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Responses in leaf water status of *Quercus castaneifolia* C.A.Mey and *Carpinus betulus* L. exposed to cement dust pollution in Northern Iran

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Abstract: Industrial air pollution, particularly cement dust, affects the leaf water status and resource utilisation and finally decreases primary production. Evaluating the relative water content (*RWC*), leaf mass per unit area (*LMA*), specific leaf area (*SLA*), and leaf water per unit area (*LWA*) helps selecting more tolerant species for dusty polluted areas. In this study, we compare two species of *Quercus castaneifolia* C.A.Mey and *Carpinus betulus* L. in a polluted site (PL) around a cement factory, and a unpolluted site (UPL) in Mazandaran province, Northern Iran. Ten individual trees of each species were tagged at each site, and twenty fully developed leaves were collected for further analysis and calculation. Based on the results, *RWC* and *LWA* were significantly lower in the PL site (61.0% and 0.0075 $g\cdot cm^{-2}$, respectively) compared to the UPL site (71.1% and 0.0114 $g\cdot cm^{-2}$, respectively) for *Q. castaneifolia*. However, no significant differences were observed in selected variables between PL and UPL sites for *C. betulus*. Among the studied variables, *SLA* was significantly higher in *C. betulus* (259.1 $cm^2\cdot g^{-1}$) compared to *Q. castaneifolia* (189.8 $cm^2\cdot g^{-1}$). Our results indicated that *C. betulus* responds better to dust pollution in terms of leaf water variables.

Keywords: Hyrcanian forest; primary production; resource utilisation; specific leaf area; transpiration

Dust particles are a significant part of air pollutants arising due to industrial processes and posing a severe threat to the ecosystem (Sett 2017). Dust is a collection of fine particles of natural or industrial origin and is considered one of the most widespread air pollutants. Reports indicate that about 30 million tons of dust enter the atmosphere and disperse annually worldwide (Van Jaarsveld 2008). Cement industries are among the most polluting industries globally (Lamare, Singh 2020). Cement dust pollution not only affects the regular operation of plant net photosynthesis, transpiration,

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and respiration, but also leads to the disturbance of plant metabolism and growth, shortening plants' growth cycle (Zhu et al. 2020).

Leaves are frequently used to assess pollution response and tolerance of trees in natural or artificial environments due to their role in biochemical, physiological, and ecological responses (Arena et al. 2014). The deposition of dust particles on the tree leaves causes numerous chemical or biochemical changes, ultimately decreasing its primary production (Heydarnezhad, Ranjbar-Fordoie 2014). For instance, Chaturvedi et al. (2013) showed the stopping and withdrawal of physiological processes due to dust pollution. Some other reports indicate the absorption of some dust particles through the stomata and the outer membrane of the leaf, which includes the destructive effect of destroying chlorophyll, reducing photosynthetic activity, reducing stomatal diffusion resistance, increasing leaf temperature, and stopping plant growth (Wijayratne et al. 2009; Rai et al. 2010; Chaturvedi et al. 2013).

In recent years, research on the relationship between dust pollution and plant responses has increased. Among the studies, leaf water status and resource utilisation are reliable and repeatable markers that are responsive to biotic and abiotic stress conditions (Brandão et al. 2017). Ade-Ademilua et al. (2008) reported a significant reduction in shoot length, total leaf area, and dry weight of plants affected by cement dust pollution. Similarly, Mukherjee and Agrawal (2018) showed significant declines of 3–16% in relative water content for the species of *Eucalyptus citriodora* Hooker and *Caesalpinia sappan* Linn. with increasing the traffic pollution load. Many studies had reported that dust pollution caused relative water content changes (Joshi, Chauhan 2008; Ogunkunle et al. 2015; Gupta 2016; Rai 2016) and the reduction of the specific surface area of leaves (Petkovšek et al. 2008). Nevertheless, the toxic impacts of dust on plants depend on the chemical composition of dust, the size of the particles, the shelf life, and the amount of its deposition (Van Jaarsveld 2008).

