

Research Article

Morphological responses of plants to air pollutants: A comparative study on leaf changes in five species

Ali Abdulrahman FADHIL, Samar Jasim MOHAMMED, Aswan AL-ABBOODI*
Department of Biology, College of Science, University of Misan, Maysan, Iraq.
*Email: aswan.abboodi@uomisan.edu.iq

Abstract: This study investigates the impact of long-term exposure to sulfur dioxide (SO₂), nitrogen dioxide (NO₂), and suspended particulate matter (SPM) on leaf morphology and physiology in five plant species. The pollutants were measured at concentrations of 1.20ppm for SO₂, 1.40 ppm for NO₂, and 230µg/m³ (12 hours daily) for SPM, representing annual average exposures. Leaf measurements were conducted on *Myrtus communis*, *Ziziphus spina-christi*, *Nerium oleander*, *Sesbania sesban*, and *Eucalyptus camaldulensis*. The results revealed variations in leaf length and width across exposure conditions. Notably, exposure to pollutants led to significant changes in leaf morphology, with SPM showing the most pronounced effects. Leaf area rates were calculated for each plant species, indicating the impact of pollutants on overall leaf development. Control groups exhibited higher leaf area rates compared to pollutant-exposed groups, with *E. camaldulensis* particularly sensitive to SO₂ and NO₂ exposures. Additionally, stomatal density was assessed, revealing pollutant-induced alterations in stomatal patterns. Epidermal cell numbers were quantified, showcasing the sensitivity of *N. oleander* and *S. sesban* to pollutant exposures. These findings contribute to our understanding of the complex interactions between air pollutants and plant physiology, emphasizing the importance of considering multiple morphological parameters. The results have implications for environmental management and plant health in polluted regions.

Keywords: Leaf morphology, Stomatal density, Epidermal cells, Plant physiology, Environmental impact.

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Introduction

The escalating levels of air pollution in contemporary urban and industrial landscapes represent a formidable challenge to both human health and ecological integrity. Among the plethora of pollutants, sulfur dioxide (SO₂), nitrogen dioxide (NO₂), and suspended particulate matter (SPM) emerge as significant contributors to environmental degradation, with repercussions extending beyond the confines of urban areas. While the detrimental effects of air pollution on human health are extensively documented, the intricate interplay between air pollutants and plant physiology remains

a focal point of investigation due to the indispensable role that plants play in terrestrial ecosystems (Husen 2021; Oksanen & Kontunen-Soppela 2021; Leisner et al. 2023).

As primary producers and contributors to oxygen production, plants are integral components of ecosystems, influencing climate regulation and providing habitats for diverse organisms. However, their sessile nature makes them particularly vulnerable to the adverse effects of air pollutants, which they absorb directly from the atmosphere. This vulnerability necessitates a thorough examination of the impact of long-term exposure to air pollutants on

various morphological and physiological aspects of plant leaves (Agrawal & Agrawal 2023).

This study undertakes a comprehensive exploration of the consequences of prolonged exposure to SO₂, NO₂, and SPM on plant species selected for their ecological significance and ubiquity: *Myrtus communis*, *Ziziphus spina-christi*, *Nerium oleander*, *Sesbania sesban*, and *Eucalyptus camaldulensis*. The selected pollutants were measured at concentrations reflecting annual averages, mirroring realistic exposure scenarios prevalent in urban and industrial settings.

While previous research has shed light on the broad impacts of air pollutants on plant growth and leaf morphology, this study adopts a nuanced approach by concurrently considering multiple morphological parameters. Leaf length and width measurements, leaf area rate calculations, stomatal density assessments, and the quantification of epidermal cell numbers collectively contribute to a holistic understanding of plant responses to varying pollutant exposures (Kwak et al. 2020). By elucidating the intricate interplay between air pollutants and diverse plant species, this research seeks to provide valuable insights into the resilience and adaptability of plants in the face of changing environmental conditions. The multifaceted nature of the study aims to not only address immediate consequences but also contribute to the broader field of ecological research, informing environmental policies and strategies for sustainable management (Becker 2023).

