



Soil dust effects on morphological, physiological and biochemical responses of four tree species of semiarid regions

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Abstract

Dust pollution is expected to be a significant pollution in the urban forests, especially in semiarid environments. The purpose of this study was to evaluate the effects of soil dust on the morphological, physiological and biochemical traits of seedlings of *Fraxinus rotundifolia* Mill., *Morus alba* L., *Celtis caucasica* Willd. and *Melia azedarach* L. These species are widely grown in Iran's urban areas, particularly in semiarid regions. The seedlings were dusted at four concentrations, 0, 300, 750 and 1500 $\mu\text{g m}^{-3}$, once a week for 10 weeks in four plastic chambers, placed in a completely randomized design with four repetitions. Dust accumulation was greatest at the 750 and 1500 $\mu\text{g m}^{-3}$ levels in the following order: *M. alba* > *C. caucasica* > *F. rotundifolia* > *M. azedarach*. In all species, dust decreased the leaf area, stem diameter, height, leaf, shoot, root and total biomass but increased the root–shoot ratio. Photosynthesis, transpiration, stomatal conductance, mesophyll conductance, leaf extract pH, F_v/F_m and $t_{1/2}$ decreased, while leaf temperature and water use efficiency increased. Likewise, chlorophyll *a*, chlorophyll *b*, carotenoid and total chlorophyll content were diminished. *M. alba* has the highest soil dust accumulation potential but the lowest decrease in the properties studied. *C. caucasica* has a high-dust accumulation potential, but it was highly affected by dust treatment. *M. azedarach* and *F. rotundifolia* did not accumulate much dust. We conclude that among the four tree species, *M. alba* is the most suitable species for the urban forests of semiarid zones where dust pollution is high and dust reduction desired.

Keywords Biomass · Dust pollution · Fluorescence chlorophyll · Gas exchange · Persian lilac · White mulberry

Introduction

Particulate matter (PM) is considered one of the main atmospheric pollutants worldwide (Lu et al. 2018). PMs of atmosphere are classified as either primary or secondary: Primary PMs are those emitted directly as particles,

including combination of fine solids such as dirt, soil dust, pollens, molds, ashes and soot, while secondary PMs are produced by gaseous interactions in the atmosphere. Volatile organic compounds, such as sulfur dioxide and nitrogen oxides, are examples of secondary PMs (Owen et al. 2006). Dust particles are one of the primary PM; it includes all suspended fine airborne soil and/or weathered or transported rock particles removed from the Earth's surface as a result of wind erosion (Rashki et al. 2013). Atmospheric dust is a major source of pollution, especially in dry climates (Drack and Vázquez 2018). In recent years, due to climate changes, dust storms, natural disasters and human activities (mainly altered vegetation layer and land use), dust phenomenon has become the most important air pollutants (Naidoo and Chirkoot 2004). Annually, an estimated 2000 Milliard tones of dust is emitted into the atmosphere (Shao et al. 2011). The highest amount of atmospheric dust originates from arid and semiarid regions of the world, particularly from subtropical latitudes. The most active dust centers in the world include

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the great deserts of Africa, Middle East, Southwest Asia, the Central Australia, Mongolia and parts of Europe and the Americas (Middleton and Kang 2017).

The dust pollution not only threatens human health, but it also has detrimental effects on ecosystem services such as biomass production and biodiversity (Lovett et al. 2009). Plants are particularly susceptible to dust pollution due to increased exposure. Dust stress may impact various aspects of plant life through physical and chemical effects on the plant's aerial parts (e.g., increased stomatal clogging, diffusive resistance, shading, leaf loss and tissue death, energy absorption in the infrared spectrum, leaf pigments content and leaf temperature and reduced photosynthetic gas exchange). Dust is also implicated in alternating soil pH and soil chemistry. It may also cause or exacerbate secondary stresses such as drought stress, pathogen and insects stress or allow penetration of toxic metals into the plant tissues (Grantz et al. 2013; Moradi et al. 2017).

Various morpho-physiological and biochemical changes were observed in different plants under dust condition. As a result, some species were shown to be more tolerant to pollution than others (Shah et al. 2018). Dust stress did not affect physiological properties of *P. eldarica* and *C. sempervirens*, but *L. ovalifolium* responded to air dust pollution with increased chlorophyll content, stem length and number of leaves (Taheri Analojeh et al. 2016). Stomatal obstruction by dust particles took place at a rate of 38.1%, 48.4% and 61.6%, respectively, in *Quercus brantii*, *Q. libani* and *Q. infectoria* (Moradi et al. 2017). In *Olea europaea*, formation of a dust layer on leaf surfaces decreased the most physiological properties and increased the leaf temperature without significantly changing the internal CO₂ concentration (Nanos and Ilias 2007). Washing leaves with water at 3-day intervals or after a natural dust fall increased the stomatal conductance by 30% in *Gossypium hirsutum* (Zia-Khan et al. 2015). *Sophora japonica* seedlings toward increasing experimental road dust concentrations from 0 to 16.44 g m⁻² indicated a decline in gas exchange, pigment content and height growth as well as a logarithmical increase in root–shoot biomass ratio (Bao et al. 2016). In *Euphorbia milii*, *Gardenia jasminoides* and *Hibiscus rosa-sinensis*, dust deposition on leaf surfaces, especially at high doses, reduced the pigment content, in *H. sinensis* and *G. jasminoides*, which are considered to be the most resistant and sensitive species, respectively, to dust stress (Shah et al. 2018). The responses *Solanum tuberosum* plants exposed to high cement dust for 60 days were reduction in growth, biomass and photosynthetic pigment contents (Tomar et al. 2018).

Abatement actions to reduce anthropogenic and natural sources of dust particles are generally costly, and natural dust sources are difficult or impossible to control (McDonald et al. 2007). Currently, controlling and decreasing dust events are the most important activities to achieve

sustainable land use and development. Tree planting and creating forest belts are important ways of combating the different types of air pollutions including dusty air pollution in urban environments (Okunlola et al. 2016). Actually, trees with characteristic such as large size, high foliage surface–volume ratio, hairy or rough leaves and bark surfaces are effective biological filters for capturing airborne particles (Onkar 2006). Many studies indicate that urban trees improve air quality by capturing the dust particles. For example, Zhang et al. (1997) estimated that trees covering an area of 1639 ha at eight residential regions in Beijing could capture 2170 tons of dust. Trees covering an area of 103 km² were able to retain 8600 tons of dust per year in Zhengzhou, China (Zhao et al. 2002). In a study of Hofman et al. (2014), average dust accumulation by tree leaves in Antwerp, Belgium, was estimated 747 mg m⁻² during throughout the in-leaf season. In Guangzhou, China, urban trees vegetation could capture 8013 tons of dust per year (Liu et al. 2013). Although the potential of dust accumulation on leaves of different tree species has been evaluated (Prusty et al. 2005; Prajapati and Tripathi 2008; Rai et al. 2010; Rai and Panda 2014; Hariram et al. 2018), questions on morpho-physiological and biochemical plant responses remain.

Iran is subjected to dust because of natural factors, human activities and its vicinity to the deserts of countries such as Iraq, Syria and Saudi Arabia. The extent of areas affected by dust in Iran was initially limited to parts of the Khuzestan and Bushehr provinces, but in recent years, the southwest, west and central regions have been affected. Currently, more than half of the Iranian provinces face a dust air pollution challenge. However, concentration, size and number of suspended particles, duration and the frequency of this phenomenon vary greatly among regions (Amiraslani and Dragovich 2011). For example, the daily maximum dust concentration reached up to 5337 µg m⁻³ (Shahsavani et al. 2012) in south (Ahvaz) and 3094 µg m⁻³ in southeast (Zabol) Iran (Rashki et al. 2012). In the cities of these regions, dust was reported on about 166 and 188 days per year, respectively. Data provided by Iranian Meteorological Organization (IMO) revealed that in the period 2001–2005, average dusty days in Dezful, Kermanshah and Abadan was 87.8, 73 and 58.2 days, respectively.