The temperate regions of Iran, located between the Caspian Sea and the Alborz Mountains, provide suitable temperatures and ample rainfall (Eslamdoust 2022). Hyrcanian temperate forests form a unique forested stripe that stretches 850 km along the southern coast of the Caspian Sea in Iran. Hyrcanian forests are significant forests among the five vegetation regions in Iran due to their density, canopy cover, and diversity (Deljouei et al. 2018; Rahbarisisakht et al. 2021). Chestnut-leaved oak (*Quercus castaneifolia* C.A.Mey) is one of the valuable species of native oaks of Iran that is widely distributed in the Hyrcanian temperate forests. This species is the second most important commercial species in Iran after oriental beech (*Fagus orientalis* Lipsky) (Rouhi-Moghaddam et al. 2008). *Q. castaneifolia* makes up 6.6% of the area and 8% of the standing volume of Hyrcanian temperate forests. Some individual trees of *Q. castaneifolia* can measure up to 50 m in height and 3 m in diameter at breast height (DBH) (Azaryan et al. 2015). Hornbeam (*Carpinus betulus* L.) is one of the most valuable tree species in the Hyrcanian forest that occurs across many elevations from the coastal plain at sea level to an altitude of around 1 800 m a.s.l. (Sagheb-Talebi et al. 2003). Hornbeam is a native species in Caspian forests and grows in mixed stands with oak, beech, and hardwood species. The species requires a warm climate for good growth and occurs at elevations up to 1 000 m a.s.l. (Abdi et al. 2009).

With the population increase and the rapid development of industries around forest ecosystems on the one hand, and considering the wide distribution of *Q. castaneifolia* and *C. betulus* on the temperate regions of Iran on the other hand, the resistance ability of these species to dust pollution was investigated by collecting samples from cement dust-polluted and unpolluted sites in Northern Iran. This study aimed to evaluate the influence of cement dust on *Q. castaneifolia* and *C. betulus* leaves in terms of leaf water status and resource utilisation.

MATERIAL AND METHODS

Study area. This study was performed in a part of the Hyrcanian mixed forests near the Mazandaran cement factory located at 36.63°N and 53.34°E. The area around the factory is mainly plain and 45 m a.s.l. The soil of the site is sandy loam in texture. The region has a subtropical climate type. The average annual humidity, temperature, and rainfall are 61%, 17.4 °C, and 676 mm, respectively. Mazandaran cement factory was established in Hyrcanian mixed forests in 1981, mainly based on the availability of raw materials and also the strategic position in Northern Iran. This factory is located 6 km southeast of the centre of Naka city and covers an area of 4.51 km^2 (website address: http://www.mazandarancement.ir).

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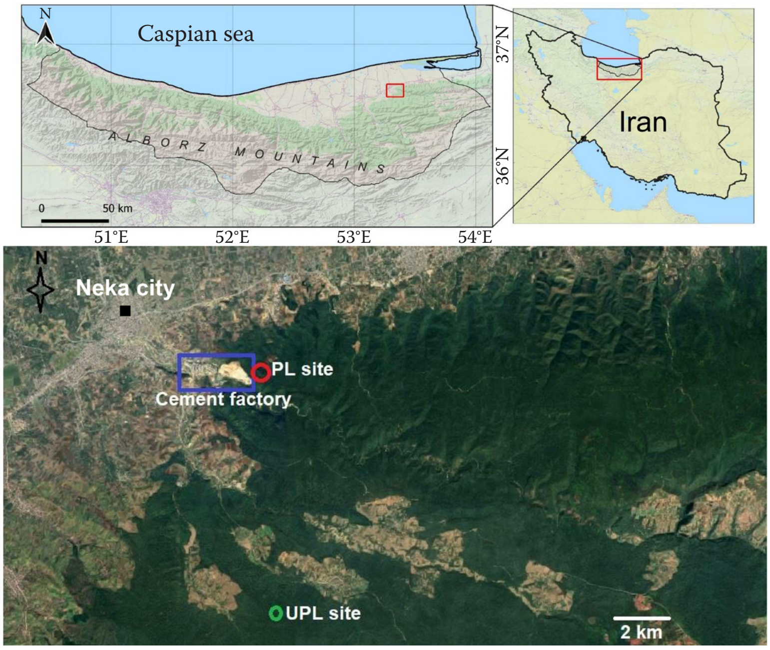

