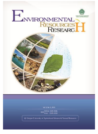

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Effects of atmospheric dust deposition on leaf chlorophyll fluorescence parameters of cow-tail shrubs (*Smirnovia iranica*) in the desert regions of Kashan, Iran

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Abstract

Smirnovia iranica, a native valuable woody species from *Fabaceae*, is an adaptable plant from the central sandy areas of Iran. Changes in chlorophyll fluorescence and photosynthetic pigment characteristics were analysed in the course of dust accumulation during April (Ap); April and May (AM); and April, May and June (AMJ) periods, respectively. The results obtained from the analysis of variance indicated that there was a significant difference among different dust-loading periods of *S. iranica* in terms of the characteristics mentioned. Extending dust accumulation resulted in a significant reduction in the F_v/F_m ratio in AM and AMJ. A significant effect of dust deposition on thermal dissipation of the light energy (*D*) value was observed in AM and reached the highest value in AMJ. In all evaluated plants, during the course of dust accumulation, there was a decrease in the photochemical efficiency of photosystem II (ΦPSII), and from AM. A similar influence of the extending dust accumulation periods on ΦPSII was observed for the electron transport rate (*ETR*). Our results proved that dust deposition decreased overall plant performance through its severe effect on pigment content and resulted in a significant inhibition of PSII efficiency throughout this study period.

Keywords: Dust, Electron transport rate, Photosynthetic apparatus, Thermal dissipation, Pigment

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Introduction

Arid environments have undergone irreparable damage due to anthropological activities. The changed ambience due to dust storms in arid regions has exerted a profound influence on the physiological, biochemical, and morphological status of plants, and therefore their responses (Prabhat and Lalita, 2014). Many factors threaten the survival of desert plant species. One of the most prevalent is aeolian dust, generated by soil surface disturbance (Upekala *et al*., 2009), which not only affects species directly through plant injury but generates many indirect threats as well (Elnap and Warren, 2002). Dust may settle on leaves, twigs, and bark surfaces of plants for extended periods of time, especially in desert environments where low rainfall frequency prevents removal of dust particles from leaves and other plant surfaces (Grantz *et al*., 2003). Excessive dust deposition in arid lands is known to cause low photosynthesis (Van Heerden *et al*., 2006) and changes in biochemical parameters like chlorophyll, protein, and soluble sugar are observed in the leaves (Santosh and Tripathi, 2008; Heydarnezhad and Ranjbar, 2014).

It is well established that photosystem II (PSII) plays a key role in the photosynthetic response to dust (Prusty *et al*., 2005) and unfavourable environmental conditions (Misra *et al*., 2012). Chlorophyll fluorescence measurements have become a widely used method to study the functioning of the photosynthetic apparatus and are a powerful tool to study the plant's response to environmental stress (Stirbet and Govindjee, 2011). However, the functionality of PSII can be assessed by measuring different attributes of chlorophyll fluorescence. For example, drought-induced decline in F_v/F_m is a promising indicator of the functionality of the photosynthetic apparatus of plants (Ranjbar *et al*., 2011; Misra *et al*., 2012). Similar is the case with the electron transport rate (*ETR*) (Hina *et al*., 2011).

Another plant response to unfavourable environmental conditions is the change in photosynthetic pigment content. The content of both chlorophylls *a* and *b* changes under adverse circumstances (Farooq *et al*., 2009). The carotenoids play fundamental roles and help plants resist drought stress (Jaleel *et al*., 2009).

Smirnovia iranica (Fabaceae) is one of the valuable shrub species with high resistance to arid conditions and only appears on sand dunes. The plant is important for forage production, soil conservation, and medicinal values (Sabeti, 1994). Very little information is available on the functioning of the photosynthetic apparatus in *S. iranica* plants under dust deposition conditions. Hence, this study was carried out to evaluate the concurrent changes of chlorophyll *a* fluorescence and leaf pigments in species.

