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Oxidative Damage and Content of Photosynthetic Pigments in Morus Alba Leaves in the Urban Area of Mendoza, Argentina

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Abstract

The activity of plants in an urban environment is under stress due to multiple environmental factors that have an adverse effect on their growth, productivity and reproductive capacity. Our hypothesis considers that any process of environmental stress can be detected early through the chlorophyll and ROS production analysis. The objective was to evaluate the lipid oxidative damage and the photosynthetic pigment content in leaves of Morus alba in two urbanistically contrasting sites in the urban area of Mendoza (Argentina). Ten trees were randomly sampled per site (micro-town and park). In each sample, 10 leaves were randomly collected, including the entire canopy. Each determination included 10 repetitions per site. Considering that M. alba is a deciduous species, leaf collection was made at the beginning of summer, when an important exposition period of leaves to many environmental factors has passed. The chlorophyll content analysis results effective in evaluating the answer to environmental stress under urban conditions. The relationship chlorophyll a and b showed significant differences among sites, minor in the micro-town, implying a decrease of chlorophyll a in relation to chlorophyll b, a possible indicator of damage by photo-oxidation. The results suggest the generation of oxidative stress due to accumulation of H2O2 in leaves, affecting cell structures, as evidenced by the major malonaldehyde content.

Key Words: Urban-Stress, Photosynthetic Pigments, Ros, Bioindicator.

Introduction

The adverse environmental conditions that are typical of urban areas present multiple challenges for plants, affecting their physiology, growth and survival. In rapidly growing cities, high levels of atmospheric pollution and constant changes in soil and water quality create continuous stress for vegetation [1-4]. Plants respond to these stress factors through physiological and biochemical adaptations that aim to maintain their integrity and functioning; however, these responses are also affected depending on the intensity and persistence of the pollutant [5, 6]. Pollution in urban areas typically includes a combination of organic and inorganic compounds, such as heavy metals, hydrocarbons, and fine particles, which have harmful effects on plant cellular structures and limit their basic functions, including photosynthesis, consequently plants tend to lose photosynthetic capacity and reduce the chlorophyll content in leaves [7-11]. This phenomenon is particularly evident in countries with high urbanization and industrial development rates, where urban growth and agricultural-industrial activity exert pressure on the plant environment [12-18]. This trend is not an exception in Argentina, where most environmental pollution issues are linked to mobile, industrial and agricultural sources [19, 20]. Specifically, in the Mendoza region, urban pollution is intensified by vehicular traffic and agro-industrial practices, which emit large amounts of gases and particulate pollutants, exposing vegetation to chronic stress conditions [21-23].

In plants, the photosynthetic pigment content, especially chlorophyll, is a variable that allows for evaluating their response to pollution, as these pigments are directly related to the plants' ability to perform photosynthesis and adapt to environmental conditions; when plants are subjected to stress conditions the carotene/chlorophyll total increases due to a greater synthesis of carotene than chlorophyll [24-27]. Chlorophyll a is the main pigment for light capture in photosynthesis, while chlorophyll b and carotenoids perform accessory and photoprotective roles [28].

It has been observed that plant exposure to environmental pollution reduces chlorophyll a content, which decreases photosynthetic efficiency and affects biomass production, in addition to altering the ratio between chlorophyll a and b, which may indicate oxidative damage in foliar cells [29-35]. Under such conditions, the plant's capacity to respond to stress is limited, weakening its role in the urban ecosystem [36, 37]. In aerobic plant metabolism, ROS (reactive oxygen species) are produced to use oxygen as the final electron acceptor. Oxygen to be reduced produces its singlet activated form (O21) or by transference of one, two or three electrons, forming a superoxide radical (O2.-), peroxide of hydrogen (H2O2) or the hydroxyl radical (HO•-), molecules produced due to normal cellular metabolism, and that under normal cellular conditions are rapidly metabolized [38, 39]. Atmospheric pollution also promotes the production of reactive oxygen species, such as hydrogen peroxide (H_2O_2). At elevated concentrations, these can cause lipid peroxidation and cellular membrane damage, which affects the overall health of plants and limits their capacity to regenerate [40, 41]. Cellular production of ROS is stimulated as a response to these metabolic imbalances generated by a stress condition that breaks the cellular homeostasis if the intracellular concentration of ROS is not controlled the direct consequence is damage to cellular structures due to peroxidation of lipids, proteins and NDA components oxidation [42-45]. In plants, ROS are produced in several cellular compartments, principally in chloroplasts and mitochondria, by the efflux of e~ from the chain conveyor to O2.