Figure 1. Location of the study sites in Northern Iran

PL – polluted site at 300 m from the cement factory location; UPL – unpolluted site at > 5 km

Two sites were selected: a cement dust polluted site (hereafter, PL) at 300 m, and an unpolluted site (hereafter, UPL) at > 5 km from the cement factory location (Figure 1). To determine the total deposited dust, 10 filter papers (Whatman No. 42) were fixed on randomly selected trees (2 m height of stem) at each site for two weeks in a no rain period. To calculate the total deposited dust, the initial and final weight of filters was measured and the results were reported in terms of the weight of dust collected, divided by the surface area of the filter papers used (mg·cm^{-2} in 14 days) (Pfeiffer 2005; Alam, Mohiuddin 2022). Table 1 shows the total deposited dust (*Tdust*) in PL and UPL sampling sites.

Sampling. Leaf samples were collected from tree species of *Q. castaneifolia* and *C. betulus* in two polluted and unpolluted sites. At each site, ten individual trees of each species were tagged, and twenty fully developed healthy and disease-free leaves were collected randomly from the four points of the horizon around the tree crown. The leaves were mixed to form a representative sample for each tree. After collection, fresh leaves were kept in zipper packs, preserved in a cool box, and brought to the laboratory for further analysis.

Leaf water and leaf mass status. A total of 10 leaves per species (20 leaves per species at each sampling site) were washed thoroughly with tap water and then distilled water. After that, the excess water was removed with filter paper. The area of fresh leaves (*LA*, cm^2) was measured using a leaf area meter, model LI-3000 (Li-Cor, USA). Then the weight of the fresh leaf was taken as fresh weight. Turgid weight was taken after immersion of fresh leaf in water overnight. Then the leaf was smudged, placed in a dryer at 70 °C for 24 h, and reweighed to obtain the dry weight. Finally, the leaf variables of relative water content (*RWC*), leaf mass per unit area (*LMA*), specific leaf area (*SLA*), and leaf water per unit area (*LWA*) were calculated using the Equations (1–4) shown in Table 2.

Statistical analysis. Statistical analysis was conducted using R software (Version 4.1.3, 2022). Shapiro-Wilk test was used to check the normality of the data. Differences in leaf variables between sites and species were tested separately using the independent samples *t*-test. Pearson correlation analysis was performed to evaluate the relationship between deposited dust and leaf variable concentrations of species.

Table 1. Mean (± standard error) total deposited dust (*Tdust*) in PL and UPL sites in 14 days

Site	PL	UPL
Deposited dust (mg·cm^{-2})	0.649 (± 0.152)	0.032 (± 0.007)

PL – polluted site; UPL – unpolluted site

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Table 2. Measured leaf variables for *Q. castaneifolia* and *C. betulus*

Abbreviation	Variable	Unit	Formula	Equation
RWC	relative water content	%	$RWC = \frac{(FW - DW)}{(TW - DW)} \times 100$	(1)
LMA	leaf mass per unit area	g·cm^{-2}	$LMA = \frac{DW}{LA}$	(2)
SLA	specific leaf area	cm^2·g^{-1}	$SLA = \frac{LA}{DW}$	(3)
LWA	leaf water per unit area	g·cm^{-2}	$LWA = \frac{FW}{LA}$	(4)

