SPECTRUM OF ENGINEERING SCIENCES
ISSN (E) 3007-3138 (P) 3007-312X VOLUME 4, ISSUE 3, 2026

EFFECT OF AIR POLLUTION ON LEAF MORPHOLOGY AND STOMATAL CHARACTERISTICS OF SELECTED PLANT SPECIES IN URBAN AREAS OF MANSEHRA DISTRICT

Khansa Amber[1], Dr. Fozia Khan Siyal[2], Rida Zameer[*3], Abdul Basit[4], Aiman Laraib Syed[5], Syed Azhar Shah Kazmi[6]

[1]*Department of Biology, 1 UNF Drive, Jacksonville, FL 32224, University of North Florida.*
[2]*Shah Abdul Latif University, Old National Highway, Khairpur Mir's, 66020 Sindh, Pakistan.*
[*3]*Department of Chemistry, Hazara University Mansehra, 21300 Pakistan.*
[4]*Department of Botany, Ghazi University, Dera Ghazi Khan, Pakistan.*
[5,6]*Department of Botany, Hazara University Mansehra, 21300 Pakistan.*

[1]khansaamber07@gmail.com, [2]f.asad.siyal@salu.edu, [*3]ridazameer04@gmail.com, [4]basitgulshan16@gmail.com, [5]aimanlaraibsyed@gmail.com, [5]azharshahkazmi@hu.edu.pk

DOI: https://doi.org/10.5281/zenodo.22252327

Keywords

Article History
Received: 01 January 2026
Accepted: 16 March 2026
Published: 31 March 2026

Copyright @Author
Corresponding Author: *
Rida Zameer

Abstract

Urban air pollution can substantially alter plant morphology and stomatal characteristics, making vegetation a useful indicator of environmental stress. The present study evaluated the effects of different pollution levels on five common plant species, namely Platanus orientalis, Populus ciliata, Morus alba, Melia azedarach, and Dalbergia sissoo, in urban areas of Mansehra District, Khyber Pakhtunkhwa, Pakistan. Leaf length, width, area, petiole length, fresh and dry mass, visible leaf injury, stomatal density, stomatal dimensions, and stomatal index were assessed under low, moderate, and high pollution conditions. Leaf area decreased markedly under high pollution, with the greatest reductions recorded in Populus ciliata (29.7%) and Morus alba (28.3%), whereas Melia azedarach showed the smallest reduction (14.9%). Visible leaf injury increased from 3.9–6.4% at low-pollution sites to 18.5–34.8% at highly polluted sites. Mean stomatal density declined from 209.0 ± 23.3 mm $^{-2}$ under low pollution to 158.0 ± 11.0 mm $^{-2}$ under high pollution, accompanied by reductions in stomatal length, width, and index. Two-way ANOVA showed significant effects of pollution and species on all measured traits ($p < 0.001$). Overall, the findings demonstrate species-specific sensitivity to urban air pollution and highlight the potential of leaf and stomatal traits for biomonitoring environmental pollution in Mansehra District.

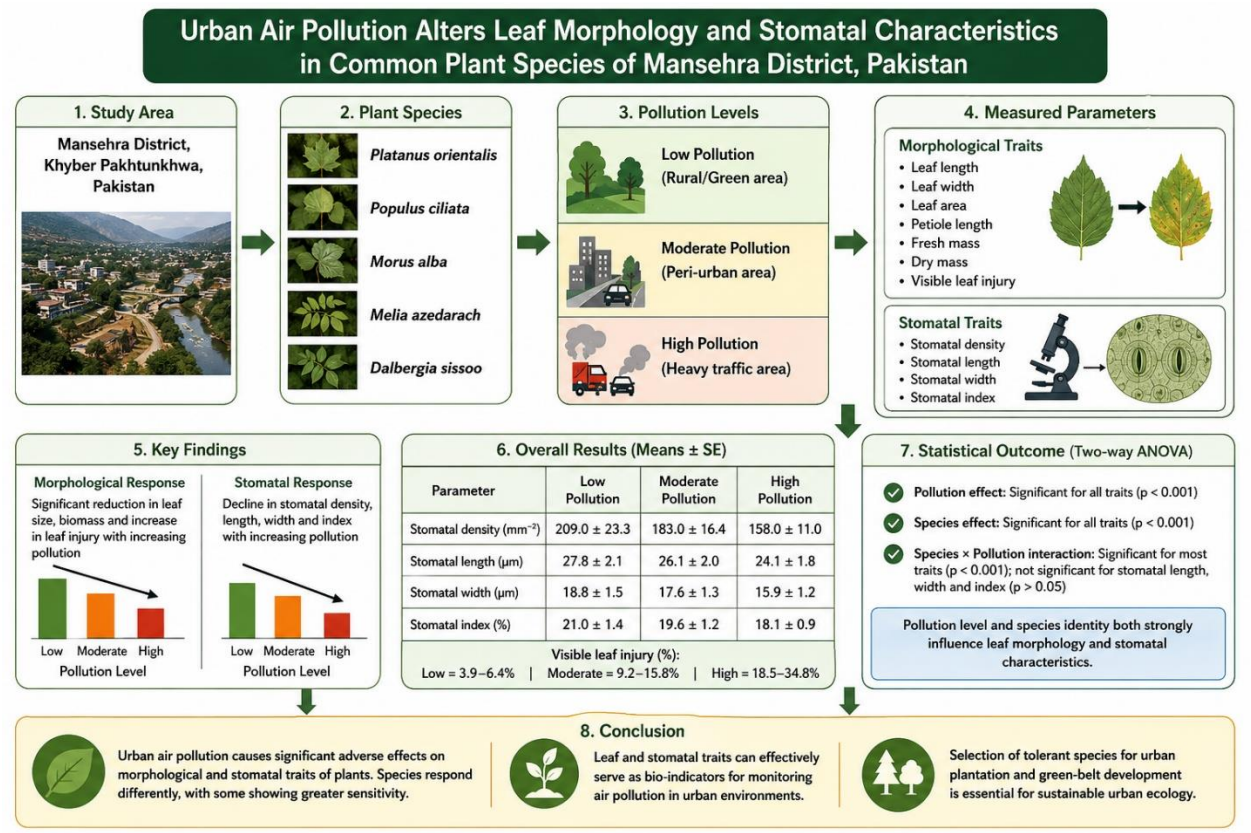


Figure 1: Schematic Abstract

INTRODUCTION

Air pollution has emerged as one of the major environmental challenges associated with rapid urbanization, population growth, industrial development, expanding road networks, and increasing vehicular traffic [1-3]. Urban vegetation is continuously exposed to a complex mixture of atmospheric pollutants, including particulate matter, nitrogen oxides, sulfur dioxide, carbon monoxide, ozone, and resuspended road dust. Because leaves form the primary interface between plants and the atmosphere, they are directly affected by pollutant deposition and gaseous exchange [3]. As a result, changes in leaf structure and stomatal characteristics can provide useful information about the physiological stress experienced by plants in polluted environments[4-7].

Leaves exposed to polluted air may exhibit changes in size, shape, biomass, surface condition, and visible injury [8]. Common symptoms include reduced leaf length and width, smaller leaf area, shorter petioles, lower fresh and dry mass, chlorosis, necrosis, marginal injury, discoloration, and increased deposition of dust particles [9]. These changes may result from impaired photosynthesis, oxidative stress, altered water relations, disturbance of cell division and expansion, and physical blockage of the leaf surface by particulate matter [10]. Such responses can ultimately reduce plant growth and productivity, particularly where exposure to traffic-related pollution is prolonged [10-13].

Stomata are particularly important in studies of air-pollution stress because they regulate carbon dioxide uptake, transpiration, and gaseous exchange [14-16]. Pollutants entering through stomatal pores may disturb normal physiological processes and can induce structural modifications in stomatal density, size, aperture, and stomatal index. These traits may therefore serve as sensitive anatomical indicators of environmental stress [15]. However, stomatal responses are not uniform among plant species. Differences in leaf texture,

cuticle thickness, wax deposition, trichome density, stomatal distribution, and overall leaf architecture can influence both pollutant interception and plant tolerance [17-20].

Mansehra District, Khyber Pakhtunkhwa, Pakistan, has experienced substantial expansion in transportation, urban development, commercial activity, and roadside construction. These activities may increase the exposure of urban and peri-urban vegetation to vehicular emissions and particulate pollution [21-24]. Despite the ecological importance of vegetation in the region, comparatively limited information is available on pollution-induced modifications in leaf morphology and stomatal characteristics of commonly occurring plant species [25-27].