According to the World Health Organization reports (WHO 2006), the air quality is considered to cause dangerous if the dust concentration exceeds 425 µg m⁻³. Comparing this value with the concentrations mentioned above indicates crucial conditions. Moreover, urban green space per capita in Iran (7–12 m² per person) is low compared to the standard (20–25 m² per person, according to United Nations Environment) or compared to the ideal global index (50 m² per person based on WHO 2010). Hence, increasing the urban green space may be seen a promising

nature-based strategy for improving the air quality. The present study used four tree species that are commonly recommended for planting in dust-polluted urban areas. The objectives of the present research were as follows:

- (i) To estimate the dust deposition potential of leaves of four common urban green spaces species (*Fraxinus rotundifolia* Mill., *Morus alba* L., *Celtis caucasica* Willd. and *Melia azedarach* L.) under greenhouse conditions.
- (ii) To study the morpho-physiological and biochemical responses of these species to artificial dusting.
- (iii) To derive recommendations for tree species suitable for planting in dusty regions.

Materials and methods

Preparation of seedlings

Seedlings used in the experiment were grown in polypropylene pots containing soil mixture (sand, clay, animal manure and cocopeat in 2:1:1:1 proportion) at a nursery before being transferred to a greenhouse at the Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran (35°44'N, 51°10'E and 1215 m a.s.l.). Twenty days after transferring the seedlings to the greenhouse, they were transplanted individually to 5-L plastic pots containing the soil mixture used during nursery production. The seedlings were then arranged in a 4 × 4 randomized complete block design with four replications of four seedlings per treatment combination. The experiment used 256 uniformly sized seedlings, each of *F. rotundifolia*, *M. alba*, *C. caucasica* and *M. azedarach*. The local climate is arid and semiarid with average annual relative humidity of 34% and average annual rainfall of 161 mm.

Preparation of dust

Dust sources were identified using a combination of remote sensing, GIS and sedimentology in the Khuzestan Province (Heidarian et al. 2015). Soil samples were collected in dust-producing regions in the south and southeast of Ahvaz City, Khuzestan Province. This region is susceptible to wind erosion. The samples were taken at depths of 0–10 cm and blended. Then, the mixed soil was poured into a sieve device with 106 µm sieve width. The resulting particles < 106 µm were oven-dried at 80 °C for 48 h and used as dust. The physical and chemical compositions of the soil dust samples are shown in Table 1.

Table 1 Some of physical and chemical properties of soil dust samples (0–10 cm depth)

Texture	Clay loam
EC (ds m ⁻¹)	7.29
K (ppm)	292
TiO ₂ (%)	0.41
MnO (%)	5.11
NiO (%)	0.01
ZrO ₂ (%)	0.02
Sand (%)	44
pH	6.65
OM (%)	0.68
Fe ₂ O ₃ (%)	4.21
K ₂ O (%)	0.35
Cr ₂ O ₃ (%)	0.03
P ₂ O ₅ (%)	0.12
Clay (%)	48.66
N (%)	0.04
SiO ₂ (%)	30.42
Na ₂ O (%)	2.25
P ₂ O ₂ (%)	0.12
MgO (%)	15.01
CdO (%)	0.0002
Silt (%)	7.33
P (ppm)	13
Al ₂ O ₃ (%)	6.2
CaO (%)	24.15
V ₂ O ₂ (%)	0.01
CuO (%)	0.01
Sb ₂ O ₃ (%)	0.00001

EC = electrical conductivity, OM = organic matter

Dust application

Experimental dust concentrations of 0, 300, 750 and 1500 µg m⁻³ were used. The concentrations were based on the findings of Shahsavani et al. (2012) and Hatami et al. (2018). Data on dust concentrations recorded in the dust-affected Khuzestan Province and total suspended particles (TSPs) in the air were obtained from the Iran's Environmental Protection Organization (IEPO).

Three chambers with plastic sheets in the dimensions of 5 × 2 × 3 m were built according to Siqueira-Silva et al. (2016b) with some modifications for the dust application. Sieved soil dust was applied to a group of 64 seedlings (four replications of four seedlings for per species) in any chamber. Dust was applied using a dust simulator (Fig. 1) once per week from 09:00 to 12:00 h for 10 weeks. Sixty-four seedlings (16 seedlings per species) were placed in a similar plastic chamber but not exposed to dust as a control group. Seedlings were irrigated every 2 days to near 100% field capacity, according to the procedure described by Zarik et al.

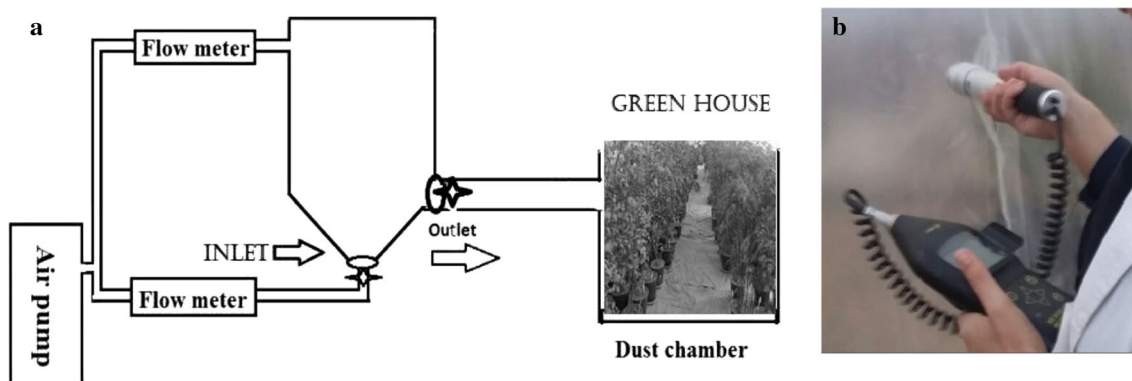


Fig. 1 Schematic presentation of the dust simulator (a) and dust meter (b) used in the present study

(2016) being careful not to wet the foliage. Dust concentration and particle size distribution in the air were monitored on each dust level by a 176000A Microdust Pro Dust Monitoring apparatus. No spraying with deionized water was done on the aboveground parts of the seedlings following the dust application.

Dust collection potential measurement

At the end of the experiment, five leaves from five randomly selected seedlings per species were removed, placed individually in plastic bags and transferred to the laboratory. Individual leaf samples were weighed using an electric digital balance. Leaves were then carefully cleaned using a fine brush and reweighed. Individual leaf area was measured by Leaf Area Meter (Model LI-3000, Li-Cor, Lincoln, NE, USA). Dust accumulation, on a unit leaf area, was calculated using Eq. (1), after the method of Prusty et al. (2005):

$$W = (W_2 - W_1)/a, \quad (1)$$

where W = the amount of dust accumulation (mg cm^{-2}), W_1 = weight of leaf without dust (mg), W_2 = weight of leaf with dust (mg) and a = total area of leaf in cm^2 .

Growth and biomass characteristics measurement

At the beginning and at the end of dust treatments, shoot heights (cm) and diameters of individual seedlings at 1 cm above the soil mix surface were measured. Seedling height was measured on the main shoot, from the soil mix surface to the seedling's apex with a ruler. The increase in seedling height and diameter was calculated by subtracting the initial measurements from the final size measurements. Average leaf area was estimated from the average of the five expanded leaves from five randomly selected seedlings per species and dust level. The area of individual leaves was determined by a leaf area meter (Model LI-3000, Li-Cor, Lincoln, NE, USA) as in Parad et al. (2013).

At the end of the experiment, four seedlings from each treatment combination were randomly selected and the soil was carefully washed from the root systems. Individual plants were separated into roots, shoots and leaves. All plant materials were oven-dried at 80°C for 48 h until constant mass was obtained. Total individual seedling biomass (after dust removal) was obtained by summing leaf, shoot and root dry weights and root–shoot ratio calculated.

Physiological characteristics measurement

At the end of the dust treatment period, gas exchange characteristics (net photosynthesis rate (P_n), transpiration rate (E), stomatal conductance (G_s), intercellular CO_2 concentration (C_i) and leaf temperature) were measured using a portable infrared gas analyzer LI-6400 (Li-Cor Inc., Lincoln, USA). The seedlings were randomly selected from each treatment combination and fully expanded leaves from the lower part of these seedlings (branches were selected from the same part in all seedlings) for photosynthetic gas exchange measurements. These were performed between 09:00 and 12:00 h on clear day, with a light intensity of $1400 \mu\text{mol m}^{-2} \text{s}^{-1}$ (LED, Red-Blue 6400-02B, Li-Cor Inc). Dust was not removed from the leaves before gas exchange measurements.

