	0.8 <sup>a-d</sup>	29.94 <sup>c-j</sup>	0.57 <sup>a-f</sup>	0.8033 <sup>ef</sup>	0.46 <sup>b-h</sup>	4.093 <sup>a-d</sup>
		Non-covered	26020 <sup>a-h</sup>	0.19 <sup>f-h</sup>	0.8 <sup>a-e</sup>	29.08 <sup>c-j</sup>	0.57 <sup>a-f</sup>	0.8 <sup>fg</sup>	0.46 <sup>b-h</sup>	4.089 <sup>a-d</sup>
	1200	Covered	27260 <sup>a-g</sup>	0.2 <sup>e-h</sup>	0.8 <sup>c-e</sup>	28.89 <sup>c-j</sup>	0.5667 <sup>b-f</sup>	0.8 <sup>fg</sup>	0.46 <sup>b-h</sup>	4.089 <sup>a-d</sup>
		Non-covered	25120 <sup>b-h</sup>	0.19 <sup>f-h</sup>	0.8 <sup>ab</sup>	28.89 <sup>c-j</sup>	0.5567 <sup>b-f</sup>	0.8 <sup>fg</sup>	0.45 <sup>c-i</sup>	4.089 <sup>a-d</sup>
	1800	Covered	26090 <sup>a-h</sup>	0.19 <sup>f-h</sup>	0.8 <sup>e</sup>	27.84 <sup>f-j</sup>	0.55 <sup>b-f</sup>	0.8 <sup>fg</sup>	0.4333 <sup>d-j</sup>	4.067 <sup>a-d</sup>
		Non-covered	25380 <sup>b-h</sup>	0.19 <sup>f-h</sup>	0.8 <sup>a-c</sup>	27.8 <sup>f-j</sup>	0.54 <sup>b-g</sup>	0.8 <sup>fg</sup>	0.4267 <sup>f-j</sup>	3.986 <sup>b-e</sup>
	2400	Covered	24280 <sup>c-h</sup>	0.19 <sup>g-i</sup>	0.8 <sup>a-c</sup>	27.73 <sup>f-j</sup>	0.5333 <sup>c-g</sup>	0.8 <sup>fg</sup>	0.42 <sup>g-k</sup>	3.986 <sup>b-e</sup>
		Non-covered	23960 <sup>c-h</sup>	0.19 <sup>f-h</sup>	0.79 <sup>b-e</sup>	27.23 <sup>g-j</sup>	0.5167 <sup>d-g</sup>	0.7933 <sup>hi</sup>	0.4133 <sup>g-k</sup>	3.977 <sup>b-e</sup>
	3000	Covered	24690 <sup>c-h</sup>	0.19 <sup>g-i</sup>	0.79 <sup>a-c</sup>	26.5 <sup>hi-j</sup>	0.5033 <sup>efg</sup>	0.7833 <sup>j</sup>	0.3933 <sup>ijk</sup>	3.812 <sup>c-g</sup>
		Non-covered	24280 <sup>c-h</sup>	0.19 <sup>f-h</sup>	0.78 <sup>ab</sup>	26.5 <sup>hi-j</sup>	0.4933 <sup>fgh</sup>	0.78 <sup>j</sup>	0.3933 <sup>ijk</sup>	3.639 <sup>d-g</sup>
	3600	Covered	24690 <sup>c-h</sup>	0.19 <sup>h-j</sup>	0.78 <sup>b-e</sup>	25.14 <sup>hi-j</sup>	0.49 <sup>gh</sup>	0.78 <sup>j</sup>	0.3867 <sup>jk</sup>	3.578 <sup>efg</sup>
		Non-covered	24280 <sup>c-h</sup>	0.19 <sup>g-i</sup>	0.78 <sup>b-e</sup>	24.66 <sup>hi-j</sup>	0.41 <sup>hi</sup>	0.77 <sup>k</sup>	0.32 <sup>l</sup>	3.574 <sup>efg</sup>
	4200	Covered	22070 <sup>i</sup>	0.18 <sup>j-i</sup>	0.77 <sup>a-e</sup>	23.42 <sup>ij</sup>	0.4 <sup>i</sup>	0.77 <sup>k</sup>	0.32 <sup>l</sup>	3.43 <sup>fg</sup>
		Non-covered	20810 <sup>f-i</sup>	0.19 <sup>g-i</sup>	0.75 <sup>a-c</sup>	23.42 <sup>ij</sup>	0.39 <sup>i</sup>	0.7567 <sup>l</sup>	0.3033 <sup>l</sup>	3.386 <sup>g</sup>

The mean with the common alphabet shows no significant difference in each column. Area: area between the fluorescence curve and F = FM, Fo/Fm: Minimal fluorescence (all PSII RCs are assumed to be open)/Maximal fluorescence, when all PSII RCs are closed Fv/Fm: Maximal variable fluorescence/maximum fluorescence, when all PSII RCs are closed, N: The number of turnovers, Sm/t(Fm): Normalized total area above the OJIP curve,  $\psi_o$  Po: Maximum quantum yield for primary photochemistry,  $\psi_o$  Eo: Efficiency/probability for electron transport (ET),  $\psi_o$  Po/(1-  $\psi_o$  Po): Quantum efficiencies or flux ratios or yields.