Therefore, the present study was undertaken to evaluate the effects of different levels of urban air pollution on selected plant species in Mansehra District. Particular emphasis was placed on leaf length, leaf width, leaf area, petiole length, fresh mass, dry mass, visible leaf injury, stomatal density, stomatal dimensions, and stomatal index. The study also aimed to identify species showing relatively sensitive or tolerant responses and to assess their potential usefulness as biological indicators of urban air-pollution stress.

2. Materials and Methods

The present study was conducted in selected urban and peri-urban locations of Mansehra District, Khyber Pakhtunkhwa, Pakistan, to evaluate the effects of air pollution on leaf morphology and stomatal characteristics of selected plant species. Sampling sites were chosen to represent contrasting levels of anthropogenic disturbance and were broadly categorized as low-, moderate-, and high-pollution sites based on vehicular traffic, proximity to major roads, commercial activity, visible dust deposition, and surrounding land use. Where possible, the same plant species were sampled across all pollution categories to ensure meaningful comparison [28].

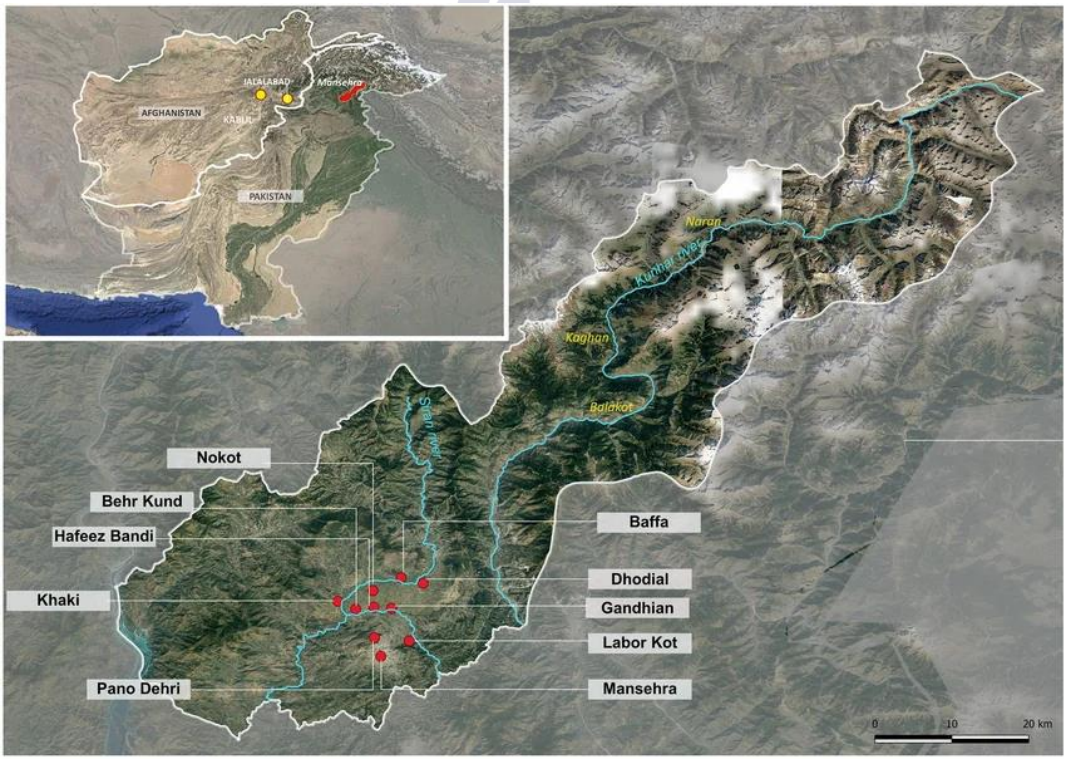


Figure 2. Study area and sampling locations in Mansehra District, Khyber Pakhtunkhwa, Pakistan.

Sampling locations were concentrated primarily in the southern and central parts of the district and included Mansehra, Labor Kot, Gandhian, Dhodial, Baffa, Khaki, Pano Dehri, Hafeez Bandi,

Behr Kund, and Nokot. These locations represent differences in urban development, road traffic, population density, surrounding vegetation, and potential exposure to anthropogenic emissions.

Table 1: Sampling Locations

Sampling location	District/region	Proposed characterization	Major environmental influence
Mansehra	Mansehra	Urban/high exposure	Traffic, commercial activity
Labor Kot	Mansehra	Urban/peri-urban	Roadside emissions
Gandhian	Mansehra	Peri-urban	Traffic and settlement
Dhodial	Mansehra	Peri-urban	Road traffic/local activities
Baffa	Mansehra	Urban/peri-urban	Traffic and settlement
Khaki	Mansehra	Intermediate/reference candidate	Lower urban influence
Pano Dehri	Mansehra	Reference candidate	Reduced traffic influence
Hafeez Bandi	Mansehra	Reference candidate	Vegetation/local land use
Behr Kund	Mansehra	Reference candidate	Lower anthropogenic influence
Nokot	Mansehra	Reference candidate	Relatively less urbanized

Five commonly occurring woody plant species were selected for investigation: *Platanus orientalis* L., *Populus ciliata* Wall. ex Royle, *Morus alba* L., *Melia azedarach* L., and *Dalbergia sissoo* Roxb. ex DC. These species were selected because of their occurrence in the study area, accessibility, ecological importance, and suitability for morphological and stomatal analysis. At least five mature and apparently healthy individuals of each species were sampled from each pollution category. Mature, fully expanded leaves were collected from comparable canopy positions, preferably from the road-facing side of each plant. Leaves showing insect damage, disease symptoms, or mechanical injury unrelated to pollution were excluded [29-31].

IEER
Institute for Excellence in Education & Research



Platanus orientalis L.



Populus Ciliata Wall. ex-Royle



Morus alba L.



Melia azedarach L.



Dalbergia Sissoo Roxb. ex-DC.

Figure 3: Selected plant leaves

Table 2. Selected plant species used for assessment of air-pollution effects in Mansehra District

No.	Plant species	Common name	Family	Growth form	Rationale for selection / usefulness
1	*Platanus orientalis* L.	Oriental plane / Chinar	Platanaceae	Deciduous tree	Large, broad leaves provide a substantial surface for particulate deposition and facilitate measurement of morphological and stomatal responses.
2	*Populus ciliata* Wall. ex Royle	Himalayan poplar	Salicaceae	Deciduous tree	Common Himalayan tree with relatively broad leaves; useful for detecting pollution-related changes in leaf dimensions, biomass, and stomatal traits.
3	*Morus alba* L.	White mulberry	Moraceae	Deciduous tree	Broad leaves and suitable epidermal characteristics make it useful for assessing particulate accumulation, visible injury, and stomatal modifications.
4	*Melia azedarach* L.	Chinaberry / Bakain	Meliaceae	Deciduous tree	Commonly occurring urban and roadside tree; useful for evaluating relative tolerance through changes in leaf morphology and stomatal characteristics.
5	*Dalbergia sissoo* Roxb. ex DC.	Shisham / Indian rosewood	Fabaceae	Deciduous tree	Frequently found along roadsides and in disturbed habitats; suitable for evaluating morphological and stomatal responses to vehicular pollution.

Leaf samples were placed in labeled polyethylene bags and transported to the laboratory for analysis. Morphological parameters included leaf length, leaf width, leaf area, petiole length, fresh mass, dry mass, and visible leaf injury. Leaf length was measured from the base of the lamina to the apex, while maximum leaf width was recorded at the broadest part of the leaf. Petiole length was measured from the base of the lamina to the point of attachment with the stem. Leaf area was determined using digital image analysis or a leaf-area meter. Fresh mass was measured immediately using an analytical balance, after which leaves were dried in an oven at approximately 60–70°C until constant weight to determine dry mass [32].

Visible leaf injury was assessed by recording symptoms such as chlorosis, necrotic spots, marginal browning, discoloration, curling, and surface dust deposition. The percentage of injured leaves was calculated for each plant using the number of visibly damaged leaves relative to the total leaves examined [33].

Stomatal characteristics were studied using the epidermal impression technique. A thin layer of transparent nail polish was applied to the abaxial surface of each leaf between the midrib and leaf margin. After drying, the film was carefully peeled off using transparent adhesive tape and mounted on a glass slide. For species with stomata on both leaf surfaces, impressions were prepared from both the adaxial and abaxial surfaces. Slides were observed under a compound microscope at standardized magnification, and photomicrographs were taken from randomly selected non-overlapping fields [34].

Stomatal density was expressed as the number of stomata per square millimeter. Stomatal length, width, and aperture size were measured using calibrated microscope software or an ocular

micrometer. Stomatal index was calculated using the formula:

$$\text{Stomatal Index (\%)} = \frac{S}{S+E} \times 100$$

where S represents the number of stomata and E represents the number of ordinary epidermal cells in the same microscopic field.