Materials and methods

Field survey

The study was conducted in a typical habitat of *S. iranica* in Kashan, Isfahan Province, Iran (34°00–34°10 N, 51°27–51°35 E, 800–950 m a.s.l.). The annual average precipitation based on a 30-year record is 130 mm, which shows uneven distribution in the form of storms. The region lies between 2000 and 2800 iso-potential evaporation lines, and according to the ombrothermic diagram, the study area has nine dry months annually. The region is mostly placed between 15–17.5 °C iso-temperature lines. Also, the climate of this region is extremely warm with dry summers (Azarnivand *et al*., 2006) and experiences several indigenous and exogenous dust storms during the year.

Experimental design and data analysis

Cow-tail species was selected for the study because the species was present in vast areas that were exposed to dust pollution, both spatially and temporally. Ease of sampling due to small-sized shrubs (1.5–2m tall) made the species a feasible material.

Four populations of the species (in Maranjab region) were selected for measuring chlorophyll fluorescence yields and photosynthetic pigment content. The measurements were carried out at the end of each month during April–June 2014 because during these months the weather remains dry and the frequency of dust storms is high. In each point, four cow-tail bushes of nearly the same size (in view of height and canopy) were selected. The first one was allocated to control and the dust deposited on leaves was rinsed thrice a month with distilled water. The second, third, and fourth ones were sampled at the end of April, May, and June, respectively. Since the points of sampling were not so far from each other, the effect of location was ignored.

Chlorophyll fluorescence analysis

We chose and marked fully expanded leaves of approximately equal size on each stock for monitoring chlorophyll fluorescence yields (CFYs) and photosynthetic pigment contents. The leaves chosen were washed in a pre-weighted beaker with 20 ml of distilled water to determine dust concentration. Droplets that remained on the leaves were dried with blotting paper. Immediately after washing the leaves, CFYs were measured using a portable fluorometer *PAM-2500* (*H. Walz*, Effeltrich, Germany). Prior to the measurement of CFYs, the leaves were put in a dark-adapted state (DAS) for 30 minutes (Genty *et al*., 1989) using light exclusion clips. During DAS, all reaction centres and electron carriers of the PSII are re-oxidized; this situation is essential for rapid fluorescence induction kinetics and for recording chlorophyll fluorescence parameters.

The following CFYs were measured: minimum CFY in the dark and light-adapted states (F_0 and F_0'), maximum CFY in the dark and light-adapted states (F_m and F_m'), and steady-state CFY in the light-adapted state (F_s) (Zhang *et al*., 2011). Some basic and mutually independent chlorophyll fluorescence parameters (CFPs) such as maximum quantum yield of PSII photochemistry (F_v/F_m), photochemical efficiency of photosystem II (ΦPSII), effective quantum yield (F_v'/F_m'), dissipation (*quenching*) of absorbed light energy to heat ($D = 1–F_v'/F_m'$), and electron transport rate (*ETR*) can be calculated from CFYs that give insights into the photosynthetic processes in chloroplasts and can be used effectively in photosynthesis research (Ranjbar *et al*., 2006).

The measurement of pigment contents in the leaves of *S. iranica* bushes were performed during the experimental period, from May until July. Analyses were accomplished in samples collected from the same leaves upon which the CFYs were determined.

Leaf sampling and pigment analysis

The leaf samples were collected in polythene bag kept in an ice box and transferred to the laboratory for pigment analysis. Fresh leaf tissues were homogenized with 100% acetone. The extracts were centrifuged for 10 minutes at 2000 × g. Pigment contents were determined spectro-photometrically at 470, 645, and 662 nm using equations described by Lichtenthaler (1987).

The mix was rinsed in each baker, as described above, and dried at a constant mass in an oven at 50°C and weighed. The amount of dust was calculated by taking the initial and final weights of the beaker. It was calculated by using the formula (Prabhat *et al*., 2014):

$$W = \frac{W2 - W1}{A} \quad (1)$$

Where, W is dust content (mg cm^2), $W1$ is weight of the beaker before washing leaves (without dust), $W2$ is weight of the beaker with dust, and A is total area of leaves in cm^2.