As urbanization and industrialization continue unabated, the findings of this study are poised to make significant contributions to our understanding of the complex relationship between air pollutants and plant physiology (Darrall 1989; Goyal et al. 2020). Ultimately, this knowledge is pivotal for the formulation of effective strategies to mitigate the environmental consequences of anthropogenic activities, ensuring the continued health and sustainability of terrestrial ecosystems. The aim is to comprehensively assess how varying concentrations

of long-term exposure to sulfur dioxide (SO₂), nitrogen dioxide (NO₂), and suspended particulate matter (SPM) pollutants influence the selected plant species: *M. communis*, *Z. spina-christi*, *N. oleander*, *S. sesban*, and *E. camaldulensis*.

Materials and Methods

Plant selection and preparation: Five plant species were selected for this study *M. communis*, *Z. spina-christi*, *N. oleander*, *S. sesban*, and *E. camaldulensis*. Healthy and uniform plant specimens were obtained and acclimated in a controlled greenhouse environment for two weeks before the start of the experiment. Standardized potting soil was used for planting, and consistent watering and fertilization practices were maintained throughout the study.

Experimental setup: A controlled environment chamber (greenhouse) was utilized to mimic real-world conditions while allowing precise control over temperature, humidity, and light. Air pollution sources were installed to introduce controlled concentrations of air pollutants: SO₂, NO₂, and SPM (Okure et al. 2022). Sensors and monitoring equipment were set up to continuously measure pollutant concentrations and environmental parameters within the chamber using specialized digital devices GFG–Quality control G460MICROTECTOR II/ Germany for gaseous pollutants (SO₂ and NO₂) and Particle mass counter Met One Instrument/ USA for particulate pollutants.

Experimental design: A randomized complete block design was employed to account for potential variations among plant individuals. Each plant species was divided into multiple groups, with each group exposed to a specific pollutant at varying concentrations, including control groups maintained in a pollutant-free environment. Replicates were maintained for each pollutant concentration, ensuring statistical robustness.

Air pollution exposure: Air pollutants were introduced into the chamber by predetermined concentration levels based on real-world pollution data from the study area. Concentrations were

Table 1. Concentrations of applied air pollutants for long-term.

Pollutant	Concentration (ppm)	Exposure Type	Duration
Sulfur Dioxide (SO ₂)	1.20	Long-Term	Annual Average
Nitrogen Dioxide (NO ₂)	1.40	Long-Term	Annual Average
Suspended Particulate Matter (SPM)	230 (µg/m ³)	Long-Term	Annual Average (12 hours daily)

Table 2. Comparison of the rates of leaf length (cm) in plants for the study period.

Plant Species	leaf length measurements from lowest to highest (cm)			
	Control Group	SO ₂ Exposure	NO ₂ Exposure	SPM Exposure
<i>Myrtus communis</i>	2-5	0.75-3.8*	1.4-4.2*	1.8-4.4
<i>Ziziphus spina-christi</i>	2-8	0.5-5.1*	1.5-5*	1.4-6.6*
<i>Nerium oleander</i>	5-15	2.2-10.2*	4-12.8*	4.7-13.6
<i>Sesbania sesban</i>	5-20	1.5-12*	2.3-14.8*	3.9-17.3*
<i>Eucalyptus camaldulensis</i>	5-15	2.1-8.2*	2.8-11.6	4.2-12.9

* $P < 0.05$

adjusted regularly to simulate varying pollution scenarios over a two-year study period. Table 1 displays the concentrations of the investigated air pollutants, namely SO₂, NO₂, and SPM. For sulfur dioxide, concentrations of 1.20ppm were selected to represent long-term exposure, characterized by the annual average. Similarly, nitrogen dioxide concentrations were set at 1.40ppm, representing long-term exposure with an annual average. In the case of suspended particulate matter, the chosen concentration for the current experiment is 230µg/m³, signifying long-term exposure over a 12-hour daily period.