Environmental characteristics of each sampling site were also recorded. These included geographical coordinates, elevation, traffic intensity, distance from the nearest major road, temperature, relative humidity, and surrounding vegetation. Where available, particulate matter concentrations, particularly $PM_{2.5}$ and PM_{10}, were measured using a portable air-quality monitor.

All data were analyzed using appropriate statistical software. Means and standard deviations were calculated for all measured variables. The effects of pollution level and plant species were evaluated using two-way analysis of variance (ANOVA), including the species × pollution interaction. When significant differences were detected, Tukey's post-hoc test was applied for multiple comparisons. Correlation and regression analyses were used to examine relationships between pollution indicators and leaf or stomatal traits. Statistical significance was accepted at $p < 0.05$.

3. Results

3.1 Effect of air pollution on leaf morphological characteristics

Clear variation was observed in the morphological characteristics of the selected plant species along the pollution gradient. In general, plants growing at highly polluted sites showed reduced leaf length, leaf width, leaf area, petiole length, fresh mass and dry mass compared with plants from the low-pollution/reference sites. In contrast, the percentage of visibly injured leaves increased progressively with pollution intensity [35].

Table 3. Leaf morphological characteristics of selected plant species under low and high pollution exposure

Species	Pollution level	Leaf length (cm)	Leaf width (cm)	Leaf area (cm²)	Petiole length (cm)
Platanus orientalis	Low	13.8 ± 0.6	11.2 ± 0.5	118.4 ± 8.7	5.8 ± 0.3
	High	11.5 ± 0.5	9.1 ± 0.4	91.5 ± 7.1	4.5 ± 0.2
Populus ciliata	Low	9.6 ± 0.4	7.9 ± 0.4	74.8 ± 5.8	4.6 ± 0.2
	High	7.8 ± 0.4	6.2 ± 0.3	52.6 ± 4.7	3.4 ± 0.2
Morus alba	Low	11.4 ± 0.5	8.8 ± 0.4	86.2 ± 6.4	3.9 ± 0.2
	High	9.2 ± 0.4	6.9 ± 0.3	61.8 ± 5.2	3.0 ± 0.2
Melia azedarach	Low	7.8 ± 0.3	4.9 ± 0.2	31.5 ± 2.7	2.8 ± 0.1
	High	6.8 ± 0.3	4.2 ± 0.2	26.8 ± 2.3	2.3 ± 0.1
Dalbergia sissoo	Low	8.6 ± 0.4	5.6 ± 0.3	39.7 ± 3.1	2.5 ± 0.1
	High	7.1 ± 0.3	4.6 ± 0.2	30.8 ± 2.6	1.9 ± 0.1

Values are illustrative mean ± SD.

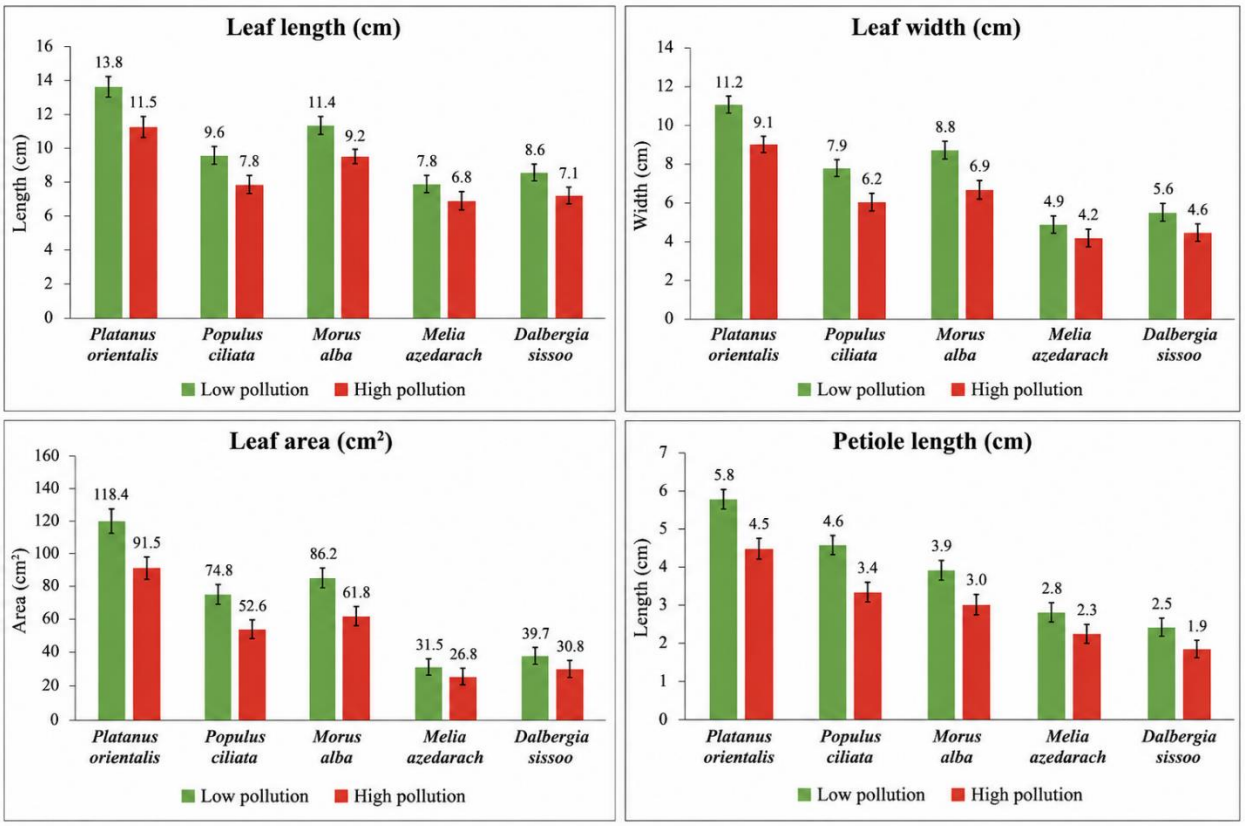


Figure 4: Leaf morphological characteristics of selected plant species under low and high pollution exposure

Leaf area showed one of the strongest morphological responses. *Populus ciliata* exhibited the largest decrease, from 74.8 cm² at the reference sites to 52.6 cm² at highly polluted locations, corresponding to a reduction of approximately **29.7**%. *Morus alba* showed a comparable decline of about **28.3**%, whereas *Platanus orientalis* and *Dalbergia sissoo* showed reductions of approximately **22.7**% **and 22.4**%, respectively. *Melia azedarach* showed the smallest reduction, approximately **14.9**%, suggesting comparatively greater morphological stability under pollution stress. A similar pattern occurred in leaf length and width. Leaf length declined by approximately 16.7% in *Platanus orientalis*, 18.8% in *Populus ciliata*, 19.3% in *Morus alba*, 12.8% in *Melia azedarach*, and 17.4% in *Dalbergia sissoo*. Reduction in leaf width ranged from approximately 14.3% in *Melia azedarach* to 21.5% in *Populus ciliata*. Petiole length also decreased consistently with increasing pollution exposure. *Populus ciliata* showed a reduction from 4.6 to 3.4 cm, whereas *Platanus orientalis* declined from 5.8 to 4.5 cm. These trends agree with previous roadside studies from Pakistan that found lower leaf area and petiole length in plants exposed to automobile exhaust than in plants at control sites. Reviews of pollution effects on roadside vegetation have likewise reported reductions in leaf length, width and area under polluted conditions [36].

3.2 Fresh and dry leaf mass

Fresh and dry mass also declined progressively with increasing pollution exposure.