mycorrhiza suppress the oxidation, post-QA reduction, and electron transfer reactions that occur between QA and QB (Schansker *et al.*, 2005). The disparity between the gathering of light and the decline in electron transfer following QA is demonstrated by a decline in the capacity to transfer electrons from QA<sup>-</sup> to QB (Lepedus *et al.*, 2010), which in turn leads to a decrease in the origin of plastoquinone (Force *et al.*, 2003). The findings of Kruger *et al.* (2014) pertaining to soybean (*Glycine max*) subjected to a prolonged period of cold stress during the night for a duration of three nights, suggest a reduction in the parameter  $\psi_o$ . In accordance with the interaction of the various treatments, the highest mean value of  $\psi_o$  Eo was achieved through the combination of inoculation, 0 cars per hour, and covered

conditions, with a value of 0.53. The parameter  $\phi$  Eo which is equivalent to  $\phi$  Eo =  $\phi$  Po.  $\Psi_o$ , provides information about electron transfer at the acceptor site. The inability to transfer electrons from QA, and the rate of transfer after QA lead to disruption of electron transfer flow and reduces  $\phi$  Eo (Zhang *et al.*, 2010). On the other hand, stress due to air pollution causes damage to intermediate transporters of electron transfer such as QB acceptor, cytochrome b6-f complex and plastocyanin, and reduction of area eventually reduces  $\phi$  Eo. Based on the interaction of treatments, it was found that the non-inoculation treatment combined with a rate of 4200 cars/h and non-covered conditions resulted in the lowest mean value of  $\psi_o$  Po/(1-  $\psi_o$  Po), with a value of 3.38. Conversely, the highest mean

value was observed when the inoculation treatment was combined with a rate of 0 cars/h and covered conditions, yielding a value of 4.55. Additionally, the efficiency of light reactions in photosystem II provides insights into the parameters within the electron transfer chain that were implicated at its inception. The results of research by (Koller *et al.*, 2013) on *Quercus robur* in drought stress showed that the efficiency of light reactions decreases with the application of stress. Based on the results of this experiment, it was found that plants inoculated with mycorrhizal fungi had a higher efficiency of the biochemical reaction of electron transfer compared to the control. Placement inoculated rosemary plants in the traffic paths of 0, 120, 300, 600, 950 and 1200 vehicles per hour increased the efficiency of the biochemical reaction of electron transfer. Plants that were in the path of 1800, 3000, 3600, and 4200 vehicles per hour were significantly different in terms of the efficiency of the biochemical reaction of electron transfer with the control treatment (without traffic). Reduction of all intermediate parameters of the electron transfer chain such as  $\phi E_0$  and  $\psi_0$  can reduce the efficiency of primary biochemical reactions, and a reduction of electron transfer current in the electron transfer chain can be a reason for reducing this parameter (Pereira *et al.*, 2000).

The phenomenon of pollution emerges as an outcome of progress in industrialization and urban development. A substantial number of heavy metals, which possess the potential to impact both human well-being and the ecological surroundings, are found in traffic dust. Consequently, the discharge of heavy metals from automobiles emerges as a principal contributor to the contamination of metal substances in urban settings, owing to the heightened traffic volume. The presence of heavy metals can adversely influence photosystem reactions, disrupt the normal course of photosynthetic reactions, and ultimately lead to a decline in plant performance. This study was carried out to evaluate the traffic's effects on chlorophyll a fluorescence measurements of the rosemary plant under Mycorrhiza symbiosis conditions. As a result of this experiment, it was found out that increased traffic has affected the activity of Area, Fv/Fm, Fo/Fm, N,  $\phi(E_0)$ ,  $\phi(P_0)$ , and Sm/t (Fm). Finally, heavy metals have affected the light reactions ( $\phi(P_0)/(1-\phi(P_0))$ ). Therefore, the increase in traffic, in addition to the electron donor sections, has also affected the electron acceptor sections. The findings of this study indicate that the utilization of mycorrhiza and the application of soil surface covering on pots have effectively mitigated the adverse consequences of traffic.