FW – fresh weight; *DW* – dry weight; *TW* – turgid weight; *LA* – leaf area

RESULTS

Leaf water status of *Q. castaneifolia*. *RWC* was significantly higher in the UPL site (71.1%) compared to the PL site (61.0%) for *Q. castaneifolia* ($t = -3.57$; *P*-value = 0.002) (Figure 2A). *LMA* was not significantly different between PL and UPL sites (Figure 2B). However, *SLA* was significantly higher in PL site (188.1 cm^2·g^{-1}) compared to UPL site (171.5 cm^2·g^{-1}) ($t = 2.22$; *P*-value = 0.040) (Figure 2C). *LWA* was significantly higher in the UPL site (0.0114 g·cm^{-2}) compared to the PL site (0.0092 g·cm^{-2}) ($t = -2.86$; *P*-value = 0.010) (Figure 2D). Also, Pearson correlation shows a significant relationship between the leaf variables of *Q. castaneifolia* (Figure 3). The highest negative relationship was found between *LMA* and *SLA* ($r = -0.99$). A significant negative relationship was observed between total deposited dust and *RWC* ($r = -0.58$), and no significant correlation was found between the total deposited dust and *LMA*, *SLA*, and *LWA*.

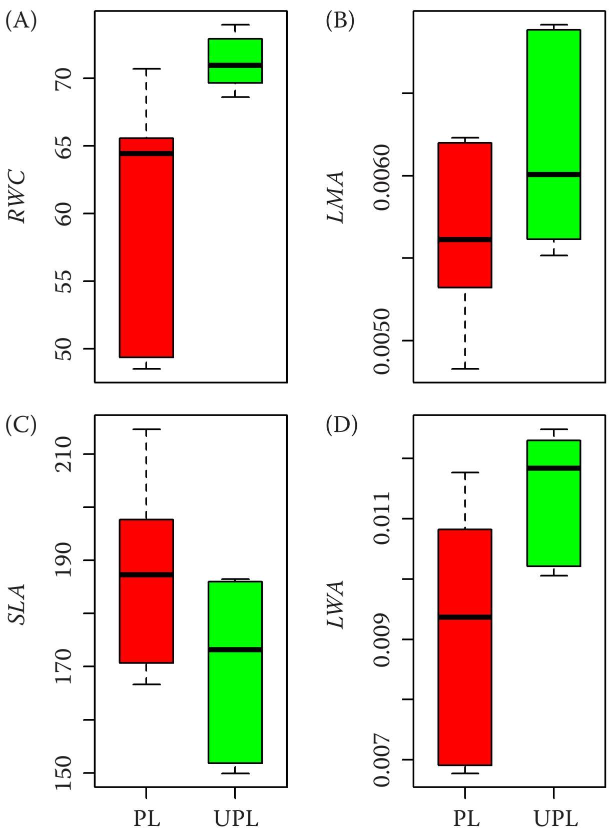

Figure 2. (A) Leaf *RWC*, (B) *LMA*, (C) *SLA*, and (D) *LWA* of *Q. castaneifolia* in PL and UPL sites

RWC – relative water content; *LMA* – leaf mass per unit area; *SLA* – specific leaf area; *LWA* – leaf water per unit area; PL – polluted site; UPL – unpolluted site

Leaf water status of *C. betulus*. Leaf variables of *RWC*, *LMA*, *SLA*, and *LWA* were not significantly different between PL and UPL sites for *C. betulus* (Figure 4A–D). Although *RWC*, *LMA*, and *LWA* were slightly higher in the PL site, *SLA* was slightly higher in the UPL site. Figure 4 shows significant correlations between the leaf variables of *C. betulus*. The highest relationship was between *LMA* and *SLA* (–0.88). However, total deposited dust and leaf variables of *RWC*, *LMA*, *SLA*, and *LWA* were not significantly correlated (Figure 5).

Species comparison. *RWC* was significantly higher in *Q. castaneifolia* (66.1%) compared to *C. betulus* (57.5%) ($t = 4.69$, *P*-value = 0.000) (Figure 6A). Also, *LMA* was significantly higher in *Q. castaneifolia* (0.0059 g·cm^{-2}) compared to *C. betulus* (0.0041 g·cm^{-2}) ($t = 40.57$; *P*-value = 0.000) (Figure 6B). However, *SLA* was significantly higher in *C. betulus* (259.1 cm^2·g^{-1}) compared