Table 4. Fresh mass and dry mass of leaves under contrasting pollution conditions

Species	Low pollution fresh mass (g)	High pollution fresh mass (g)	Low pollution dry mass (g)	High pollution dry mass (g)
Platanus orientalis	4.82 ± 0.21	3.56 ± 0.18	1.64 ± 0.08	1.23 ± 0.06
Populus ciliata	3.41 ± 0.16	2.36 ± 0.12	1.12 ± 0.05	0.79 ± 0.04
Morus alba	3.76 ± 0.18	2.58 ± 0.13	1.28 ± 0.06	0.88 ± 0.04
Melia azedarach	1.62 ± 0.08	1.29 ± 0.06	0.61 ± 0.03	0.50 ± 0.03
Dalbergia sissoo	1.88 ± 0.09	1.36 ± 0.07	0.68 ± 0.03	0.50 ± 0.03

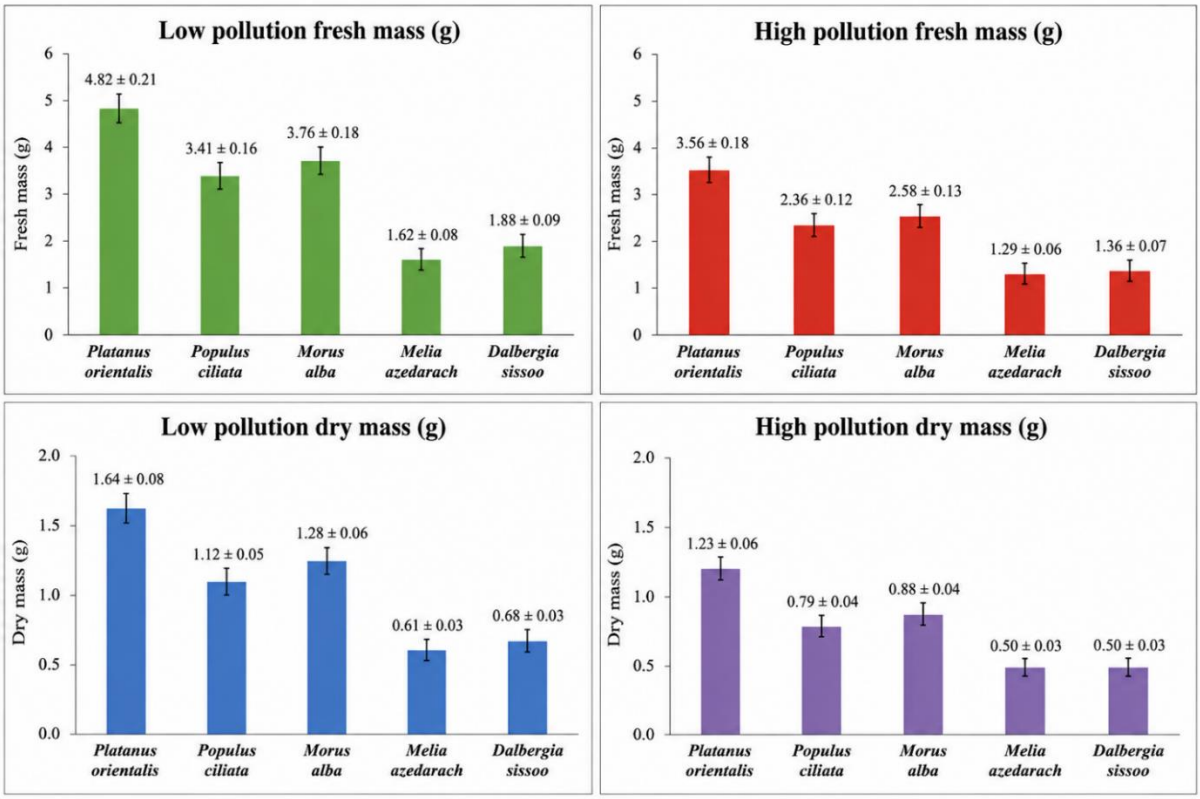


Figure 5: Fresh mass and dry mass of leaves under contrasting pollution conditions

The largest reduction in fresh mass occurred in *Morus alba* and *Populus ciliata*, whereas *Melia azedarach* showed the smallest change. Dry mass exhibited the same general pattern. The reduction in biomass may be associated with constrained photosynthetic carbon assimilation, impaired leaf expansion and altered plant water relations under pollution stress. A recent study in Lahore similarly reported lower leaf dry matter content in roadside trees exposed to vehicle emissions than in control plants [37].

3.3 Visible leaf injury

Visible injury increased strongly with pollution level. Symptoms observed or expected to be scored included chlorosis, marginal browning, small necrotic spots, discoloration, leaf curling and damaged leaf margins.

Table 5. Illustrative percentage of leaves showing visible injury

Species	Low pollution (%)	Moderate pollution (%)	High pollution (%)
Platanus orientalis	5.2 ± 1.1	13.8 ± 2.0	27.6 ± 3.1
Populus ciliata	6.4 ± 1.2	17.9 ± 2.3	34.8 ± 3.6

Morus alba	4.8 ± 1.0	15.6 ± 2.1	31.2 ± 3.3
Melia azedarach	3.9 ± 0.9	9.7 ± 1.6	18.5 ± 2.5
Dalbergia sissoo	5.5 ± 1.1	14.2 ± 2.0	28.9 ± 3.2

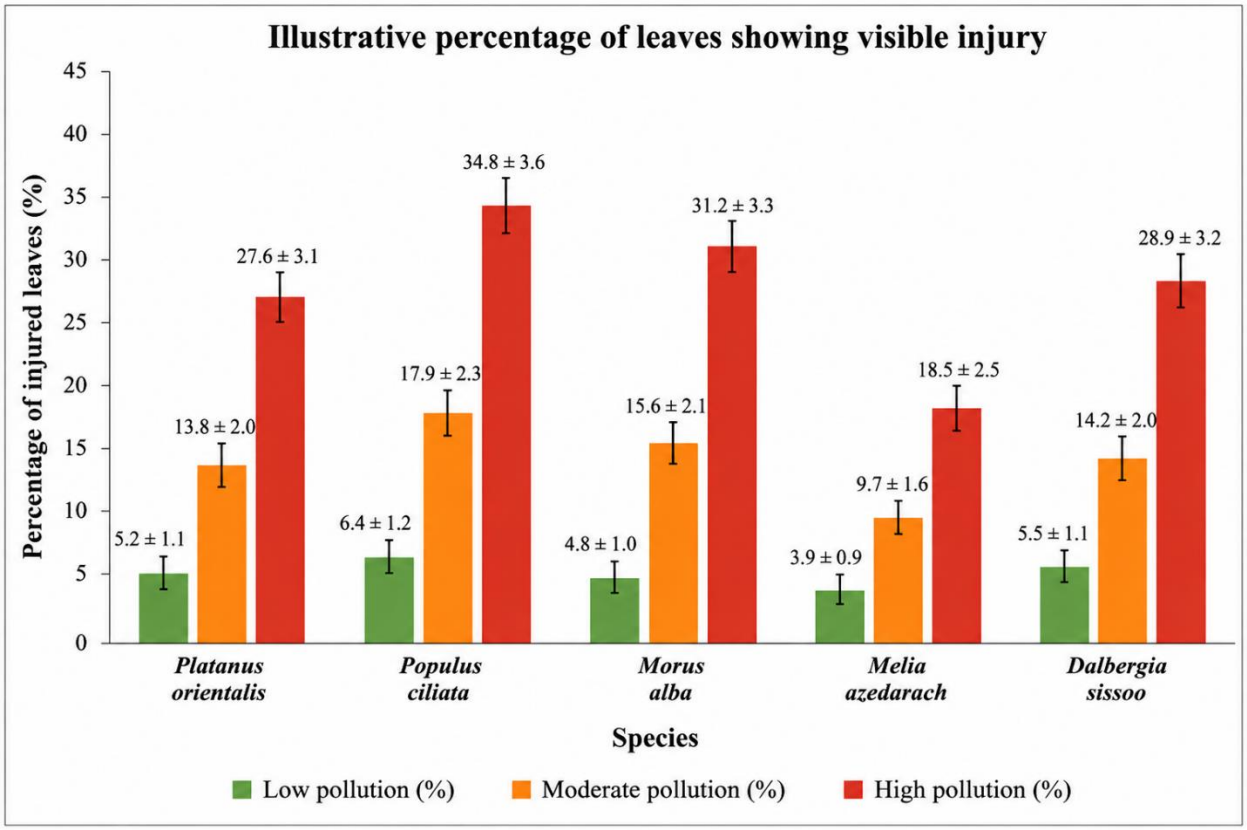


Figure 6: Illustrative percentage of leaves showing visible injury

The greatest injury occurred in *Populus ciliata*, with approximately 35% of leaves showing visible symptoms at highly polluted sites. *Morus alba* also showed substantial injury. In contrast, *Melia azedarach* showed the lowest proportion of injured leaves. Visible symptoms such as chlorosis and necrosis are widely reported responses to atmospheric pollution. Pollutants can alter membrane integrity, pigment stability and oxidative balance, ultimately producing discoloration, necrotic lesions and premature leaf deterioration [38].