Water use efficiency and mesophyll conductance were calculated according to Eqs. (2) and (3):

$$\text{Water use efficiency} = P_n/E \quad (2)$$

$$\text{Mesophyll conductance} = P_n/C_i, \quad (3)$$

where P_n = photosynthesis rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$), E = transpiration rate ($\text{mmol m}^{-2} \text{s}^{-1}$) and C_i = intercellular CO_2 concentration ($\mu\text{mol mol}^{-1}$).

Determination of leaf extract pH was conducted by the protocol of Rai and Panda (2014). For any treatment combination, three replicates of 0.5 g of the fresh leaves were homogenized in 50 mL deionized water and centrifuged at 7000 rpm for 10 min. In addition, pH of supernatant was

measured using a digital pH meter (Systronics; model μ pH system 361).

Fluorescence chlorophyll traits measurement

Prior to measuring chlorophyll fluorescence attributes, fully expanded healthy leaves from the upper part of the seedlings were marked and dust particles were removed with a soft-bristle brush. The maximum quantum yield of Photosynthesis II (PSII), $(F_v/F_m = (F_m - F_0)/F_m)$, where F_v = the variable fluorescence, F_m = the maximum fluorescence of the leaves adapted to darkness and F_0 = the minimum fluorescence yield in leaves adapted to darkness) and $t_{1/2}$, half-rise time from F_0 to F_m , were measured using a portable pulse-modulated fluorometer (PSM-2000, H Waltz GmbH, Effeltrich, Germany), between 11 am and 14 pm (Öquist and Wass 1988). Prior to measurements, leaves were placed in the dark for 45 min. F_0 and F_m were measured by applying a low-intensity red-measuring light source and a saturating light pulse (5 s) of $400 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Determination of plant pigments content

For measuring photosynthetic pigment contents, at the end of experiment, leaves of seedlings from each treatment combination were collected and perfectly cleaned by a soft brush according to Siqueira-Silva et al. (2016a). 0.1 g of fresh leaves (three replicates for each treatment combination) was extracted with chilled 80% acetone. The liquid portion was decanted into another test tube and centrifuged at 4 °C in the dark at 4000 rpm for 10 min. The supernatant was then collected, and the absorbance was read at 470, 645 and 663 nm using a spectrophotometer. Specific absorption coefficients of chlorophyll *a*, chlorophyll *b* and carotenoids reported by Lichtenthaler (1987) were used. The total chlorophyll content was calculated by the addition of chlorophyll *a* and chlorophyll *b* values.

Statistical analysis

Data were analyzed using GLM with two factors, soil dust at four concentrations (0, 300, 750 and $1500 \mu\text{g m}^{-3}$) and tree species. Duncan's multiple range test was used to compare the means of the treatments. All statistical analyses were done by the SPSS software, version 23.

Results

Dust collection potential by leaf of different tree species

Dust covered completely and uniformly the leaf surfaces of *M. azedarach*, *F. rotundifolia* and *C. caucasica*, but dust deposition on abaxial surface of *M. alba* was higher than the adaxial surface (Fig. 2). In all species, as the dust stress increased, the amount of deposition on the leaf increased. Dust accumulation at concentrations of 750 and $1500 \mu\text{g m}^{-3}$ was: *M. alba* > *C. caucasica* > *F. rotundifolia* > *M. azedarach* (Fig. 3).

Effects of dust accumulation on growth and biomass parameters

Leaf, shoot, root and total biomass and root–shoot ratio were significantly affected by dust concentration and tree species (Table 2). The interaction of dust concentration and tree seedling significantly affected the leaf area, diameter growth and height growth.

For the four studied species, leaf area, diameter growth and height growth were reduced with increasing dust concentration (Fig. 4). The reduction range of leaf area among species treated with $1500 \mu\text{g m}^{-3}$ of dust as compared with control plants was 39–54%, while the reduction range of diameter growth and height growth varied between 25–41 and 45–61%, respectively (Fig. 4).

Elevating the dust level resulted in a decrease in leaf, shoot, root and total biomass and an increase in root–shoot ratio of all species (Fig. 5). The reduction in leaf biomass at the highest dust treatment is relative to the control varied among the species (from 42 to 51%). The reduction rate for shoot, root and total biomass ranged between 35–43, 30–33 and 34–38%, respectively. Root–shoot ratio of soil-dusted plants with $1500 \mu\text{g m}^{-3}$ of concentration to control increased from 0.12 to 0.26 among the species. Also, regardless of dust concentration effect, *M. alba* showed the highest leaf, shoot, root and total biomass (19.3, 48, 69.9 and 137.2 g, respectively). The lowest values were observed for *C. caucasica* (9, 37.64, 44.88 and 91.6, respectively). The maximum root–shoot ratio was obtained jointly for *M. alba*, *M. azedarach* and *F. rotundifolia*, and then it is lowest in *C. caucasica* (Fig. 5).

Effect of dust accumulation on physiological traits

The effect of dust concentration and tree species on photosynthesis, transpiration and stomatal conductance was significant (Table 3). Dust stress alone was significant on leaf

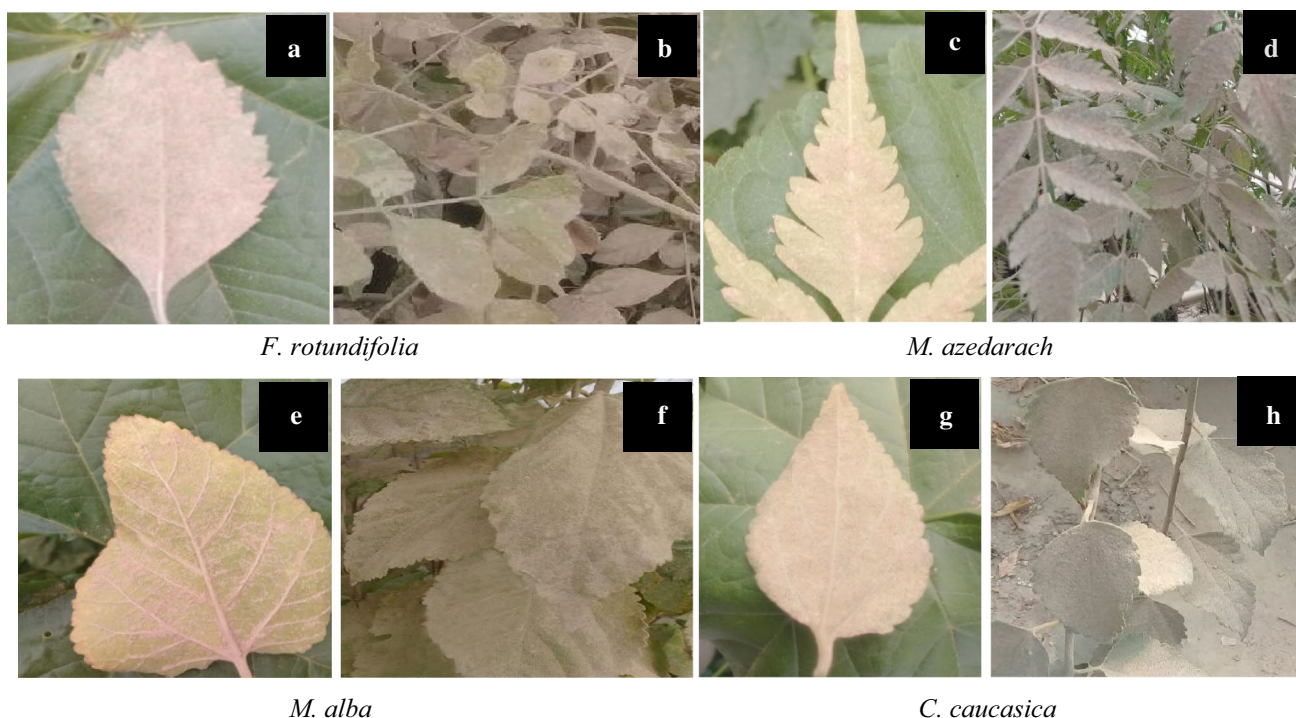


Fig. 2 Dust accumulation on abaxial (a, c, e and g) and adaxial (b, d, f and h) leaf surfaces of studied species in $1500 \mu\text{g m}^{-3}$ of dust treatment, 35 days after the beginning of the experiment