Although all ROS are highly reactive, each has different properties and reactions with different molecules. The H2O2 is relatively stable and does not react easily with lipids, carbohydrates or NDA, but can inactivate enzymes through oxidation of thiol groups In this context, hydrogen peroxide has been established as an effective marker of cellular damage, and its increase in plants exposed to urban stress is an indicator of oxidative stress generation and the potential effects of pollution). In order to prevent cellular damage, plants adopt various defense strategies, such as the seuestration of heavy metals by fitoquelatins and/or metallotioneines, compartmentalization in vacuoles, exclusion and inactivation by means of organic compound secretion [46].

Another strategy used by plants is the ROS detoxification mechanisms, including the synthesis of non-enzymatic metabolites that capture free radicals, such as glutathione, flavonoids, α-tocoferol, among others [47]. One of the best-known stress markers and easy to determine is the peroxidation of membrane lipids, a technique widely applied in the study of plants [48-50]. This technique consists in the detection of lipid peroxides through the content of malonaldehyde (ADM) generated by the oxidation and enzymatic degradation of polyunsaturated fatty acids in cells.
Stress factors elicit diverse responses in plants that support similar adverse conditions in urban areas to those experienced in laboratory, so the urban environment offers opportunities without cost and restrictions.

Morus alba was chosen for this study due to its ecological and physiological characteristics, making it a suitable model for evaluating urban stress. M. alba is a fast-growing species that has adapted well to urban areas due to its tolerance to pollution and its capacity to regenerate after pruning, making it common in cities like Mendoza, where it is often exposed to high levels of pollution and intensive management practices. This species showed moderate resistance to air pollution, with an APTI (air pollution tolerance index) value of 14.08, an index derived of the total chlorophyll, relative water content, pH, and ascorbic acid content of leaf extract, and is therefore recommended for urban planting.

The white mulberry is known for its ability to withstand various abiotic stress conditions, making it an excellent bioindicator of atmospheric pollution [51, 52]. Additionally, previous research has shown that this species exhibits detectable changes in chlorophyll content and ROS accumulation when exposed to environmental stress conditions, making it ideal for urban ecology studies focused on air quality and environmental monitoring [53-56].

This study hypothesizes that analyzing chlorophyll content and ROS production in M. alba leaves can provide early detection of urban stress impacts. Specifically, it is expected that differences in chlorophyll a and b content, as well as in hydrogen peroxide levels, will reflect the degree of environmental stress in sites with varying pollution levels in Mendoza city. The study aims to evaluate lipid oxidative damage and photosynthetic pigment content in M. alba leaves at two contrasting sites in Mendoza: a high-traffic urban micro-town and a park with less human interference. Through this approach, M. alba is proposed as a reliable bioindicator for environmental quality monitoring in urban areas, offering a scientific tool to improve environmental management strategies and select suitable plant species for urban green space planning.

Materials &Methods

Study Area

The study was conducted in the metropolitan area of Mendoza, Argentina, located at 32° 50' 11.7" S - 68° 45' 22.5" W and 32° 59' 52.7" S - 68° 52' 19.2" W. This region has an arid climate characterized by an annual precipitation of 234.7 mm and an annual average temperature of 16.8 °C, with maximum temperatures reaching 43 °C in summer and minimums of -9 °C in winter [57, 58]. The predominant winds come from the south and southeast, influencing the dispersion of atmospheric pollutants and local microclimatic conditions, factors that can affect the health and development of urban vegetation.

The urban ecosystem of Mendoza presents semi-desert conditions, where urban forestry consists almost exclusively of exotic species planted along sidewalks, in parks, and in areas close to the city. This urban forest, irrigated by ditches, includes approximately 10 to 12 trees per block, providing a green structure that partially mitigates urban stress. One of the predominant species in this region is Morus alba, a deciduous, monoecious plant with alternate, serrated leaves containing laticifers channels, adapted to urban environments due to its tolerance to pruning and moderate pollution. For the study, two contrasting urban sites were selected: the micro-town, the high-activity core of Mendoza with multiple stress sources such as heavy vehicular traffic and periodic pruning, and the General San Martín Park, a 500-hectare area with little traffic and no pruning interventions, located approximately 8 km from the center (Figure 1).