Statistical analysis

The values presented are means ± one standard error (SE) of four replicates. The significance of the same index for different dust treatments was determined using Duncan's multiple range tests. All statistical analyses were carried out on SPSS 16.0 statistical package (SPSS Inc., Chicago, IL, USA).

Results

Dust accumulation (mg cm^{-2} d^{-1}) on the leaves of *S. iranica* from different periods (April–June) is presented in Figure 1. Four levels of dust deposition-0.012, 0.057, 0.085, and 0.113 mg cm^{-2} d^{-1}-were measured for control during the periods of

April (Ap); April and May (AM); and April, May, and June (AMJ), respectively. It is evident from the figure that the species has different dust deposition with respect to various sampling periods. The minimum deposition of dust (0.057 mg cm^{-2} d^{-1}) was accumulated for *S. iranica* in Ap. In contrast, the maximum amount of dust (0.113 mg cm^{-2} d^{-1}) was collected from the leaves of the species in AMJ. The trend of dust deposition was AMJ> AM> Ap.

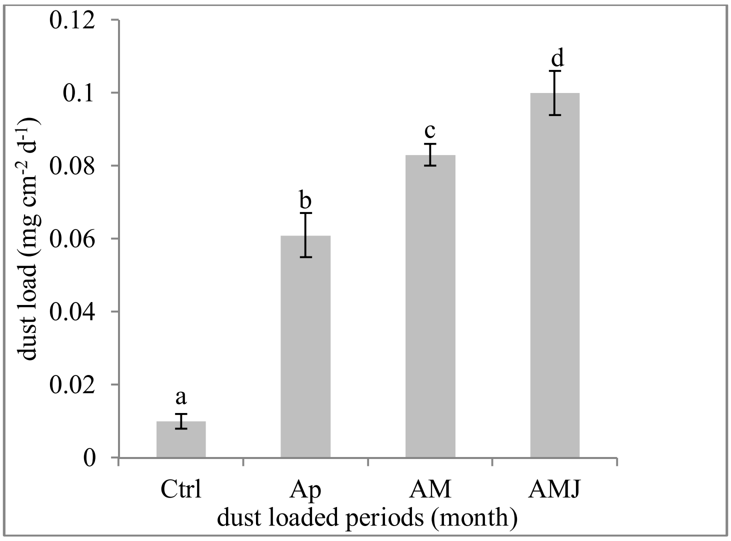

Figure 1. Variation in dust deposition during the period from Ap to AMJ over the leaves of *S. iranica*. The values are means of four replicates. The values over the bars are significantly different at $P < 0.001$ level.

Analysis of variance of the data for dust accumulation presented in Table 1 depicted that the amount of dust from various months varied significantly (P< 0.001). A significantly profound dust load was observed from Ap. However, it differed significantly in the other two periods—AM and AMJ.

Chlorophyll fluorescence parameters

Table 1 shows statistically the highly significant negative effects of dust accumulation on the values of chlorophyll fluorescence parameters in the assimilatory organs of *S. iranica* bushes during the experimental period.

The plant showed an increase in F_0 with a progressing dust load (*p<0.001*). However, in Ctrl and Ap, the photosynthetic apparatus was significantly not altered. A significant increase in F_0 was initiated in AM and reached the highest value (0.113 mg cm^{-2} d^{-1}) in AMJ. In contrast, the F_m value decreased with progressing dust load (*p<0.001*). A significant decrease in F_m was initiated in AM and reached the lowest value (863) in AM June. The increase of dust load concomitant with progressing of experimental months provided the decline in F_v/F_m. Plants in Ctrl and Ap showed an F_v/F_m ratio within the range of healthy plants (values between 0.750 and 0.850). However, extending the dust load resulted in a significant reduction in the F_v/F_m ratio in AM and AMJ. A similar influence of the extending dust load on F_v/F_m was observed for F_v'/F_m'. A significant decrease in F_v'/F_m' was initiated in AM (0.48) and reached the lowest value (0.41) in AMJ. A significant effect of dust load on thermal dissipation of light energy (*D*) value was initiated in AM and reached the highest value (0.59) in AMJ. So, the lowest measured value (0.37) was recorded in Ctrl (Table 1).