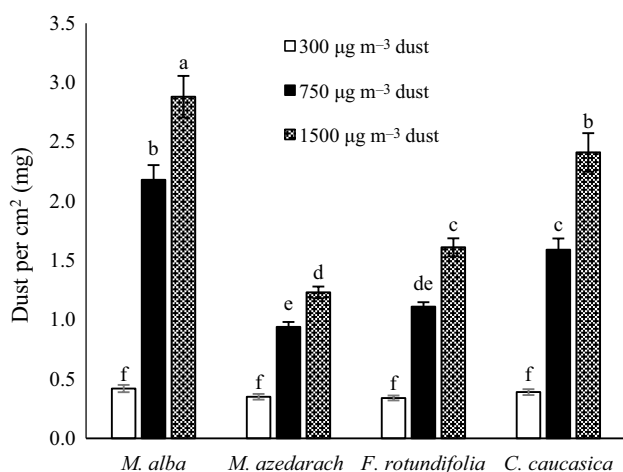


Fig. 3 Dust collection potential during the 10-week dust exposure treatment for four tree species. Different letters above bars indicate significant differences among treatments using Duncan's test when $P < 0.05$. Values are the mean \pm SE; $n = 5$ seedlings from each treatment combination (dust level–tree species)

temperature and leaf extract pH. Intercellular CO_2 concentration, mesophyll conductance, F_v/F_m and $t_{1/2}$ were significantly affected by the interaction of dust concentration and tree species (Table 3).

In all species, along with the rising dust load, the rate of photosynthesis, transpiration and stomatal conductance

showed a declining trend (Fig. 6). At $1500 \mu\text{g m}^{-3}$ of dust concentration, reduction range of photosynthesis, transpiration and stomatal conductance was 53–77%, 59–75% and 48–79%, respectively, when compared to the control plants. The maximum values of photosynthesis, transpiration and stomatal conductance ($11.1 \mu\text{mol m}^{-2} \text{s}^{-1}$, $8.1 \text{mmol m}^{-2} \text{s}^{-1}$ and $304.85 \text{mmol m}^{-2} \text{s}^{-1}$, respectively) were found for *M. alba*, and the minimum values ($6.2 \mu\text{mol m}^{-2} \text{s}^{-1}$, $3.4 \text{mmol m}^{-2} \text{s}^{-1}$ and $133.54 \text{mmol m}^{-2} \text{s}^{-1}$, respectively) did for *C. caucasica* (Fig. 6).

In *M. alba*, *M. azedarach* and *F. rotundifolia*, with increasing dust concentration from 0 to $750 \mu\text{g m}^{-3}$, the intercellular CO_2 concentration decreased and then increased, while in *C. caucasica*, 750 and $1500 \mu\text{g m}^{-3}$ of dust levels led to higher intercellular CO_2 than at 0 and $300 \mu\text{g m}^{-3}$ dust levels (Fig. 6).

Leaf temperature increased in high dust concentrations of all species. The leaf extract pH of leaf samples of all four species decreased when subjected to high dust concentrations. Leaf pH values at 0– $1500 \mu\text{g m}^{-3}$ of dust concentration were in the range of 7.6–6.35 in *M. alba*, 7.3–6.3 in *M. azedarach*, 7.7–6.5 in *F. rotundifolia* and 7.4–6.0 in *C. caucasica* (Fig. 6).

Water use efficiency was not affected by dust concentration and tree species (Fig. 7). The mesophyll conductance of all species was reduced at $1500 \mu\text{g m}^{-3}$ of dust level. The lowest value was recorded for *C. caucasica* (Fig. 7).

Table 2 Two-way analysis of variance (ANOVA) morphological traits in relation to different dust levels and tree seedling

	Growth and biomass traits		Dust concentration		Tree seedling		Dust concentration × tree seedling	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Leaf area (cm ²)	361.594	0.000	729.340	0.000	139.298	0.000		
Diameter growth (mm)	221.135	0.000	236.929	0.000	2.104	0.030		
Height growth (cm)	440.022	0.000	164.675	0.000	4.322	0.000		
Leaf biomass (g)	48.891	0.000	49.140	0.000	.593	0.797		
Shoot biomass (g)	79.165	0.000	18.615	0.000	0.410	0.924		
Root biomass (g)	47.619	0.000	50,474	0.000	0.728	0.681		
Total biomass (g)	152.955	0.000	99.925	0.000	0.876	0.552		
Root–shoot ratio	3.155	0.033	4.775	0.005	0.227	0.979		

Bold numbers indicate significant statistical differences

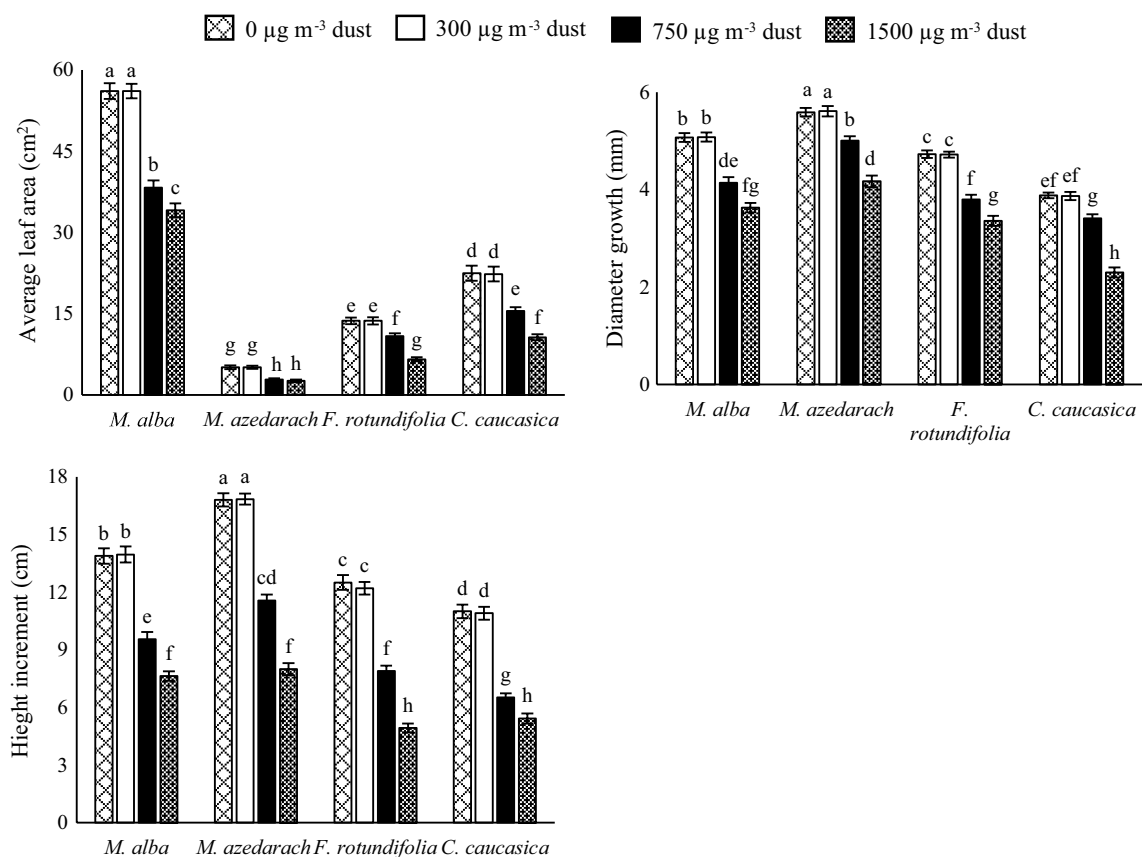


Fig. 4 Leaf area, diameter growth and height growth during the 10-week dust exposure treatment for four tree species. Different letters indicated a significant difference under the interaction between the two variability factors at 0.05 using Duncan’s test. Values are

the mean ± SE; *n* = 5 seedlings for leaf area and *n* = 16 seedlings for diameter growth and height growth from each treatment combination (dust level–tree species)