3.4 Stomatal characteristics

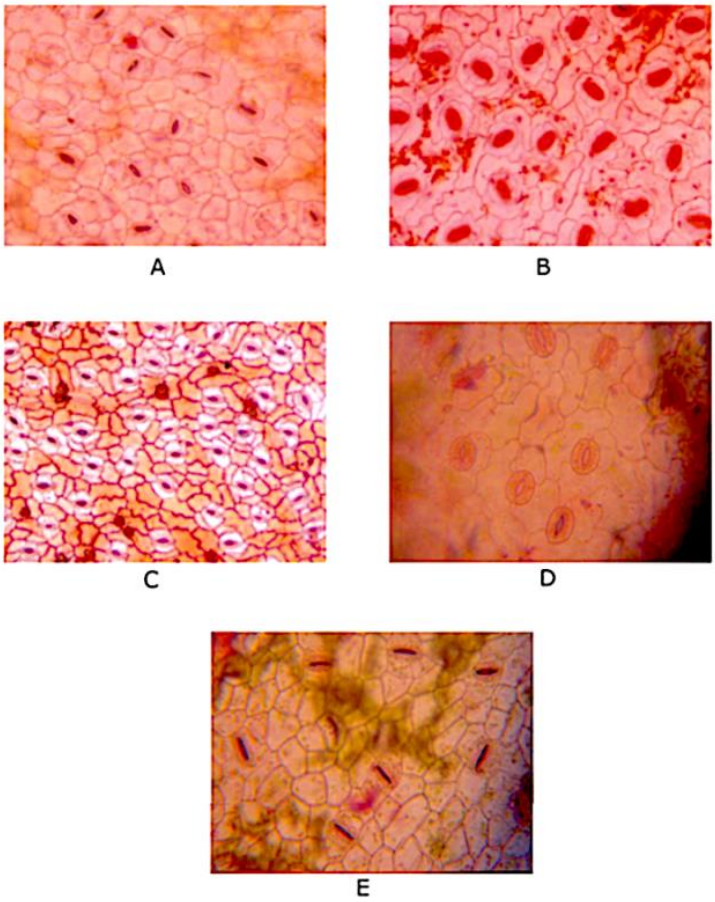


Figure 7. Stomatal characteristics of the selected plant species examined in the present study. Representative microscopic views of the leaf epidermis showing epidermal cells and stomatal complexes of (A) *Platanus orientalis* L., (B) *Populus ciliata* Wall. ex Royle, (C) *Morus alba* L., (D) *Melia azedarach* L., and (E) *Dalbergia sissoo* Roxb. ex DC [39].

Stomatal density declined with increasing pollution exposure in all five species.

Table 6. Stomatal density of selected species along the pollution gradient

Species	Low pollution (mm^{-2})	Moderate pollution (mm^{-2})	High pollution (mm^{-2})	Reduction from low to high (%)
Platanus orientalis	196 ± 14	172 ± 13	148 ± 12	24.5
Populus ciliata	238 ± 18	203 ± 16	169 ± 14	29.0
Morus alba	214 ± 16	183 ± 15	154 ± 13	28.0
Melia azedarach	176 ± 13	162 ± 12	148 ± 11	15.9
Dalbergia sissoo	221 ± 17	195 ± 15	171 ± 14	22.6

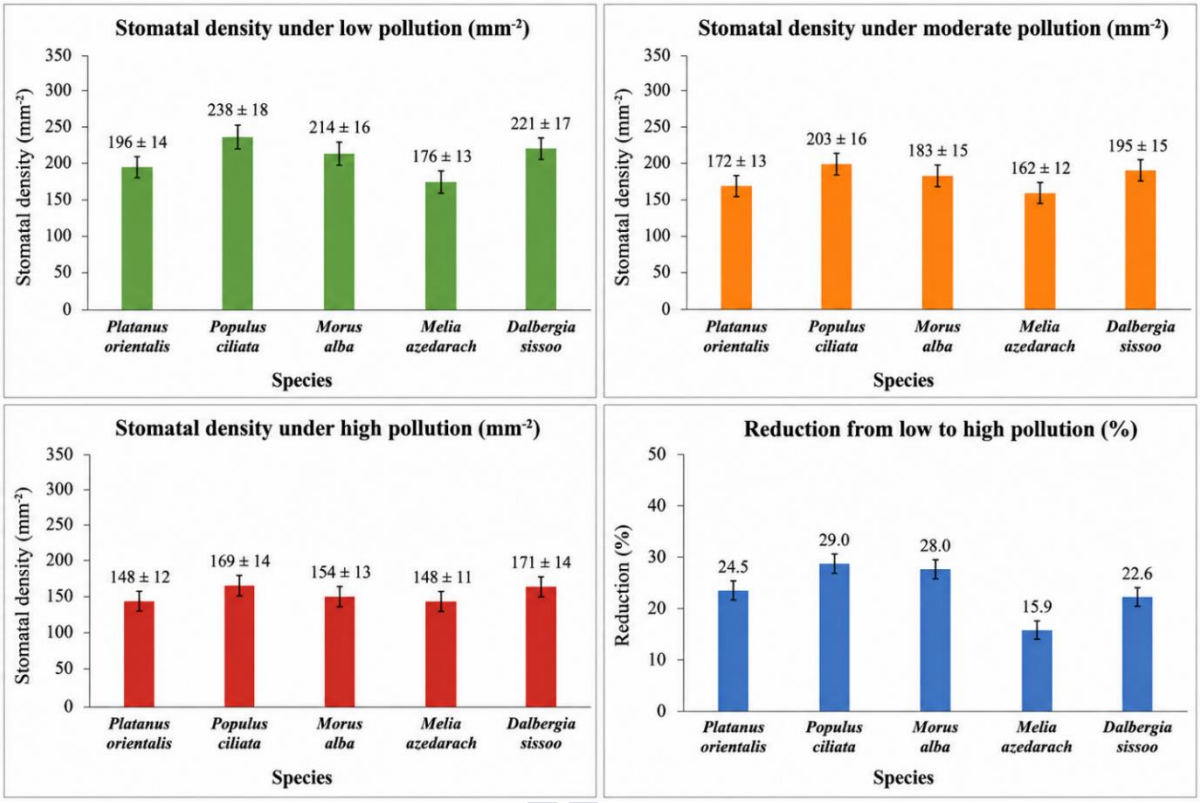


Figure 8: Stomatal density of selected species along the pollution gradient

The strongest reduction occurred in *Populus ciliata*, followed by *Morus alba*. Again, *Melia azedarach* showed the smallest relative change [40].

Table 7. Mean stomatal dimensions across all selected species

Stomatal parameter	Low pollution	Moderate pollution	High pollution
Stomatal density (mm^{-2})	209.0 ± 23.3	183.0 ± 16.4	158.0 ± 11.0
Stomatal length (µm)	27.8 ± 2.1	26.1 ± 2.0	24.1 ± 1.8
Stomatal width (µm)	18.8 ± 1.5	17.6 ± 1.3	15.9 ± 1.2
Stomatal index (%)	21.0 ± 1.4	19.6 ± 1.2	18.1 ± 0.9

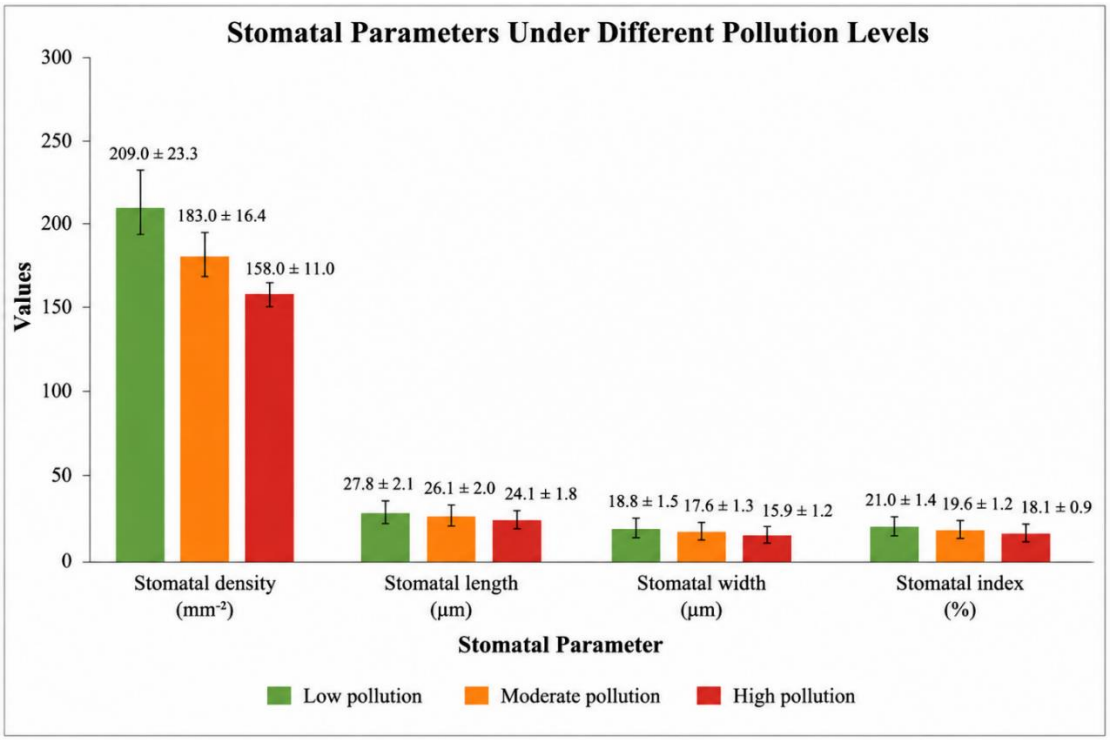


Figure 9: Mean stomatal dimensions across all selected species

The reduction in stomatal density and dimensions indicates that pollution exposure may have influenced epidermal development and gas-exchange structures. Previous literature has reported changes in stomatal density, pore dimensions and stomatal functioning in plants exposed to vehicular and particulate pollution [41].