Data collection: Continuous monitoring of pollutant levels was conducted using calibrated sensors for SO₂, NO₂, and SPM. Plant parameters were measured periodically, including leaf length (cm): measured using a ruler from the leaf base to the leaf tip, and leaf width (cm): measured at the widest point of the leaf. The rates of leaf area (cm²) according to Wood & Roper (2000) and Yang et al. (2023). Number of Stomata (stomata/mm²): Counted under a microscope. Annual rates of epidermal cell number in plant leaves during the study period were counted under a microscope according to Redha et al. (2011).

Statistical Analysis: Collected data were subjected to appropriate statistical analysis, including ANOVA, t-tests, and regression analysis, to determine the

significance of differences among pollutant-exposed and control groups. Post-hoc tests were conducted to identify specific differences between groups. Statistical significance was set at $P < 0.05$.

Results

In the control group of *M. communis*, planted in a typical, non-polluted environment, the leaf length exhibited a range from approximately 2cm as the lowest length to 5cm as the highest length. This variability is attributed to factors such as age, health, and specific environmental conditions (Lyu et al. 2021; Venegas Hargous et al. 2023). In contrast, the *M. communis* group exposed to SO₂ displayed a significant impact on leaf length, with a range from 0.75cm to 3.8cm as the lowest and highest lengths, respectively, measured over the two-year study period. A one-way ANOVA revealed a statistically significant difference in leaf lengths among the control and SO₂-exposed groups ($P < 0.05$). Similarly, the group exposed to NO₂ showed a range of leaf lengths from 1.4 to 4.2cm, and the group exposed to SPM recorded leaf lengths ranging from 1.8 to 4.4cm measured over the same two-year period. ANOVA indicated statistically significant differences in leaf lengths among the control and NO₂-exposed groups ($P < 0.05$) and the control and SPM-exposed groups ($P < 0.05$) (Table 2).

Table 3. Comparison of the average rates of leaf width (cm) in plants for the study period.

Plant Species	leaf width measurements (n=20) (cm)			
	Control Group	SO ₂ Exposure	NO ₂ Exposure	SPM Exposure
<i>Myrtus communis</i>	1.88	0.95 *	1.16*	1.32*
<i>Ziziphus spina-christi</i>	4.12	1.35*	2.44*	3.12*
<i>Nerium oleander</i>	3.76	2.11*	2.46*	3.33
<i>Sesbania sesban</i>	8.00	4.65*	5.96*	6.12*
<i>Eucalyptus camaldulensis</i>	9.47	4.55*	5.86*	7.22

* $P<0.05$ **Table 4.** Comparison of the rates of leaf area (cm²) in plants for the study period.

Plant Species	The rates of leaf area (cm ²)			
	Control Group	SO ₂ Exposure	NO ₂ Exposure	SPM Exposure
<i>Myrtus communis</i>	3.75	0.905 *	1.731 *	2.073*
<i>Ziziphus spina-christi</i>	8.244	1.747*	4.996*	7.001
<i>Nerium oleander</i>	14.1	7.981*	12.229	12.415
<i>Sesbania sesban</i>	96.0	69.6*	78.61*	87.01
<i>Eucalyptus camaldulensis</i>	71.235	53.004 *	54.824*	68.265*

* $P<0.05$

The trends observed in *M. communis* were mirrored in *Z. spina-christi*, *N. oleander*, *S. sesban*, and *E. camaldulensis*. In the control groups of these plant species, leaf lengths varied within expected ranges. ANOVA tests demonstrated significant differences in leaf lengths among the control and pollutant-exposed groups for each plant species ($P<0.05$). For each plant species, exposure to pollutants demonstrated a consistent pattern of reduced leaf lengths compared to the control groups. Notably, the impact was most pronounced in the SO₂-exposed groups across all plant species. For instance, in *N. oleander* exposed to SO₂, leaf lengths ranged from 2.2 to 10.2cm, indicating a significant deviation from the control group. Similar trends were observed in groups exposed to NO₂ and SPM, further emphasizing the adverse effects of air pollutants on leaf morphology (Table 2).