In all species, F_v/F_m and $t_{1/2}$ decreased with rising dust loads (Fig. 8). F_v/F_m reduction range in 1500 µg m⁻³ of dust concentration in comparison with the control was

24%, 43%, 54% and 68%, respectively, in *M. alba*, *M. azedarach*, *F. rotundifolia* and *C. caucasica*. The reduction

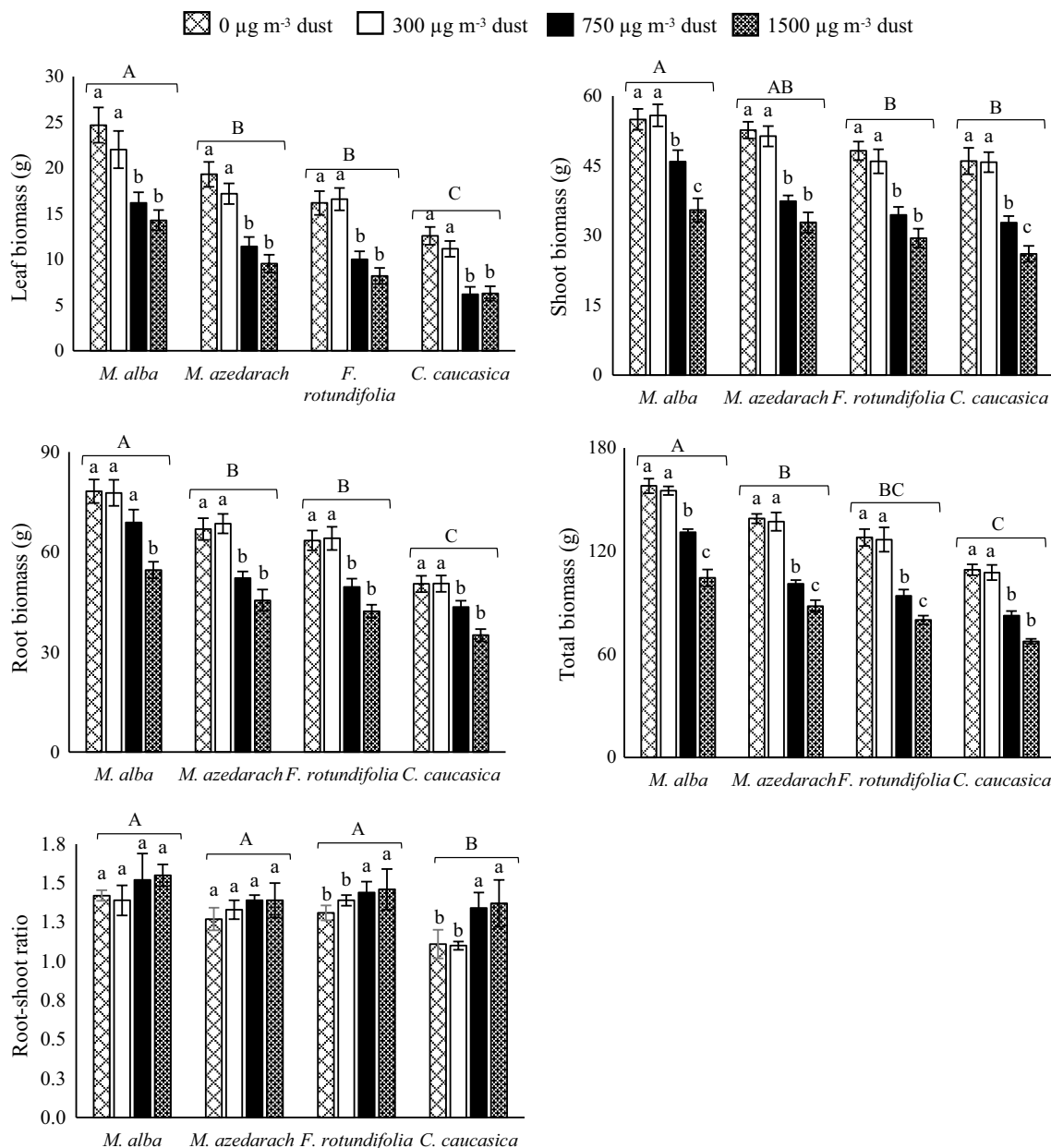


Fig. 5 Leaf, shoot, root, total biomass and root–shoot ratio during the 10-week dust exposure treatment for four tree species. Different letters indicate a significant difference depending on four dust levels (lowercase letters) and tree species (capital letters), analysis

of variance (ANOVA) and Duncan’s test; $P < 0.05$. Values are the mean \pm SE; $n = 4$ seedlings from each treatment combination (dust level–tree species)

range of $t_{1/2}$ for the above species was 40%, 42%, 46% and 55%, respectively (Fig. 8).

Effect of dust accumulation on biochemical traits

Chlorophyll *a*, chlorophyll *b* and carotenoid were significantly affected by dust concentration and tree species (Table 4). The chlorophyll *a/b* ratio was influenced by dust

concentration. Total chlorophyll was significantly affected by combination of dust concentration and tree species (Table 4).

With increasing dust concentration to 750 and 1500 µg m⁻³, a significant decline was observed in the pigments (chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid) of all species (Fig. 9). Among species, plants treated with 1500 µg m⁻³ of dust level as compared with control indicated 43–66%, 38–55%, 41–62% and 54–64% reduction in chlorophyll *a*, chlorophyll *b*, total chlorophyll and

Table 3 Two-way analysis of variance (ANOVA) for physiological properties in relation to different dust levels and tree species

Physiological properties	Dust concentration		Tree seedling		Dust concentration × tree seedling	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Photosynthesis	143.447	0.000	49.932	0.000	0.998	0.461
Transpiration	80.944	0.000	46.813	0.000	1.082	0.402
Stomatal conductance	264.910	0.000	182.412	0.000	1.385	0.236
Intercellular CO ₂ concentration	33.501	0.000	84.321	0.000	22.710	0.000
Leaf temperature	5.198	0.005	0.536	0.661	0.115	0.999
Leaf extract pH	19.842	0.005	2.634	0.054	0.258	0.984
Water use efficiency	1.924	0.146	2.047	0.127	0.963	0.487
Mesophyll conductance	70.930	0.000	7.542	0.001	7.750	0.000
F_v/F_m	166.112	0.000	45.681	0.000	7.119	0.000
$t_{1/2}$	410.568	0.000	20.506	0.000	2.509	0.012

Bold numbers indicate significant statistical differences

carotenoid, respectively. The highest values of chlorophyll *a*, chlorophyll *b* and carotenoids (1.4, 0.9 and 0.24 mg g⁻¹, respectively) were in *M. alba*, and the lowest (1.0, 0.7 and 0.17 mg g⁻¹, respectively) in *C. caucasica*. Chlorophyll *a/b* ratio reduction range of 1500 µg m⁻³ of dust level compared to control was 11%, 10%, 31% and 39%, respectively, in *M. alba*, *M. azedarach*, *F. rotundifolia* and *C. caucasica* (Fig. 9).

Discussion

Dust accumulation on leaves of different tree species

In the present study, dust collection potential and dust accumulation differed among the species at dust levels of 750 and 1500 µg m⁻³ (*M. alba* > *C. caucasica* > *F. rotundifolia* > *M. azedarach*). In general, dust-retention capability of leaves varies depending on age, structure, surface geometry, phyllotaxy, epidermal and cuticular features, presence or absence of hairs and wax on leaf surface, height of plant, orientation of the leaf and length of petiole (sessile or semisessile) (Pranjapati and Tripathi 2008; Chaturvedi et al. 2013).

Factors such as the amount of dust deposition and the distribution of particles on the leaf surfaces, alterations in stomatal morphology and physical protection structures can cause changes in the amount of dust entering the stomata and interfere with stomatal functions (Siqueira-Silva et al. 2016b; Moradi et al. 2017; Shi et al. 2017). Number and type of trichomes and type of epicuticular waxes in some sclerophyll species lead to less stomatal clogging and low disruption in gas exchange processes (Moradi et al. 2017). In different plant species, particularly trees, the stomata are often on the abaxial of leaf surface. If only the adaxial surface of the leaves is dust-covered, gas exchange can still

continue and plant growth is less affected. However, if both leaf surfaces are exposed to dust, stomatal functions can be significantly reduced and plant growth will be greatly affected (Taheri Analojeh et al. 2016).