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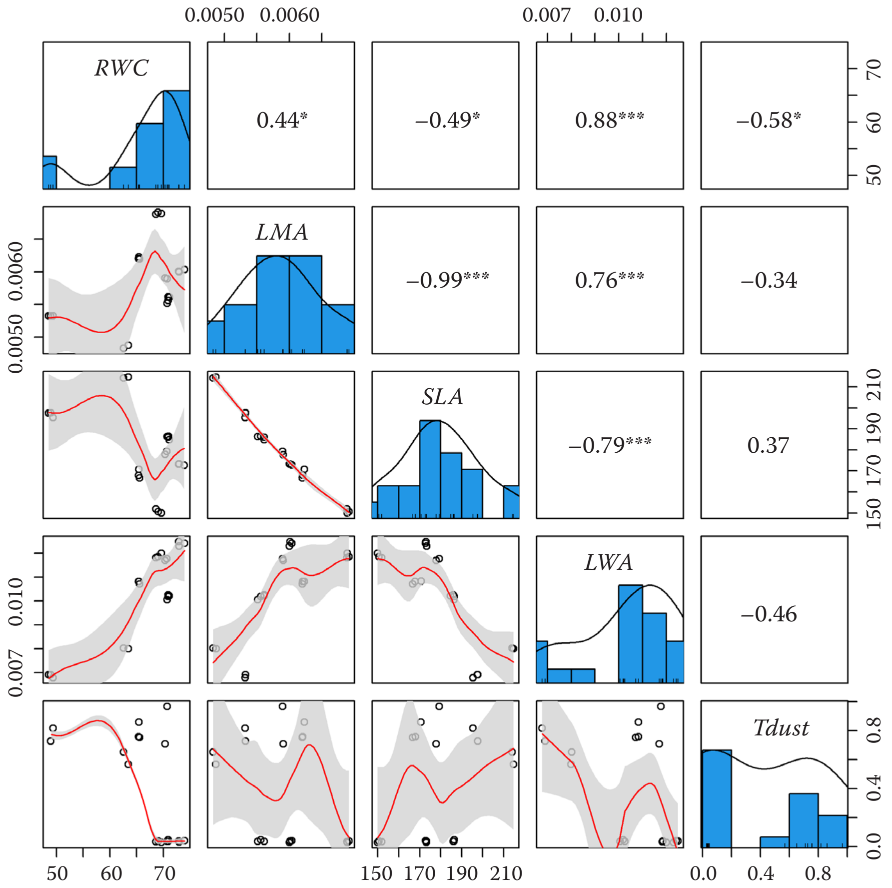

Figure 3. Pearson correlations between the leaf variables of *Q. castaneifolia*

*, *** significant correlations at 0.05 and 0.001, respectively; *RWC* – relative water content; *LMA* – leaf mass per unit area; *SLA* – specific leaf area; *LWA* – leaf water per unit area; *Tdust* – total deposited dust

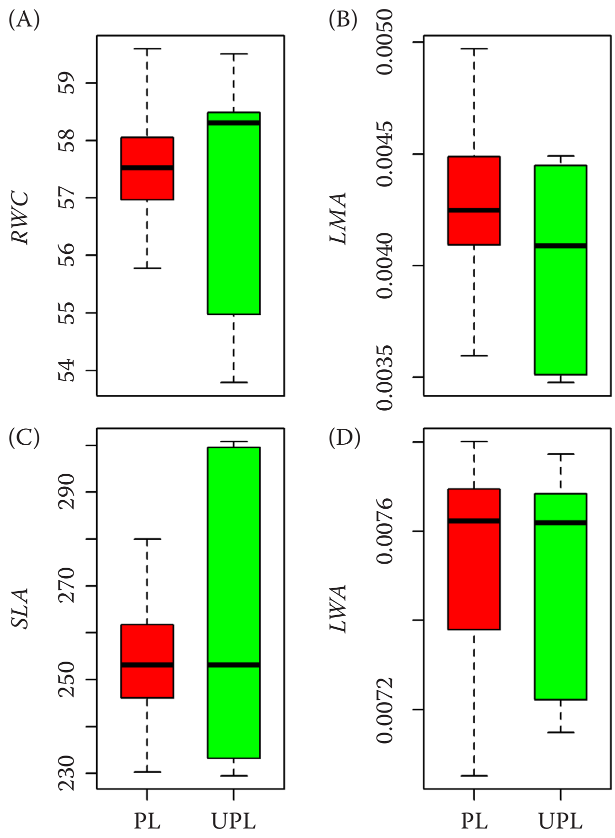

Figure 4. (A) Leaf *RWC*, (B) *LMA*, (C) *SLA*, and (D) *LWA* of *C. betulus* in PL and UPL sites