The dataset reveals the growth responses of various plant species under the influence of different air pollutants—SO₂, NO₂, and SPM. All measurements, presented in centimeters, are based on an average of n=20 leaf samples per condition, providing a statistically robust foundation for analysis. Regarding *M. communis*, the mean growth

in the SO₂ group significantly reduced to 0.95cm ($P<0.05$) while in the NO₂ group, it shows a significant reduction to 1.16cm ($P<0.01$). The SPM group for the same plant exhibits a significant decrease to 1.32cm ($P<0.001$). The same results findings were recorded for *Z. spina-christi*, *N. oleander*, *S. sesban*, and *E. camaldulensis*. These findings contribute valuable insights into the intricate relationships between air quality and the growth dynamics of diverse plant species (Table 3).

The data illustrates the leaf area rates (in square centimeters) for various plant species under different exposure conditions, including the control group and exposures to SO₂, NO₂, and SPM. In terms of *M. communis*, the control group leaf area rate was 3.75cm², however, in the SO₂ exposure group, the calculated rate was 0.9025cm², reflecting the impact of SO₂ on leaf area. In the NO₂ exposure group, the results in a calculated rate of 1.7312cm², indicating changes in leaf area under NO₂ influence, and the SPM Exposure group shows a rate of 2.0736 cm² (Table 4).

To assess the stomatal density, the data is presented as the number of stomata per square millimeter (stomata/mm²). The results indicated a

Table 5. Comparison between the rates of the number of stomatal in plant leaves for the study period.

Plant Species	The rates of the number of stomata (mm ²)			
	Control Group	SO ₂ Exposure	NO ₂ Exposure	TSP Exposure
<i>Myrtus communis</i>	245	84 *	116*	157*
<i>Ziziphus spina-christi</i>	180	72*	93*	112*
<i>Nerium oleander</i>	266	125*	156*	178*
<i>Sesbania sesban</i>	365	174*	193*	200*
<i>Eucalyptus camaldulensis</i>	122	36*	49*	61*

* $P < 0.05$ **Table 6.** Comparison of the rates of epidermal cell number in plant leaves for the study period.

Plant Species	Number of epidermal cells/mm ²			
	Control Group	SO ₂ Exposure	NO ₂ Exposure	TSP Exposure
<i>Myrtus communis</i>	16000	7456*	8215*	14500
<i>Ziziphus spina-christi</i>	12000	7002*	9213*	11012
<i>Nerium oleander</i>	17000	11125*	14055*	15000*
<i>Sesbania sesban</i>	22000	17077*	19003*	20200
<i>Eucalyptus camaldulensis</i>	11000	3006*	4900*	7500*

* $P < 0.05$

significant difference among the groups ($P < 0.05$). Post-hoc pairwise comparisons using Tukey's HSD test revealed that the control group had a significantly higher stomatal density compared to the SO₂, NO₂, and SPM groups. The results confirmed significant variations in stomatal density among control and pollutant-exposed groups for each plant species, emphasizing the impact of pollutants on stomatal development (Table 5).

The differences in epidermal cell numbers is presented as the number of epidermal cells per square millimeter (cells/mm²) for each group. The results indicated a significant difference among the groups ($P < 0.05$). The control group had a significantly higher number of epidermal cells compared to the SO₂, NO₂, and SPM groups. The findings confirmed significant variations in the number of epidermal cells among control and pollutant-exposed groups for each plant species, indicating the impact of pollutants on epidermal cell density (Table 6).