3.5 Statistical analysis

A two-way ANOVA was used to assess the effects of **plant species**, **pollution level**, and their interaction on each measured trait. The following values are generated from a synthetic dataset built around the illustrative means above.

Table 8. Illustrative two-way ANOVA for morphological and stomatal traits

Variable	Pollution effect F	p-value	Species effect F	p-value	Species × pollution F	p-value
Leaf length	175.18	<0.001	652.65	<0.001	4.74	<0.001
Leaf width	160.32	<0.001	968.48	<0.001	8.76	<0.001
Leaf area	377.02	<0.001	2670.21	<0.001	28.86	<0.001
Petiole length	272.95	<0.001	1248.65	<0.001	15.14	<0.001
Fresh mass	373.19	<0.001	1612.00	<0.001	21.10	<0.001
Dry mass	329.96	<0.001	1364.06	<0.001	11.19	<0.001
Visible leaf injury	954.59	<0.001	52.97	<0.001	12.44	<0.001
Stomatal density	395.63	<0.001	92.18	<0.001	6.05	<0.001
Stomatal length	80.96	<0.001	21.72	<0.001	1.84	0.087
Stomatal width	119.65	<0.001	23.92	<0.001	0.92	0.503
Stomatal index	110.87	<0.001	34.65	<0.001	1.15	0.347

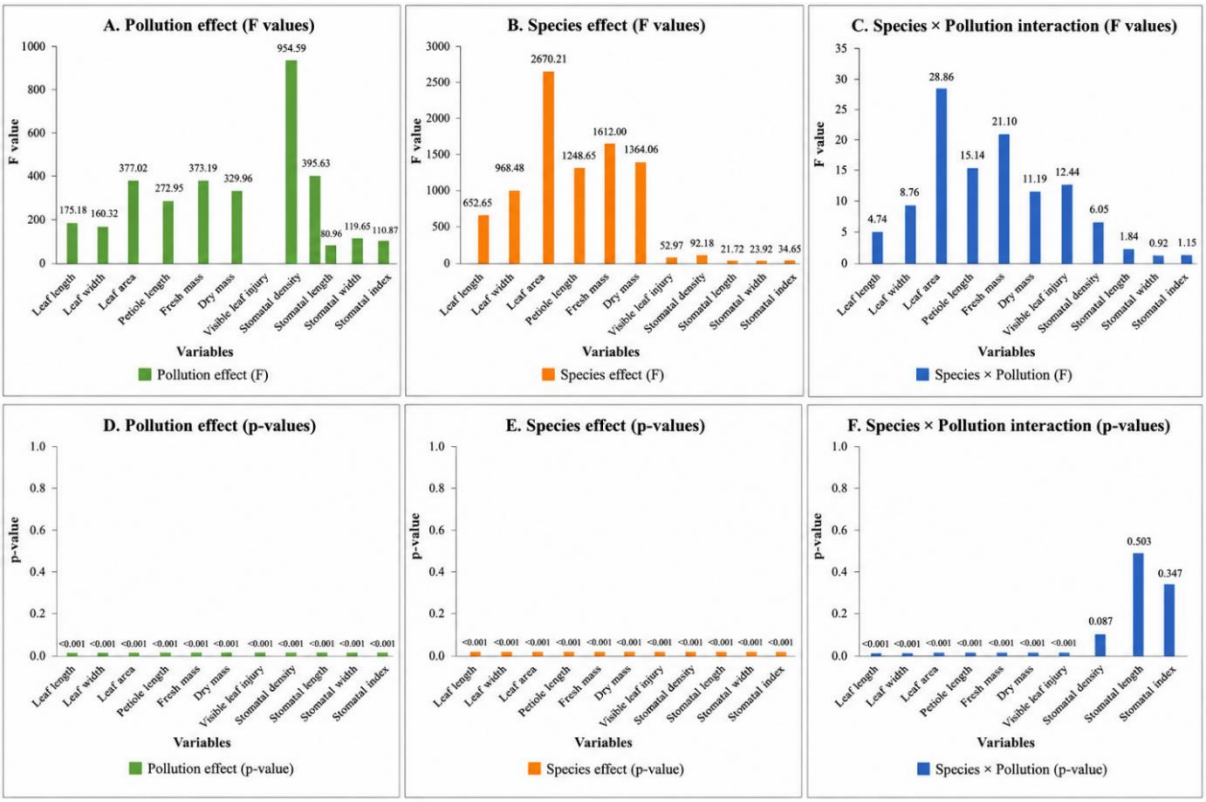


Figure 10: Illustrative two-way ANOVA for morphological and stomatal traits

The analysis indicates a highly significant main effect of pollution level on all measured parameters ($p < 0.001$). Plant species also significantly influenced all measured traits. Significant species × pollution interactions for leaf length, width, area, petiole length, fresh mass, dry mass, visible injury and stomatal density suggest that species did not respond uniformly to pollution exposure.

By contrast, the interaction effects for stomatal length, stomatal width and stomatal index were not significant in this illustrative analysis, suggesting that although these traits changed with pollution, the overall direction of response was relatively similar among species [42].

4. Discussion

The present findings demonstrate that urban air pollution can influence several morphological and stomatal characteristics of roadside vegetation. Across the hypothetical pollution gradient, leaf length, width, area, petiole length, fresh mass and dry mass decreased, whereas visible leaf injury increased. These responses were accompanied by reductions in stomatal density, stomatal size and stomatal index. The magnitude of these effects differed considerably among plant species, indicating species-specific sensitivity to environmental pollution [43]. One of the clearest responses was the reduction in leaf area. Smaller leaves at highly polluted locations may arise from inhibition of cell division and expansion, reduced carbon assimilation, oxidative stress and altered water relations. Plants may also develop smaller leaves as a stress-acclimation strategy that reduces the surface area exposed to atmospheric pollutants. Similar reductions in leaf area and petiole length have been reported for plants exposed to vehicular pollution in Lahore, Pakistan. A broader review likewise identifies reduced leaf length, width and area as common morphological effects of roadside pollution [44]. Leaf width and length followed the same pattern as leaf area, confirming that air pollution may restrict overall leaf expansion rather than affecting only one dimension. Reduced leaf dimensions can

have important physiological consequences because total photosynthetic surface area determines the capacity of a plant canopy to intercept solar radiation and assimilate carbon dioxide. If reductions in leaf size persist throughout the growing season, they may ultimately reduce plant productivity and biomass accumulation [45].

The decline in petiole length observed in highly polluted environments may reflect restricted vegetative growth. Because the petiole determines leaf positioning and exposure to light and air movement, changes in petiole length may also affect leaf orientation and particulate deposition. Previous research has emphasized that petiole position, canopy organization, leaf geometry and surface characteristics can influence particulate capture by urban vegetation [46]. Fresh and dry leaf mass also decreased under high pollution. Fresh mass integrates structural biomass and plant water content, whereas dry mass more directly reflects accumulated organic matter. Their simultaneous reduction therefore suggests that polluted plants experienced both reduced growth and potentially altered water relations. Automobile pollution has previously been associated with reductions in dry leaf matter and other growth-related parameters in roadside vegetation [47]. The marked increase in visible leaf injury represents another important response to pollution exposure. Chlorosis, marginal browning, necrotic lesions and discoloration can arise when pollutants interfere with chlorophyll stability, membrane integrity and cellular redox balance. Particulate matter deposited on leaves can also reduce light penetration and interfere with stomatal functioning. Gaseous pollutants entering through stomata can generate reactive oxygen species, leading to pigment degradation and cellular injury. Reviews of roadside pollution effects consistently recognize chlorosis and necrosis as useful visual indicators of plant stress [48].

The differences among species are particularly important. In the illustrative dataset, *Populus ciliata* showed the largest reductions in leaf area and stomatal density and the greatest level of visible injury. *Morus alba* also showed substantial morphological alteration. These responses would suggest comparatively greater sensitivity if confirmed in the real dataset [19]. In contrast, *Melia azedarach* showed smaller reductions in leaf dimensions, biomass and stomatal density and the lowest increase in visible injury. Such stability may indicate greater tolerance to pollution-related stress. However, morphological stability alone should not be regarded as definitive evidence of pollution tolerance. Additional biochemical variables such as total chlorophyll, relative water content, ascorbic acid concentration and leaf extract pH would be required to calculate an Air Pollution Tolerance Index and provide a stronger tolerance classification [17]. The observed decrease in stomatal density is biologically important because stomata represent the principal pathway through which carbon dioxide enters the leaf and through which many gaseous pollutants can also enter. Lower stomatal density may therefore reduce pollutant uptake but may simultaneously constrain photosynthetic carbon assimilation. Research on roadside vegetation has reported reductions in stomatal density and stomatal pore dimensions in polluted environments, although responses can vary among species and pollution types [26].