RWC – relative water content; *LMA* – leaf mass per unit area; *SLA* – specific leaf area; *LWA* – leaf water per unit area; PL – polluted site; UPL – unpolluted site

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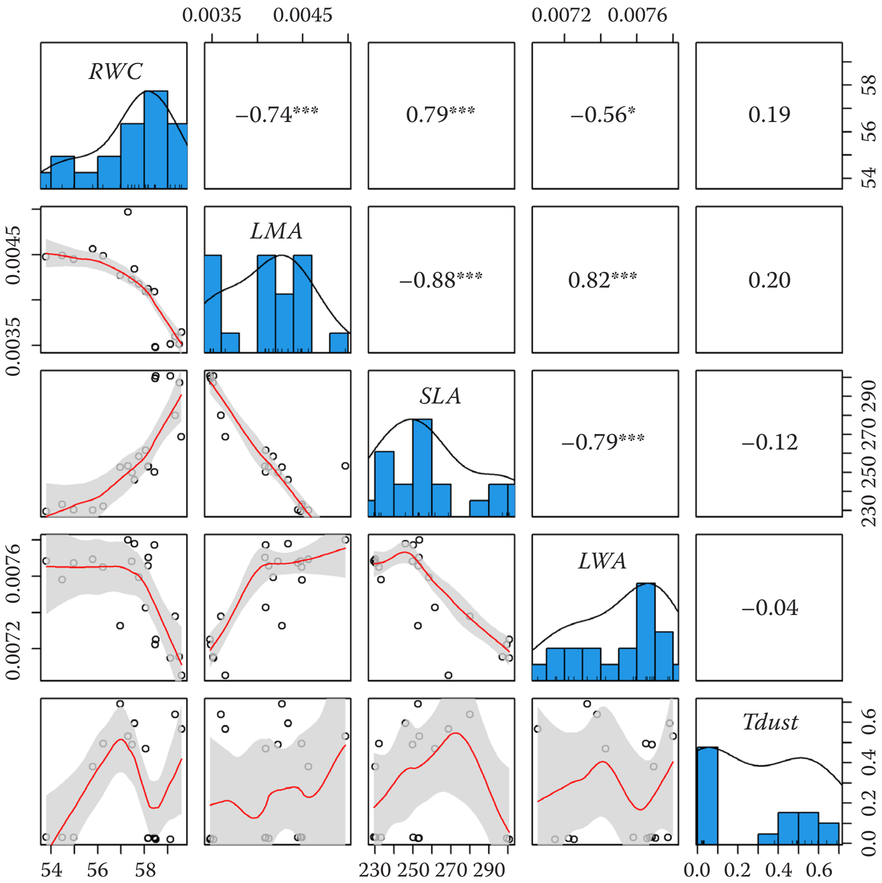

Figure 5. Pearson correlations between the leaf variables of *C. betulus*

*, *** significant correlations at 0.05 and 0.001, respectively; *RWC* – relative water content; *LMA* – leaf mass per unit area; *SLA* – specific leaf area; *LWA* – leaf water per unit area; *Tdust* – total deposited dust