Discussion

Plants experience a myriad of external stressors in their surroundings, which can manifest concurrently or sequentially with differing intensities and

frequencies. Typically, the initial signs of these stresses, including air pollution, become apparent in the leaves. Therefore, assessing morphological features such as length, width, and area stands out as a crucial aspect of monitoring initiatives (Otoide 2015; Bazgeer et al. 2022). The observed alterations in leaf morphology across plant species in response to long-term exposure to SO₂, NO₂, and SP) are noteworthy. *Myrtus communis* and *Z. spina-christi* exhibit consistent reductions in both leaf length and width. For instance, *M. communis* demonstrates a decrease in leaf length from the control group's range of 2-5 to 0.75-3.8 cm under SO₂ exposure, indicating a notable impact on growth. Similarly, *Z. spina-christi* experiences reductions in leaf width from the control group's 4.12 to 1.35cm under SO₂ exposure, showcasing a significant influence on the species' leaf dimensions. Leaf area rate calculations further illustrate the nuanced impact of pollutants on plant growth. For example, *S. sesban* exhibits a substantial decrease in leaf area under SO₂ exposure, with the calculated rate dropping from 96.0cm² in the control group to 69.6cm². This emphasizes the species' sensitivity to sulfur dioxide. Describing the decrease in plant leaf characteristics, such as length, width,

and area, can be interpreted as a mechanism of plant adaptation to endure harsh environmental conditions, particularly in the presence of intense air pollution from exhaust emissions (Uka et al. 2017; Jha & Yadav 2023). The significance of plant leaf area lies in its role as a crucial indicator, reflecting the extent of a plant's response to external stressors (Fränzle 2006; Seyyednejad et al. 2009; Zhou et al. 2015).

Stomatal density and epidermal cell number analyses provide additional insights into the physiological responses of plants to air pollutants. Reductions in stomatal density, aligned with diminished leaf dimensions, are evident across various species. Notably, *M. communis* and *Z. spina-christi* show a decrease in epidermal cell numbers, further highlighting the cellular-level impacts on growth. *Sesbania sesban*, with a substantial reduction in epidermal cell numbers, underscores the species' vulnerability to pollutant exposures. The decline in the number of stomata and epidermal cells in plant leaves can be attributed to a decrease in leaf area (Hegazi & El-Kady 2010; Chin et al. 2023). Plants exhibit resistance to drought or air pollutants through various mechanisms, including a reduced leaf area, stomatal number, water loss rate via transpiration, and plant leaf gas exchange rate. This reduction ultimately leads to a decreased penetration rate of polluting gases into the leaf (Pourkhabbaz et al. 2010). Additionally, the stomatal guide serves as a valuable anatomical adaptation to air pollution (Gostin 2016; Uka et al. 2017; Mlitan 2023). A lower stomatal number is regarded as a sign of plant adaptation to air pollution, as it results in decreased absorption of gaseous pollutants from the air (Alushi & Veizi 2020).

These numerical trends emphasize the importance of considering specific quantitative parameters when assessing the ecological consequences of air pollutants on plant physiology. The varying responses across species underscore the need for nuanced considerations in ecological assessments, providing valuable insights for environmental management strategies aimed at promoting the

sustainability of terrestrial ecosystems in the face of increasing anthropogenic activities.

Conclusion

The observed alterations in leaf morphology, encompassing changes in length, width, stomatal density, and epidermal cell numbers, reflect the multifaceted impacts of air pollutants on plant physiology. *Myrtus communis* and *Z. spina-christi* exhibit consistent reductions in both leaf length and width, indicating a vulnerability to pollutant exposures. In contrast, *E. camaldulensis* displays a more resilient response, suggesting species-specific adaptability mechanisms. The leaf area rate calculations provide quantitative evidence of the varying impacts of pollutants on plant growth. Stomatal density and epidermal cell number analyses further elucidate the cellular-level responses to pollutant exposures. The reduction in stomatal density, coupled with decreased epidermal cell numbers, accentuates the physiological stress experienced by *M. communis* and *Z. spina-christi*. *Sesbania sesban*, with a substantial decrease in epidermal cell numbers, exemplifies heightened vulnerability to pollutant exposures. These nuanced insights contribute to our understanding of the complex interactions between plants and their environment, guiding the development of targeted environmental management strategies for sustaining terrestrial ecosystems in the face of escalating anthropogenic pressures.

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