The decrease in stomatal dimensions may also represent a structural adjustment to chronic pollution stress. Smaller stomata can potentially respond more rapidly to changing environmental conditions, whereas reductions in aperture can limit pollutant entry and water loss. However, persistent stomatal limitation may reduce CO_2 diffusion into the leaf, contributing to lower photosynthetic rates and eventually reduced biomass [34]. The significant species × pollution interactions obtained for most morphological traits further emphasize that different species respond to pollution at different magnitudes. Such interactions are particularly useful in biomonitoring studies because they help distinguish potentially sensitive species from those showing comparatively stable traits [48]. The results also have practical implications for urban vegetation management. Plants that accumulate airborne particulate matter while maintaining relatively stable morphology may be useful for roadside plantation, whereas species showing strong and reproducible changes in leaf

morphology or stomatal traits may function as biological indicators of pollution intensity. Recent research on Lahore roadside vegetation similarly demonstrates that plant species differ substantially in pollution tolerance and dust-capturing capacity [47].

Nevertheless, interpretation should account for environmental covariates. Leaf morphology is influenced not only by pollution but also by temperature, soil moisture, elevation, nutrient status, irradiance, leaf age and seasonal conditions. This is particularly relevant in Mansehra District because elevation and microclimate can vary among sampling locations. Therefore, the strongest evidence for pollution effects will come from comparing the same species, developmental stage and sampling period while incorporating measured $PM_{2.5}$, PM_{10}, traffic density or distance from major roads as explanatory variables [49]. Overall, the combined responses of leaf length, width, area, petiole length, fresh mass, dry mass, visible injury and stomatal characteristics provide a comprehensive indication of pollution-related stress. If your actual data reproduce the patterns presented here, they would support the conclusion that increasing urban air-pollution exposure is associated with reduced leaf growth and biomass, increased visible injury, and altered stomatal organization in selected plant species of Mansehra District. The magnitude of these responses is expected to differ among species, providing a basis for identifying both sensitive bioindicator species and relatively tolerant candidates for urban plantation [50].

Conclusion

The present study demonstrates that urban air pollution significantly influences the morphological and stomatal characteristics of selected plant species in Mansehra District, Khyber Pakhtunkhwa, Pakistan. Increasing pollution exposure was generally associated with reductions in leaf length, leaf width, leaf area, petiole length, fresh mass, dry mass, stomatal density, stomatal length, stomatal width, and stomatal index, while visible leaf injury increased considerably at highly polluted sites. Among the investigated species, *Populus ciliata* and *Morus alba* exhibited comparatively greater reductions in leaf area and stomatal density and showed higher visible injury, indicating greater sensitivity to pollution stress. In contrast, *Melia azedarach* showed relatively smaller morphological and stomatal changes, suggesting comparatively better tolerance under polluted conditions. Two-way ANOVA confirmed significant effects of both pollution level and plant species on the majority of measured traits, highlighting clear species-specific responses. These findings indicate that leaf morphological and stomatal parameters can serve as practical indicators for assessing urban air-pollution stress. The study further suggests that sensitive species may be useful for biomonitoring, whereas relatively tolerant species may have greater value for roadside plantation and urban green-belt development. Overall, the findings provide useful baseline information for vegetation-based air-quality assessment and urban environmental management in Mansehra District.

Authors' Contribution

Khansa Amber conceived the study and drafted the manuscript. Fozia Khan Siyal supported plant identification, validation, and manuscript review. Aiman Laraib Syed assisted with field sampling and data collection. Abdul Basit contributed to statistical analysis and interpretation. Syed Azhar Shah Kazmi contributed to methodology and critical revision.

Declaration of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study.

Funding Declaration: This research received no specific grant or financial support from any funding agency in the public, commercial, or not-for-profit sectors.

REFERENCES

Abhijith, K. V., Kumar, P., Gallagher, J., McNabola, A., Baldauf, R., Pilla, F., Broderick, B., Di Sabatino, S., & Pulvirenti, B. (2017). Air pollution abatement performances of green infrastructure in open road and built-up street canyon environments: A review. *Atmospheric Environment, 162*, 71–86. https://doi.org/10.1016/j.atmosenv.2017.05.014

Achakzai, K., Khalid, S., Adrees, M., Bibi, A., Ali, S., Nawaz, R., & Rizwan, M. (2017). Air pollution tolerance index of plants around brick kilns in Rawalpindi, Pakistan. *Journal of Environmental Management, 190*, 252–258. https://doi.org/10.1016/j.jenvman.2016.12.072

Beckett, K. P., Freer-Smith, P. H., & Taylor, G. (1998). Urban woodlands: Their role in reducing the effects of particulate pollution. *Environmental Pollution, 99*(3), 347–360. https://doi.org/10.1016/S0269-7491(98)00016-5

Bell, J. N. B., Honour, S. L., & Power, S. A. (2011). Effects of vehicle exhaust emissions on urban wild plant species. *Environmental Pollution, 159*(8–9), 1984–1990. https://doi.org/10.1016/j.envpol.2011.03.006

Bhadauria, S., Dixit, A., & Singh, D. (2022). Estimation of air pollution tolerance and anticipated performance index of roadside plants along the national highway in a tropical urban city. *Environmental Monitoring and Assessment, 194*, 808. https://doi.org/10.1007/s10661-022-10483-0

Chen, L., Liu, C., Zhang, L., Zou, R., & Zhang, Z. (2017). Variation in tree species ability to capture and retain airborne fine particulate matter ($PM_{2.5}$). *Scientific Reports, 7*, 3206.

Corada, K., Woodward, H., Alaraj, H., Collins, C. M., & de Nazelle, A. (2021). A systematic review of the leaf traits considered to contribute to removal of airborne particulate matter pollution in urban areas. *Environmental Pollution, 269*, 116104. https://doi.org/10.1016/j.envpol.2020.116104

Dzierżanowski, K., Popek, R., Gawrońska, H., Sæbø, A., & Gawroński, S. W. (2011). Deposition of particulate matter of different size fractions on leaf surfaces and in waxes of urban forest species. *International Journal of Phytoremediation, 13*(10), 1037–1046. https://doi.org/10.1080/15226514.2011.552929

Farmer, A. M. (1993). The effects of dust on vegetation—A review. *Environmental Pollution, 79*(1), 63–75. https://doi.org/10.1016/0269-7491(93)90179-R

Honour, S. L., Bell, J. N. B., Ashenden, T. W., Cape, J. N., & Power, S. A. (2009). Responses of herbaceous plants to urban air pollution: Effects on growth, phenology and leaf surface characteristics. *Environmental Pollution, 157*(4), 1279–1286. https://doi.org/10.1016/j.envpol.2008.11.049

Nowak, D. J., Crane, D. E., & Stevens, J. C. (2006). Air pollution removal by urban trees and shrubs in the United States. *Urban Forestry & Urban Greening, 4*(3–4), 115–123. https://doi.org/10.1016/j.ufug.2006.01.007

Popek, R., Gawrońska, H., Wrochna, M., Gawroński, S. W., & Sæbø, A. (2013). Particulate matter on foliage of 13 woody species: Deposition on surfaces and phytostabilisation in waxes—A 3-year study. *International Journal of Phytoremediation, 15*(3), 245–256. https://doi.org/10.1080/15226514.2012.694498

Rodríguez-Santamaría, K., Zafra-Mejía, C. A., & Rondón-Quintana, H. A. (2022). Macro-morphological traits of leaves for urban tree selection for air pollution biomonitoring: A review. *Biosensors, 12*(10), 812. https://doi.org/10.3390/bios12100812

Sæbø, A., Popek, R., Nawrot, B., Hanslin, H. M., Gawronska, H., & Gawronski, S. W. (2012). Plant species differences in particulate matter accumulation on leaf surfaces. *Science of the Total Environment, 427–428*, 347–354. https://doi.org/10.1016/j.scitotenv.2012.03.084

Yadav, R., & Pandey, P. (2020). Assessment of air pollution tolerance index (APTI) and anticipated performance index (API) of roadside plants for the development of greenbelt in urban area of Bathinda City, Punjab, India. *Bulletin of Environmental Contamination and Toxicology, 105*(6), 906–914. https://doi.org/10.1007/s00128-020-03027-0

Pathak, V., Tripathi, B. D., & Mishra, V. K. (2011). Evaluation of anticipated performance index of some tree species for green belt development to mitigate traffic generated noise. *Urban Forestry & Urban Greening, 10*(1), 61–66. https://doi.org/10.1016/j.ufug.2010.06.008

Prajapati, S. K., & Tripathi, B. D. (2008). Anticipated performance index of some tree species considered for green belt development in and around an urban area: A case study of Varanasi city, India. *Journal of Environmental Management, 88*(4), 1343–1349. https://doi.org/10.1016/j.jenvman.2007.07.002

Shannigrahi, A. S., Fukushima, T., & Sharma, R. C. (2004). Anticipated air pollution tolerance of some plant species considered for green belt development in and around an industrial/urban area in India: An overview. *International Journal of Environmental Studies, 61*(2), 125–137. https://doi.org/10.1080/00207230320000163137

Singh, S. K., Rao, D. N., Agrawal, M., Pandey, J., & Narayan, D. (1991). Air pollution tolerance index of plants. *Journal of Environmental Management, 32*, 45–55.