Table 1. Effects of dust deposition on the values of CFPs in assimilatory organs of *S.iranica* bushes during the experimental period. Different letters in each column represent statistically significant values at $P \leq 0.0$; Means ± SD., $n = 4$; dust load (DL)

CFPs	F_0	F_m	F_v/F_m	F_v'/F_m'	*D*	ΦPSII	*ETR*
DL (mg cm^{-2} d^{-1})							
Ctrl	192±11^a	1237±20^a	0.84^a	0.63^a	0.37^a	0.73^a	47^a
Ap	211±23^a	1172±10^a	0.82^a	0.58^a	0.43^a	0.66^a	43^a
AM	264±19^b	958±17^b	0.72^b	0.48^b	0.52^b	0.51^b	34^b
AMJ	289±13^c	863±39^c	0.66^c	0.41^c	0.59^c	0.43^c	25^c
ANOVA							
F	28.406	53.273	37.374	17.136	18.94	16.253	12.094
MS	8157.333	124081.33	0.28	0.038	0.038	0.075	0.039
P	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.001*

In the current study, ΦPSII decreased significantly due to the progressing dust load. Ctrl was the highest in ΦPSII (0.73) than in the other dust deposition periods, while AMJ showed the lowest (0.43) in this attribute at the highest dust load condition.

The *S. iranica* plants showed a reduction in *ETR* with the progressing of dust load ($p<0.01$). However, a significant reduction in the *ETR* value occurred in AM (34) and reached the lowest value in AMJ (25).

Chlorophyll contents

The results on the effects of gradual dust load on the pigment parameters in leaves of *S.iranica* are presented in Table 2. A significant alteration in the chlorophyll content of the leaf was observed with increasing dust load in AM and reached the lowest value in AMJ (2.55 mg g^{-1}).

A significant decrease in carotenoids content (Cc+x) was initiated in AM and reached the lowest value (0.26 mg g^{-1}) in AMJ. However, no significant difference with (Cc+x) was observed between Ctrl and Ap. A significant alteration in the total chlorophyll content [Chl. (*a*+*b*)] of the leaf was noticed with increasing dust load. The size of the reduction varied ranging from a reduction by 12.6% in AM to a reduction by 41% in AMJ compared to Ctrl, respectively. However, dust accumulation did not result in a significant alteration in the ratio of Chl. (*a*/*b*) between Ctrl and AMJ but a significant difference was observed between Ap and AM (Table 2).

Table 2. Concentration of photosynthetic pigments in the leaves of *S. iranica* measured in three different dust loading stages

Photosynthetic pigment	Chl.a (mg g-1)	Chl.*b* (mg g^{-1})	C(c+x) (mg g^{-1})	Chl.(*a*+*b*) (mg g^{-1})	Chl.(*a*/*b*)
DL (mg cm^{-2})					
Ctrl	4.38±0.11^a	2.14±0.10^a	0.44±0.08^a	6.50^a	2.05^a
Ap	3.85±0.19^{ab}	1.83±0.18^b	0.46±0.04^a	5.68^b	2.73^b
AM	3.21±0.11^b	1.41±0.17^c	0.35±0.10^b	4.62^c	2.28^c
AMJ	2.55±0.18^c	1.28±0.08^d	0.26±0.07^c	3.83^{cd}	1.99^a
ANOVA					
F	89.053	37.850	5.680		
MS	2.511	0.751	0.034		
P	0.000*	0.000*	0.010*		