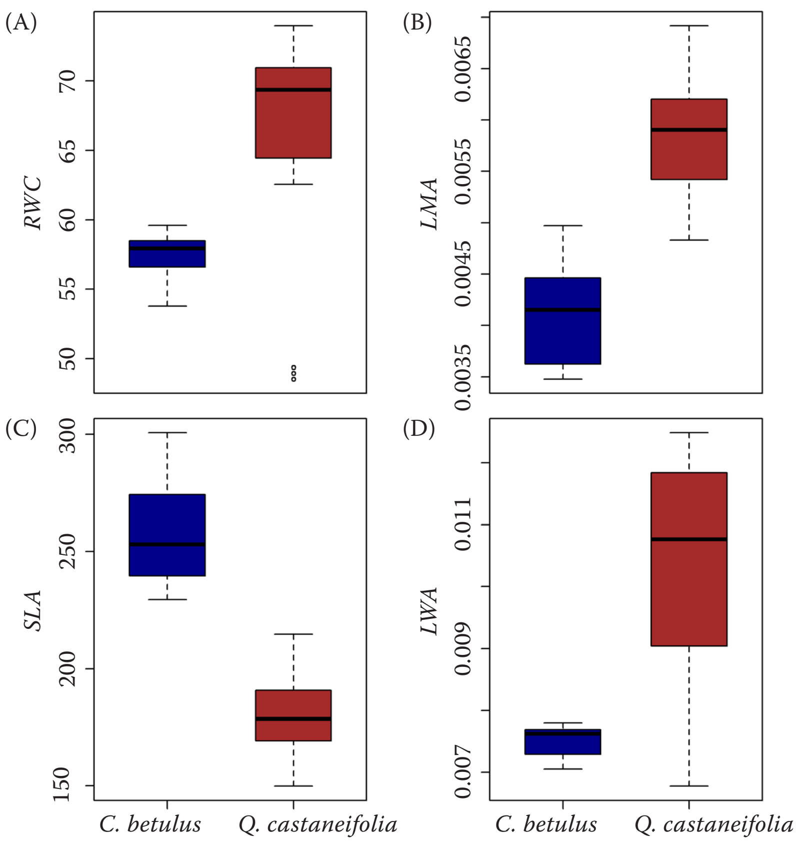

Figure 6. Leaf variables of (A) *RWC*, (B) *LMA*, (C) *SLA*, and (D) *LWA* for *Q. castaneifolia* and *C. betulus*

RWC – relative water content; *LMA* – leaf mass per unit area; *SLA* – specific leaf area; *LWA* – leaf water per unit area

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to *Q. castaneifolia* (189.8 $cm^2 \cdot g^{-1}$) (t = –11.52; P-value = 0.000) (Figure 6C). *LWA* was significantly higher in *Q. castaneifolia* (0.0103 $g \cdot cm^{-2}$) compared to *C. betulus* (0.0075 $g \cdot cm^{-2}$) (t = 6.37; P-value = 0.000) (Figure 6D).

DISCUSSION

The wax on the cuticle is the first place of impact of pollutants (Günthardt-Goerg, Vollenweider 2007) that could be damaged depending on different conditions such as the type of plant species and the climate of the site conditions (Kupčinskienė et al. 2000). Plants show more resistance to the penetration of pollutants by increasing leaf thickness (Pourkhabbaz et al. 2010). The increase in the thickness of the lower epidermis of leaves is one of the resistance mechanisms of plants to the penetration of dust particles into the internal tissues of leaves (Suganthi et al. 2013).

Based on our data, a significant decline of 14% in *RWC* was observed for *Q. castaneifolia* in the PL site. Arena et al. (2014) also observed a decline in *RWC* at a higher pollution load. Generally, high *RWC* in plants helps regulate cellular turgidity and osmotic potential (Mukherjee, Agrawal 2018). Air pollution changes the size and structure of leaves so that by changing the size of leaf cells, plants control the absorption of pollutants (Meerabai et al. 2012). For *C. betulus*, *RWC* was not significantly different between PL and UPL sites but slightly higher in the PL site. This may be because of the smaller leaves of *C. betulus* compared to *Q. castaneifolia*. Also, the comparison between species of *C. betulus* and *Q. castaneifolia* shows a significantly lower *RWC* in *C. betulus*. A decreased water content in plants denotes early stress conditions (Mukherjee, Agrawal 2018). Pearson correlations show a significant positive correlation between the *RWC* with *LMA* (0.44) and *LWA* (0.88). However, a significant negative correlation with *SLA* (–0.49) for *Q. castaneifolia* was recorded. *C. betulus* also showed significant correlations of –0.74, 0.79, and –0.56 between *RWC* with *LMA*, *SLA*, and *LWA*, respectively.