The leaves of species with thin lamina, smooth surfaces and long petioles are easily disturbed by air movement, reducing dust retention. In contrast, species with thick rough and hairy leaves and short petioles exhibit high dust deposition and are therefore favor dust retention (Prusty et al. 2005). In our study, high dust collection in *M. alba* can be attributed to the ovate shape of the leaves with deep vein depressions in the middle, a leaf-rugged surface and a high surface leaf area. In the case of *C. caucasica*, dust accumulation may be due to relatively high area and rough and uneven surface of the leaves and the presence of tiny hairs on abaxial surface. The low dust accumulation capacity of *F. rotundifolia* is probably caused by the smooth surface of its leaves and the low leaf area. The low dust-retention capacity at *M. azedarach* may be due to small leaf area, thin lamina and flat, smooth leaf surfaces.

Effects of dust accumulations on morphological traits

In our study, leaf area, diameter growth, height growth, biomass of leaf, shoot, root and total biomass were reduced with raising dust concentration for all four species which is in line with the findings of Zia-Khan et al. (2015), Bao et al. (2016) and Tomar et al. (2018). Plants need light to synthesize their own resources and thus to photosynthetically transform light energy to chemical energy. In dusty condition, the deposition of dust particles on leaf surfaces may change the leaf properties, especially the surface reflectance in the visible and short-wave infrared radiation spectrum limits the quality and quantity of light reaching the chloroplasts for photosynthesis activity of the plants (Keller and

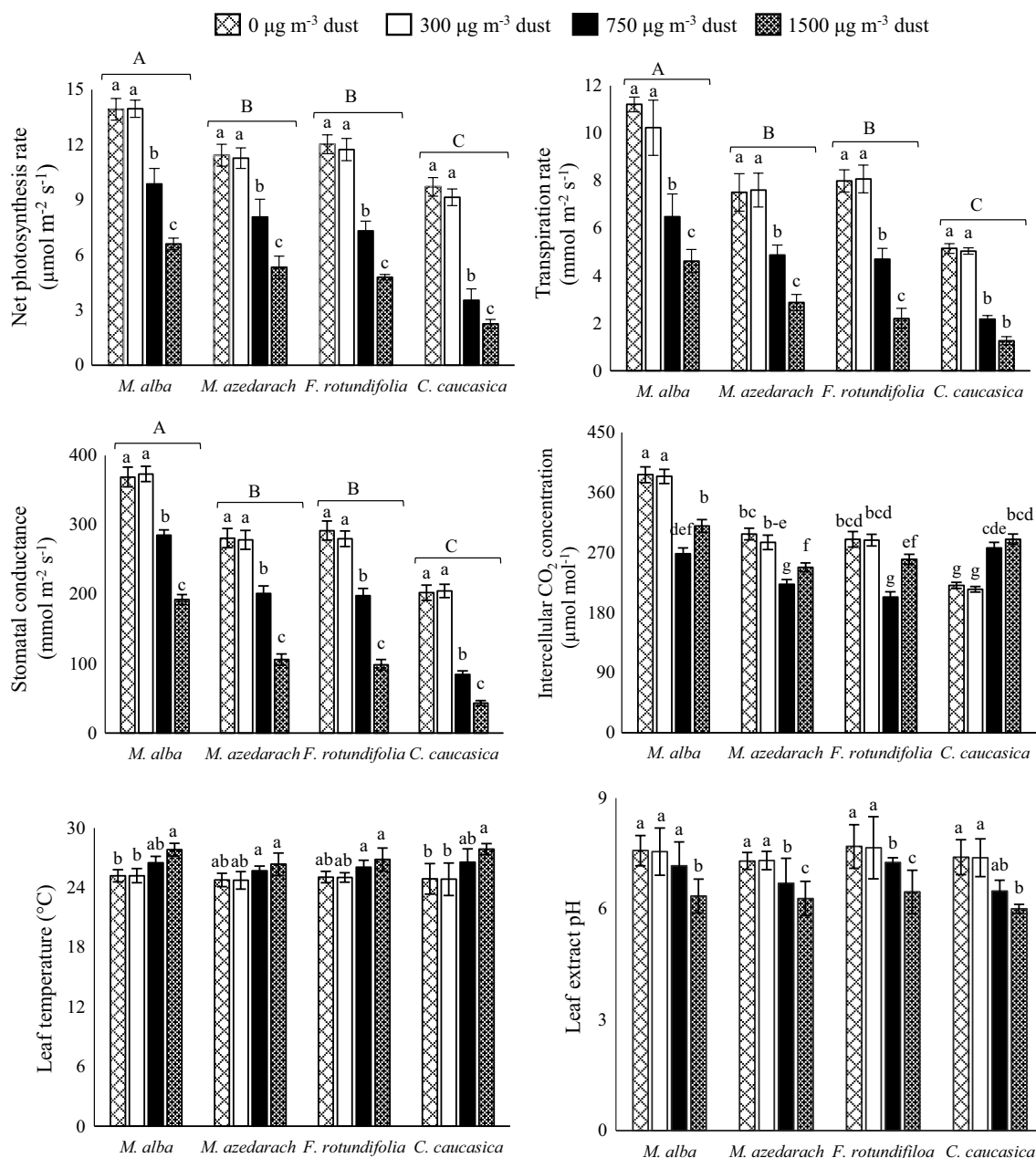


Fig. 6 Photosynthesis, transpiration, stomatal conductance, intercellular CO_2 concentration, leaf temperature and extract pH during the 10-week dust exposure treatment for four tree species. Different letters in photosynthesis, transpiration and stomatal conductance indicated a significant difference under four dust levels (lowercase letters) and tree seedling (capital letters), different letters in intercellular CO_2

concentration indicated a significant difference under the interaction between the two variability factors, and different letters in leaf temperature and extract pH indicated a significant difference under the four dust levels at 0.05 using Duncan's test. Values are the mean \pm SE, $n=4$ seedlings, except for leaf extract pH, $n=3$ seedlings from each treatment combination (dust level–tree species)

Lamprecht 1995). Furthermore, the dusty layer on the plant organs blocks the stomata and interrupts the gas exchange (Rai et al. 2010; Grantz et al. 2013). As a result, net photosynthesis and transpiration, growth and biomass production decrease (Pavlik et al. 2012), while plant tissue temperatures increase (Redondo-Gómez et al. 2011; Sett 2017). In line with our results, Leghari et al. (2014) reported that the

reduced height of *Vitis vinifera* was related to the reduction in phytomass (plant green mass), net primary production and chlorophyll content under road dust.

In the current investigation, *M. alba* showed the greatest and *C. caucasica* the lowest values in most of the growth and biomass traits. Pollution effects on the plant metabolism, such as reduced pigments contents, may block stomata

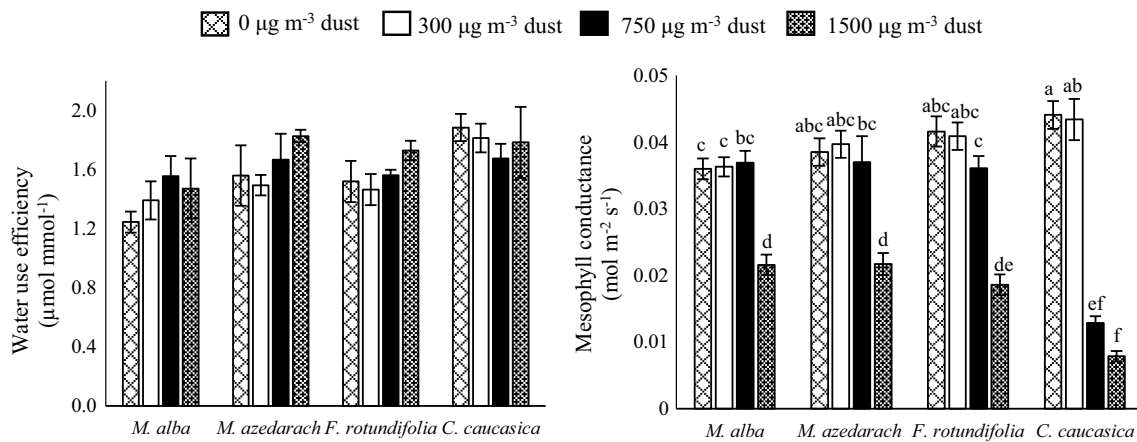


Fig. 7 Water use efficiency and mesophyll conductance during the 10-week dust exposure treatment for four tree species. The absence of letter in water use efficiency indicates not statistical significance, and different letters in mesophyll conductance indicated a significant

difference under the interaction between the two variability factors at 0.05 using Duncan’s test. Values are the mean ± SE; n=4 seedlings from each treatment combination (dust level–tree species)