Discussions

In this study, the photosynthetic apparatus of *S. iranica* L. was damaged to a certain extent at the AM and AMJ periods, as observed from leaf Chl. CFPs such as F_0, F_m, F_v/F_m, *ETR,* and ΦPSII. The F_0 value may increase if transfer of excitation energy from the antenna to the reaction centres is impaired (Zhang *et al*., 2011). Thus, the increase in F_0 observed in the *S. iranica* plants studied may be associated with damage to the photosynthetic apparatus, such as inactivation reaction centres of PSII (Ranjbar *et al*., 2013). The F_m value decreased with progressing dust accumulation ($p<0.001$). The reduction of F_m can be associated with increased non-photochemical dissipation as the heat may be related to a decrease in the activity of the water-splitting enzyme complex (Cicero *et al.*, 2012). Our results on F_0 and F_m agree with the findings of Li *et al.* (2015) on *Medicago sativa* during drought. The data obtained in this study indicates a significant alteration in F_v/F_m. The results on F_v/F_m can be interpreted in view of the arguments of Rong-Hua *et al.* (2006), who suggested that any decrease in F_v/F_m indicates that PSII suffers damage and that key reactions of photosynthesis are inhibited. A similar result was also reported by Xinghong *et al.* (2006) in wheat.

The increase in dust accumulation brought the decline of F_v'/ F_m' ($p<0.001$). The reduction of F_v'/ F_m' can be associated with a decrease in the conversion efficiency of electron energy in PII into chemical energy (Schreiber *et al.*, 1994). A similar result was also reported by Liu *et al.* (2012) in maze.

The photochemical efficiency of photosystem II (ΦPSII) decreased significantly due to the progressing dust load. The decrease in ΦPSII can be ascribed to an increase in the rate of dissipation of light energy to heat in the PII antenna complexes as well as a decrease in the proportion of photons and excitation of electrons of chlorophyll (Cicero *et al.*, 2012). Our findings are in agreement with those of Fu and Wang (2015), who reported that declines in ΦPSII could be due to progressing soil lead concentration. The *S. iranica* plants showed a reduction in *ETR* with an increase in the dust load. The *ETR* value shows the sum of all electron sinks in chloroplast such as carbon fixation, photorespiration, nitrate assimilation, and Mehler reaction. A perturbation or change in any of these parameters affects *ETR* (Vladkova *et al.*, 2011). Similar to the findings of the current study, earlier authors have reported either stable or decreased *ETR* for *Mangifera indica* (Cicero *et al.*, (2012) and *Vigna radiata* (Alavi and Sharifi, 2015).

The chlorophyll content determines the vegetation photosynthetic capacity. It is an important bio-indicator of vegetation performance, especially under unfavourable environmental conditions. In the current study, the dust exposure of *S. iranica* plants led to a substantial reduction in the content of chlorophylls *a* and *b*, which reveals a possible damage in the photosynthetic capacity of chloroplasts (Younis *et al.*, 2013). However, reduction of total pigment content, as a result of either slow synthesis or fast breakdown, has been considered as a typical symptom of oxidative stress (Mafakheri *et al.*, 2010). The results obtained are in close conformity with those reported by (Abdel-Rahman and Ibrahim, 2012) in which dusted plants showed marked changes in the accumulation of chlorophylls *a* and *b* contents.

In Ap to AMJ, decreases in Chl. (*a*+*b*) and Cc+x were paralleled by a decrease in F_v/F_m, which indicated that pigment breakdown was accompanied by a decrease in the maximum photochemical efficiency. Other authors explained this phenomenon as a photo-protection mechanism by reducing light absorbance and decreasing pigment content (Galmes *et al.*, 2007; Elsheery and Cao, 2008). A marked decrease in Chl. *a*/*b* ratio was observed in the plants studied (from Ctrl to AMJ). Our results are consistent with Abdel-Rahman and Ibrahim (2012), who reported that cement dust decreased the total leaf chlorophyll content and the chlorophyll *a*/*b* ratio.

Conclusions

It was found that dust accumulation on leaf surfaces induces stress conditions, such as reduction of photosynthetic activities. More importantly, the results of this study show that both the chlorophyll fluorescence parameters of cow-tail plants and the chlorophyll contents are adversely affected by dust deposits.

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