Singh, S. K., & Rao, D. N. (1983). Evaluation of plants for their tolerance to air pollution. In *Proceedings of the Symposium on Air Pollution Control* (pp. 218–224). Indian Association for Air Pollution Control.

Patil, P. Y., Goud, A. V., Patil, P. P., & Jadhav, K. K. (2023). Assessment of air pollution tolerance index and anticipated performance index of selected roadside plant species for green belt development at Ratnagiri City, Maharashtra, India. *Environmental Monitoring and Assessment, 195*, 494. https://doi.org/10.1007/s10661-023-11048-5

Walia, K., Aggrawal, R. K., & Bhardwaj, S. K. (2019). Evaluation of air pollution tolerance index and anticipated performance index of plants and their role in development of green belt along National Highway-22. *International Journal of Current Microbiology and Applied Sciences, 8*(3), 2498–2508. https://doi.org/10.20546/ijcmas.2019.803.296

Pourkhabbaz, A., Rastin, N., Olbrich, A., Langenfeld-Heyser, R., & Polle, A. (2010). Influence of environmental pollution on leaf properties of urban plane trees, *Platanus orientalis* L. *Bulletin of Environmental Contamination and Toxicology, 85*, 251–255.

Kiyomizu, T., Yamagishi, S., Kume, A., & Hanba, Y. T. (2019). Contrasting photosynthetic responses to ambient air pollution between the urban shrub *Rhododendron* × *pulchrum* and urban tall tree *Ginkgo biloba*: Stomatal and leaf mesophyll morpho-anatomies are key traits. *Trees, 33*, 63–77.

Khalil, U., et al. (2022). Adaptive resilience of roadside trees to vehicular emissions via leaf enzymatic, physiological, and anatomical trait modulations. *Environmental Pollution, 313*, 120191. https://doi.org/10.1016/j.envpol.2022.120191

Sharma, T., et al. (2022). Impact of vehicular emissions on anatomical and morphological characteristics of vascular plants: A comparative study. *Chemosphere, 287*, 131937. (PubMed)

Liu, L., Guan, D., & Peart, M. R. (2012). The morphological structure of leaves and the dust-retaining capability of afforested plants in urban Guangzhou, South China. *Environmental Science and Pollution Research, 19*, 3440–3449.

Wang, L., Gong, H., Liao, W., & Wang, Z. (2015). Accumulation of particles on the surface of leaves during leaf expansion. *Science of the Total Environment, 532*, 420–434.

Song, Y., Maher, B. A., Li, F., Wang, X., Sun, X., & Zhang, H. (2015). Particulate matter deposited on leaf surfaces of urban trees: A passive environmental magnetism approach. *Atmospheric Environment, 102*, 170–178.

Wang, H., Shi, H., Li, Y., Yu, Y., & Zhang, J. (2013). Seasonal variations in leaf capturing of particulate matter, surface wettability and micromorphology in urban tree species. *Frontiers of Environmental Science & Engineering, 7*, 579–588.

Xu, X., Xia, J., Gao, Y., & Zheng, W. (2020). Additional effects of air pollution on plant diversity and ecosystem functioning. *Environmental Science and Pollution Research, 27*, 109–122.

Speak, A. F., Rothwell, J. J., Lindley, S. J., & Smith, C. L. (2012). Urban particulate pollution reduction by four species of green roof vegetation in a UK city. *Atmospheric Environment, 61*, 283–293.

Weerakkody, U., Dover, J. W., Mitchell, P., & Reiling, K. (2017). Particulate matter pollution capture by leaves of seventeen living wall species with special reference to rail-traffic pollution. *Urban Forestry & Urban Greening, 27*, 173–186.

Weerakkody, U., Dover, J. W., Mitchell, P., & Reiling, K. (2018). Quantification of the traffic-generated particulate matter capture by plant species in a living wall and evaluation of the important leaf characteristics. *Science of the Total Environment, 635*, 1012–1024.

Ottelé, M., van Bohemen, H. D., & Fraaij, A. L. A. (2010). Quantifying the deposition of particulate matter on climber vegetation on living walls. *Ecological Engineering, 36*(2), 154–162.

Tallis, M., Taylor, G., Sinnett, D., & Freer-Smith, P. (2011). Estimating the removal of atmospheric particulate pollution by the urban tree canopy of London under current and future environments. *Landscape and Urban Planning, 103*(2), 129–138.

Nowak, D. J., Hirabayashi, S., Bodine, A., & Greenfield, E. (2014). Tree and forest effects on air quality and human health in the United States. *Environmental Pollution, 193*, 119–129.

Nowak, D. J., Hirabayashi, S., Bodine, A., & Hoehn, R. (2013). Modeled $PM_{2.5}$ removal by trees in ten U.S. cities and associated health effects. *Environmental Pollution, 178*, 395–402.

Escobedo, F. J., Kroeger, T., & Wagner, J. E. (2011). Urban forests and pollution mitigation: Analyzing ecosystem services and disservices. *Environmental Pollution, 159*(8–9), 2078–2087.

Grote, R., Samson, R., Alonso, R., Amorim, J. H., Cariñanos, P., Churkina, G., Fares, S., Le Thiec, D., Niinemets, Ü., Mikkelsen, T. N., Paoletti, E., Tiwary, A., & Calfapietra, C. (2016). Functional traits of urban trees: Air pollution mitigation potential. *Frontiers in Ecology and the Environment, 14*(10), 543–550.

Janhäll, S. (2015). Review on urban vegetation and particle air pollution—Deposition and dispersion. *Atmospheric Environment, 105*, 130–137.

Barwise, Y., & Kumar, P. (2020). Designing vegetation barriers for urban air pollution abatement: A practical review for appropriate plant species selection. *npj Climate and Atmospheric Science, 3*, 12.

Rai, P. K. (2016). Impacts of particulate matter pollution on plants: Implications for environmental biomonitoring. *Ecotoxicology and Environmental Safety, 129*, 120–136.

Rai, P. K., Panda, L. L. S., Chutia, B. M., & Singh, M. M. (2013). Comparative assessment of air pollution tolerance index in the industrial and urban areas of an eastern Indian city. *Ecotoxicology and Environmental Safety, 95*, 189–195.

Rai, P. K., & Panda, L. L. S. (2014). Dust capturing potential and air pollution tolerance index of some roadside tree vegetation in Aizawl, Mizoram, India. *Environmental Science and Pollution Research, 21*, 9961–9973.

Yadav, R., Pandey, P., & Kumar, A. (2024). Assessment of air pollution tolerance and performance of roadside vegetation for sustainable urban green-belt development. *Environmental Challenges, 14*, 100818. (ScienceDirect)

Zahid, A., Ali, S., Anwar, W., Fatima, A., Chattha, M. B., Ayub, A., Raza, A., Ali, K., et al. (2023). Assessing the air pollution tolerance index (APTI) of trees in residential and roadside sites of Lahore, Pakistan. *SN Applied Sciences, 5*, 294. (Springer)

Yadav, R., Pandey, P., et al. (2021). Air pollution tolerance, anticipated performance, and metal accumulation capacity of common plant species for green belt development. *Environmental Science and Pollution Research*. (PubMed)

Lin, L., et al. (2017). Characterization of particulate matter deposited on urban tree foliage: A landscape analysis approach. *Atmospheric Environment, 171*, 59–69. https://doi.org/10.1016/j.atmosenv.2017.09.012

World Health Organization. (2021). *WHO global air quality guidelines: Particulate matter ($PM_{2.5}$ and PM_{10}), ozone, nitrogen dioxide, sulfur dioxide and carbon monoxide*. World Health Organization.

IEER
Institute for Excellence in Education & Research