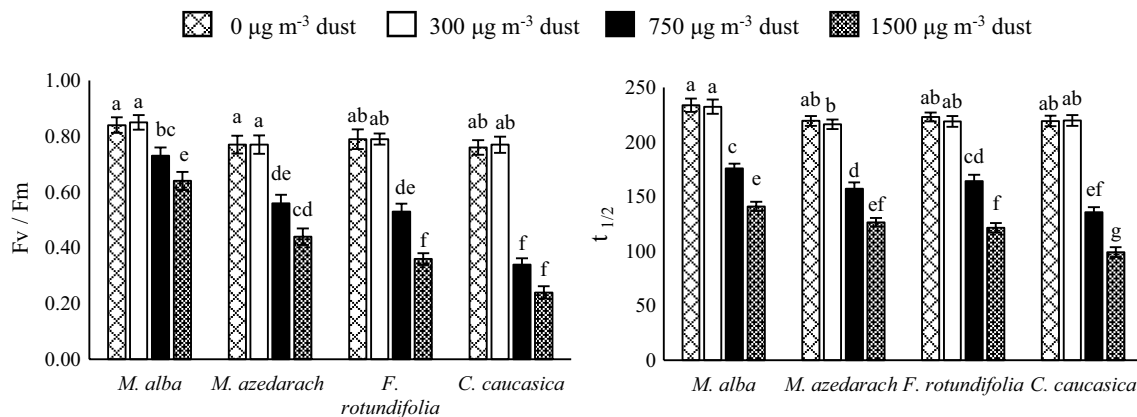


Fig. 8 F_v/F_m and t_{1/2} during the 10-week dust exposure treatment for four tree species. Different letters indicated a significant difference under the interaction between the two variability factors at 0.05 using

Duncan’s test. Values are the mean ± SE; n=4 seedlings from each treatment combination (dust level–tree species)

Table 4 Two-way analysis of variance (ANOVA) for biochemical properties traits in relation to different dust levels and tree species

Biochemical properties	Dust concentration		Tree seedling		Dust concentration × tree seedling	
	F	P	F	P	F	P
Chlorophyll a	189.345	0.000	24.620	0.000	2.477	0.330
Chlorophyll b	114.537	0.000	19.201	0.000	1.154	0.356
Chlorophyll a/b ratio	2.867	0.005	0.823	0.491	0.358	0.947
Total chlorophyll	345.832	0.000	48.824	0.000	3.952	0.002
Carotenoid	75.106	0.000	11.715	0.000	1.062	0.416

Bold numbers indicate significant statistical differences

(Ninave et al. 2001) and indicate that these variables are key regulators of growth and biomass.

Our findings indicated that elevated dust concentration increased the root–shoot ratio in all species which

is in accordance with the results of Bao et al. (2016) on *Sophora japonica*. From a physiological point of view, the root–shoot ratio reflects a different allocation of photosynthesis products between above- and belowground

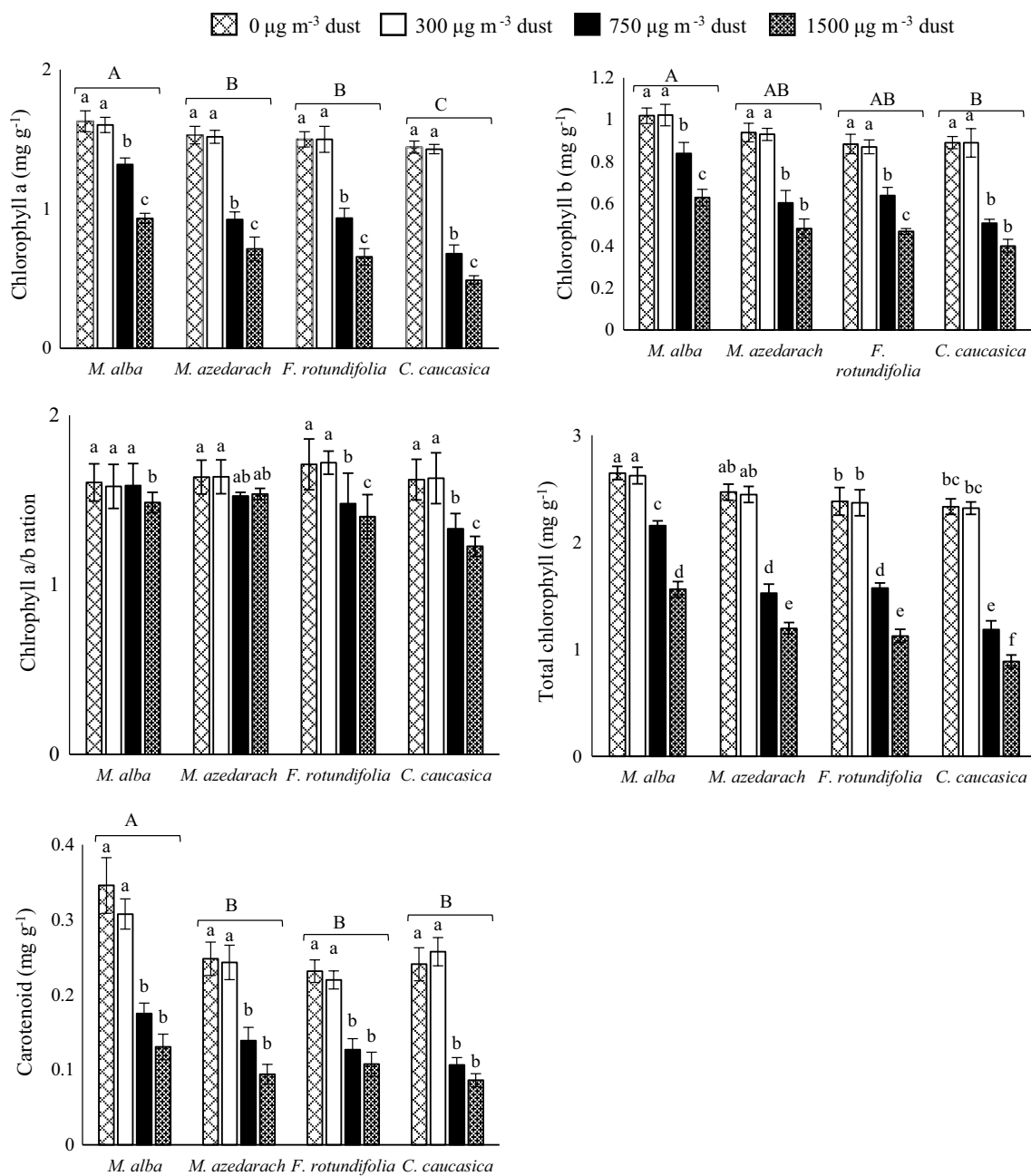


Fig. 9 Chlorophyll *a*, chlorophyll *b*, chlorophyll *ab* ratio, total chlorophyll and carotenoid during the 10-week dust exposure treatment for four tree species. Different letters in chlorophyll *a*, chlorophyll *b* and carotenoid indicated a significant difference under four dust levels (lowercase letters) and tree seedling (capital letters), different letters in chlorophyll *ab* ratio indicated a significant difference under

the four dust levels, and different letters in total chlorophyll indicated a significant difference under the interaction between the two variability factors, at 0.05 using Duncan’s test. Values are the mean ± SE; *n* = 3 seedlings from each treatment combination (dust level–tree species)

plant compartments, caused by biotic and abiotic stresses (Schoefs 2000). Plants under dust stress appear to allocate more biomass to the roots than to the shoot (Bao et al. 2016). Since plants respond in the same way to increased shade (Schall et al. 2012), this finding suggests that dust

reduces the light availability. The highest root–shoot ratio was found for *M. alba*, *M. azedarach* and *F. rotundifolia*, while the minimum value was observed for *C. caucasica*. It seems as if the latter suffers most from light availability due to soil dust.