LMA measures the partitioning of resources between leaf biomass and area. In our study, *LMA* was not significantly different in PL and UPL sites for both species. However, *LMA* was slightly lower at 8% in the PL site for *Q. castaneifolia* but slightly higher at 7% in the PL site for *C. betulus* compared to the UPL site. It has been pointed out that leaves with high *LMA* have a structure that reduces photosynthetic rates but also water losses by transpiration (Hultine, Marshall 2000). Variable response of *LMA* in three woody species in the Pearl River Delta of south China was observed by Wen et al. (2004). Mukherjee and Agrawal (2018) reported increases of 32% and 22% in *LMA* for Ilex rotunda and Ficus macrocarpa, respectively, while a reduction of 20% was observed in Machilus chinensis with increasing pollution load consisting of air pollutants PM_{10} (particulate matter ≤ 10 μm), total suspended particulate matter, SO_2, NO_2, and O_3. *LMA* of *Q. castaneifolia* showed a significantly higher 30% than *C. betulus*. Paridari et al. (2013) reported *LMA* in a range of 0.006–0.011 $g \cdot cm^{-2}$ for *C. betulus* along an altitudinal gradient ranging from 100 m to 1 150 m. *LMA* was significantly correlated with *SLA* (–0.99) and *LWA* (0.76) for *Q. castaneifolia*. Also, a significant correlation of –0.88 and 0.82 was observed between *LMA* with *SLA* and *LWA*.

A significant increment of 10% in *SLA* was observed for *Q. castaneifolia* in the PL site, which shows an increase in leaf area surface in the PL site. *SLA* was insignificantly higher at 4% in the UPL site for *C. betulus*. Our results show a significantly higher *SLA* for *C. betulus* than for *Q. castaneifolia*. However, the leaves of *C. betulus* are smaller in the case of leaf area than the leaves of *Q. castaneifolia*, but also thinner. This can be the main reason for higher *SLA* in *C. betulus*. The decrease in leaf area and specific leaf area in response to pollution causes leaf production to decrease, which leads to a decline in photosynthetic activity (Datta et al. 1987; Rai et al. 2010). Based on the Pearson correlation results, the correlation between *SLA* and *LWA* was significant but negative for both species (–0.79).

Leaf water content is a suitable index to express the water status in plants which shows a comprehensive situation of the amount of supply and evaporation. A significant decrement of 11% was observed in *LWA* for *Q. castaneifolia* in the PL site compared to the UPL site. However, although *LMA* for *C. betulus* was slightly higher in the PL site, the difference was insignificant. Between the species, *Q. castaneifolia* shows significantly higher *LWA* than *C. betulus*. Therefore, dust as an environmental pollutant factor is responsible for the change in leaf ecology (Quadir, Siddiqui 2014).

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CONCLUSION

In this study, we evaluate the leaf variables of relative water content (*RWC*), leaf mass per unit area (*LMA*), specific leaf area (*SLA*), and leaf water per unit area (*LWA*) for *Q. castaneifolia* and *C. betulus* tree species in two polluted (around cement factory) and unpolluted sites. Our results show that the leaf variables of *Q. castaneifolia*, except for *LMA*, were significantly affected by dust pollution. In contrast, the leaf variables of *C. betulus* did not show significant changes. Among the leaf valuables, only the *RWC* of *Q. castaneifolia* shows a significant negative correlation with total deposited dust. Our results indicated that *C. betulus* responds better to dust pollution in terms of leaf water variables. Between the variables, leaf water status of *RWC* and *LWA* were found to be most responsive, whereas resource utilisation of *SLA* and *LMA* were least responsive to cement dust pollution. Identification of tolerance of tree species to different air pollutants with leaf variables will be useful for further assessment of ecosystem services and the pollution removal potential of tree species. However, we suggest considering other physiological and biochemical variables in evaluating the tree responses to dust pollution in future studies.

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