## Conclusion

## References

- Bozdogan Sert, E., Turkmen, M., & Cetin, M. (2019). Heavy metal accumulation in rosemary leaves and stems exposed to traffic-related pollution near Adana-Iskenderun Highway (Hatay, Turkey). *Environmental Monitoring and Assessment*, 191, 1-12.
- Choi, D. S., Qureshi, A. M., Maruyama, Y., Jin, H. O., & Koike, T. (2005). Effect of ectomycorrhizal infection on growth and photosynthetic characteristics of *Pinus densiflora* seedlings grown under elevated CO<sub>2</sub> concentrations. *Photosynthetica*, 43, 223-229.
- Dewez, D., Eullaffroy, P., Popovic, R., & Juneau, P. (2007). Rapid chlorophyll a fluorescence transients of *Lemna minor* leaves as indication of light and exogenous electron carriers effect on photosystem II activity. *Photochemistry and Photobiology*, 83(3), 714-721.
- Force, L., Critchley, C., & Van Rensen, J. J. (2003). New fluorescence parameters for monitoring photosynthesis in plants. *Photosynthesis Research*, 78, 17-33.
- Hermans, C., Smeyers, M., Rodriguez, R. M., Eyletters, M., Strasser, R. J., & Delhay, J. P. (2003). Quality assessment of urban trees: A comparative study of physiological characterisation, airborne imaging and on site fluorescence monitoring by the OJIP-test. *Journal of Plant Physiology*, 160(1), 81-90.
- Hu, Y., Wang, D., Wei, L., Zhang, X., & Song, B. (2014). Bioaccumulation of heavy metals in plant leaves from Yan' an city of the Loess Plateau, China. *Ecotoxicology and Environmental Safety*, 110, 82-88.
- Kocheva, K., Lambrev, P., Georgiev, G., Goltsev, V., & Karabaliev, M. (2004). Evaluation of chlorophyll fluorescence and membrane injury in the leaves of barley cultivars under osmotic stress. *Bioelectrochemistry*, 63(1-2), 121-124.
- Koller, S., Holland, V., & Brüggemann, W. (2013). Effects of drought stress on the evergreen *Quercus ilex* L., the deciduous *Q. robur* L. and their hybrid *Q. × turneri* Willd. *Photosynthetica*, 51(4), 574-582.
- Kruger, G. H. J., De Villiers, M. F., Strauss, A. J., De Beer, M., Van Heerden, P. D. R., Maldonado, R., & Strasser, R. J. (2014). Inhibition of photosystem II activities in soybean (*Glycine max*) genotypes differing in chilling sensitivity. *South African Journal of Botany*, 95, 85-96.
- Lendenmann, M., Thonar, C., Barnard, R. L., Salmon, Y., Werner, R. A., Frossard, E., & Jansa, J. (2011). Symbiont identity matters: Carbon and phosphorus fluxes between *Medicago truncatula* and different arbuscular mycorrhizal fungi. *Mycorrhiza*, 21, 689-702.
- Lepedus, H., Jurkovic, V., Stolf, I., Curkovic-Perica, M., Fulgosi, H., & Cesar, V. (2010). Changes in photosystem II photochemistry in senescing maple leaves. *Croatica Chemica Acta*, 83(4), 379-386.

- Littell, R. C., Henry, P. R., & Ammerman, C. B. (1998). Statistical analysis of repeated measures data using SAS procedures. *Journal of Animal Science*, 76(4), 1216-1231.
- Mohammadi, A., Azhdarpoor, A., Shahsavani, A., & Tabatabaee, H. (2016). Investigating the health effects of exposure to criteria pollutants using AirQ2. 2.3 in Shiraz, Iran. *Aerosol and Air Quality Research*, 16(4), 1035-1043.
- Morte, A., Lovisolo, C., & Schubert, A. (2000). Effect of drought stress on growth and water relations of the mycorrhizal association *Helianthemum almeriense*-*Terfezia clavaryi*. *Mycorrhiza*, 10, 115-119.
- Pereira, W. E., de Siqueira, D. L., Martinez, C. A., & Puiatti, M. (2000). Gas exchange and chlorophyll fluorescence in four citrus rootstocks under aluminium stress. *Journal of Plant Physiology*, 157(5), 513-520.
- Pourakbar, L. & Ebrahimzade, N. (2014). Growth and physiological responses of *Zea mays* L. to Cu and Ni stress. *Applied Field Crops Research*, 27(103), 147-159.
- Reid, C. P. P., Kidd, F. A., & Ekwebelam, S. A. (1983). Nitrogen nutrition, photosynthesis and carbon allocation in ectomycorrhizal pine. *Tree Root Systems and their Mycorrhizas*, 415-431.
- Rohacek, K. (2002). Chlorophyll fluorescence parameters: The definitions, photosynthetic meaning, and mutual relationships. *Photosynthetica*, 40, 13-29.
- Rouphael, Y., Franken, P., Schneider, C., Schwarz, D., Giovannetti, M., Agnolucci, M., ... & Colla, G. (2015). Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. *Scientia Horticulturae*, 196, 91-108.
- Schansker, G., Toth, S. Z., & Strasser, R. J. (2005). Methylviologen and dibromothymoquinone treatments of pea leaves reveal the role of photosystem I in the Chl a fluorescence rise OJIP. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1706(3), 250-261.
- Zengin, F. K. & Munzuroglu, O. (2005). Effects of some heavy metals on content of chlorophyll, proline and some antioxidant chemicals in bean (*Phaseolus vulgaris* L.) seedlings. *Acta Biologica Cracoviensia Series Botanica*, 47(2), 157-164.
- Zhang, T., Gong, H., Wen, X., & Lu, C. (2010). Salt stress induces a decrease in excitation energy transfer from phycobilisomes to photosystem II but an increase to photosystem I in the cyanobacterium *Spirulina platensis*. *Journal of Plant Physiology*, 167(12), 951-958.