Effects of dust accumulation on physiological parameters

Similar to the findings of Vardaka et al. (1995) on *Quercus coccifera*, Zhan-Yi et al. (2016) on *Agropyron cristatum*, *Chenopodium album*, *Leymus chinensis* and *Melissilus ruthenicus*, Siqueira-Silva et al. (2016a) on *Cedrela fissilis* and Moradi et al. (2017) on *Quercus brantii*, *Q. infectoria* and *Q. libani*, the four species of our study responded to increased dust concentration with reductions in photosynthesis, transpiration and stomatal conductance while leaf temperature increased. Reducing photosynthesis, transpiration and stomatal conductance by dust application may be due to the blockage of leaf stomata (Siqueira-Silva et al. 2016a), or due to reduced absorption of photosynthetically active radiation (PAR) (Prajapati and Tripathi 2008; Pourkhabbaz et al. 2010). On the other hand, because of dust-loaded substrate on leaves, increased absorption of near-infrared solar spectral irradiance may result in enhanced leaf temperature and photorespiration and decreased net photosynthesis (Hirano et al. 1995).

Among the species tested, the highest and lowest values of photosynthesis, transpiration and stomatal conductance occurred in *M. alba* and *C. caucasica*, respectively. It is well known from other studies that *M. alba* is resistant to disturbances such as air pollution (Dineva 2017). The remarkable resistance of *M. alba* to polluted environments may be due to leaf anatomical and morphological alterations, for example increased leaf thickness of laminae, increased cuticle layer of the adaxial surface, detection of idioblasts on the adaxial surface epidermis and special mesophyll structure without noticeable air spaces in the spongy parenchyma.

In our study, leaf extract pH for all species decreased with rising soil dust concentrations. It seems as if the penetration of dust particles into the leaf tissues and subsequent dust accumulation on the leaf surface reduced the leaf extract pH. However, it is not clear yet how the lowered pH may influence photosynthesis and tree growth. Water use efficiency represents the rate of photosynthesis per unit transpiration and enhances with carbon assimilation and/or reduced transpiration rate. In this investigation, water use efficiency was not affected by dust concentration or tree species, as indicated by the uniform decrease in photosynthesis and transpiration rate at different dust levels. Increasing the dust concentration from 0 to 1500 $\mu\text{g m}^{-3}$ decreased the photosynthesis by 53–77% and transpiration by 59–75%.

We found that in *M. alba*, *M. azedarach* and *F. rotundifolia*, intercellular CO_2 concentration decreased and then increased with rising dust stress up to 750 $\mu\text{g m}^{-3}$. In *C. caucasica*, dust levels of 750 and 1500 $\mu\text{g m}^{-3}$ showed the highest intercellular CO_2 concentrations. The reduction in CO_2 processing and photosynthesis rate, in spite of the present high CO_2 concentration in intercellular spaces, is

attributed to low mesophyll conductance and the inability of mesophyll cells to use CO_2 (Fischer et al. 1998). In our study, the probable interference in the function of mesophyll cells and the elevation of intercellular CO_2 concentration in *C. caucasica* occurred at dust concentrations $> 300 \mu\text{g m}^{-3}$, whereas in other species, this interference was observed at $> 750 \mu\text{g m}^{-3}$.

In each of the four studied species, increased dust concentration resulted in reduction in F_v/F_m and $t_{1/2}$. The lowest values were recorded at 1500 $\mu\text{g m}^{-3}$ of dust in *C. caucasica*, which is in line with findings of Naidoo and Chirkoot (2004) on *Avicennia marina* and Maletsika et al. (2015) on *Prunus persica*. F_v/F_m ratio which indicates the quantum yield of photosynthesis commonly reaches values around 0.77–0.86 for non-stressed plants. The much lower values found in this study in the dust treatments may point toward the destruction of the reaction center of PSII. In fact, it may be assumed that photoinhibition limited the photosynthesis (Van Heerden et al. 2007). Actually, $t_{1/2}$ is an indicator for estimating the size of the plastoquinone (PQ) pool that is located in the PSII. With increasing accumulation of dust on the leaves, $t_{1/2}$ decreased, suggesting the smaller size of the PQ pool on the reducing side of PSII (Vardaka et al. 1995).

Effects of dust accumulation on biochemical traits

In our study, the highest amounts of chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid occurred in *M. alba*. In all species, increased dust concentrations caused a decline in the content of photosynthetic pigments. Our findings are in line with the results of Nanos and Ilias (2007), Lone et al. (2011), Chaturvedi et al. (2013) and Siqueira-Silva et al. (2016a). For the biosynthesis of photosynthetic pigments, light and oxygen are essential factors (Yuan et al. 2017). Dust-deposited particles form a coating layer on the leaf surface. This coat seems to block the stomata and hampers the absorption of oxygen and light. Thereby, it inhibits energy dissipation of those antenna proteins that are related to light forage (Horton and Ruban 2004). Moreover, decreases in chlorophyll pigments may be the result of pH changes inside the leaf cells. It seems that the dissolution of chemical materials originating from dust particles in the cell sap may cause chlorophyll degradation (chlorophyll *a* is converted to pheophytin by replacement of Mg^{2+} and chlorophyll *b* is degraded to chlorophyllide *b* through the removal of phytol group of the molecule) or inhibition of enzymes essential for chlorophyll biosynthesis (Prusty et al. 2005).

Due to variation in the air pollution tolerance index (APTI), different plant species do not necessarily show similar susceptibility to a same pollutant. Leaf pigments assessment is important indicator for determining the tolerance of plants against air pollution. For example, plant species having high pigments content display high

tolerance to air pollution (Ninave et al. 2001). Against this background, our study suggests that *M. alba* is more tolerant to air dust pollution than the other tree species tested here.

Chlorophyll *a/b* ratio declined with increasing dust levels. This is in close conformity with the results reported by Bao et al. (2016) and Siqueira-Silva et al. (2016a) and contrary to the findings of Maletsika et al. (2015). However, the latter also found a decrease in total chlorophyll with increasing dust accumulation. The chlorophyll *a/b* ratio displays the balance between important photosynthesis pigments involved in light capture and is an indicator of leaf shading (Maletsika et al. 2015). Hence, in the present study, it seems as if reduced light availability was the most important agent for the reduced photosynthesis rate. The chlorophyll *a/b* ratio is further used to estimate the tolerance of plants to environmental stress (Hariram et al. 2018). A reduced chlorophyll *a/b* ratio may be used to rank stress tolerance of different species. Therefore, on the basis of the changes in the chlorophyll *a/b* ratio of the tree species of this study, *M. alba* and *M. azedarach* with 11% and 10% decline, respectively, when exposed to 1500 $\mu\text{g m}^{-3}$ of dust compared to the control, were the most tolerant species. In contrast, *C. caucasica* with a 39% decline was the most sensitive species.

Conclusions

Ambient dust levels occurring in Iran affected morpho-physiological and biochemical traits of four commonly planted urban tree species under greenhouse conditions. Species differed in the amount of accumulated dust and in their ability to tolerate dust stress. At dust levels of 750 and 1500 $\mu\text{g m}^{-3}$, the following ranking of dust accumulation was found: *M. alba* > *C. caucasica* > *F. rotundifolia* > *M. azedarach*. All species exposed to soil dust showed negative responses in morpho-physiological and biochemical traits. However, while *M. alba* has the highest dust accumulating potential it was least affected by dust pollution. In contrast, *C. caucasica* which has also a relatively high capacity for dust accumulation was highly affected in morphology, physiology and biochemistry. *M. azedarach* and *F. rotundifolia* did not indicate high efficiency for dust trapping but were considerably affected in their performance. We conclude that *M. alba* seems to be a suitable tree species for urban green spaces where the dust pollution ranges between 750 and 1500 $\mu\text{g m}^{-3}$.

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Author contributions ZJ, MTK, HAB and SMH conceived and designed the experiments. ZJ, HAB and SAMMS performed the experiments. ZJ and MTK analyzed the data, prepared the figures and tables and wrote the first draft of the manuscript. DS and CA contributed to improvement in manuscript from viewpoint of technical and English language. All the authors reviewed the manuscript.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

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