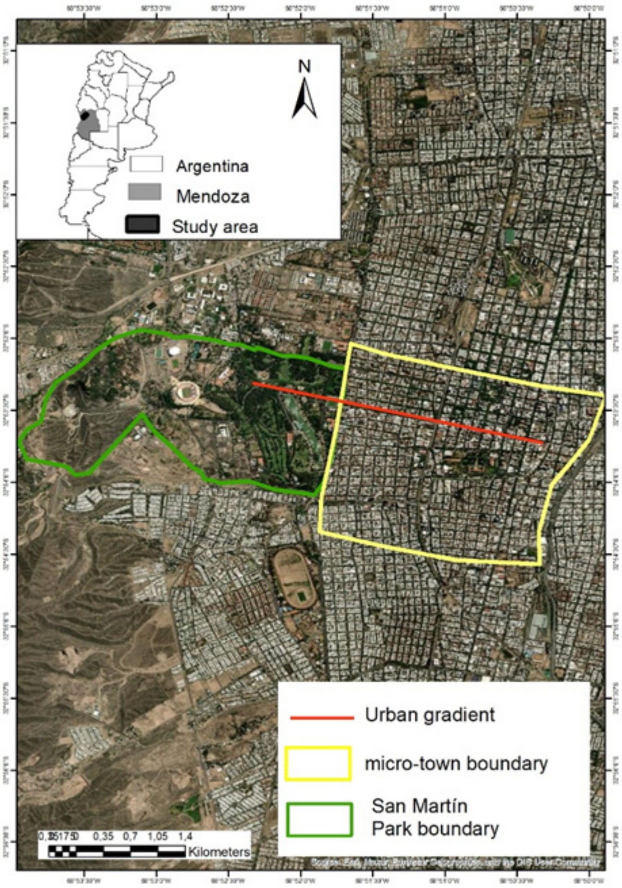

Figure 1: Sampling Areas in Mendoza Province

Leaves Sampling

Ten randomly selected M. alba trees were sampled at each site (micro-town and park). For each tree, 10 leaves were randomly collected from the entire canopy to ensure a representative sample of the environment's effects on the tree structure. Sampling was conducted in early summer, a period with environmental stability and when leaves have already been exposed to the characteristic environmental factors of each site, allowing for an assessment of the accumulated impact of urban stress. Each site included 10 replicates to enhance the statistical robustness of the results. The collected leaves were stored at -24 °C until analysis, approximately 15 days after collection, to preserve their condition and reduce the degradation of photosynthetic and oxidative compounds.

Determination of Photosynthetic Changes and Oxidative Damage Indicators

To quantify photosynthetic pigment content, chlorophyll concentration was determined using an adaptation of the Vernon (1960) technique. For the analysis, 0.1 g of fresh leaf material was immersed in 15 ml of an 80% (v/v) ethanol solution and heated at 100 °C for 15 minutes in a thermostatic bath, allowing efficient pigment extraction without damaging the molecular structure. The maximum absorbances of chlorophyll a, chlorophyll b, and carotenoids were measured at wavelengths of 665, 650, and 450 nm, respectively, in a spectrophotometer. Data were expressed as mg of chlorophyll per gram of fresh weight. To calculate pigment concentrations, the modified Vernon and MacKinney formula was applied: Chlorophyll a = 11.63 (DO665) - 2.39 (DO650); Chlorophyll b = 20.11 (DO650) - 5.18 (DO665); Total chlorophyll = Chlorophyll a + Chlorophyll b; Carotenoids = 0.02 (DO450).

Hydrogen peroxide (H_2O_2) quantification, as an oxidative stress indicator, was conducted using the Alexieva technique. For this, 0.1 g of fresh leaves were homogenized in a mortar with 0.1% trichloroacetic acid (TCA) in a 1:10 (w/v) ratio. The extract was centrifuged at 12000 g for 15 minutes, and the supernatant was used for measurements. The reaction mixture contained 160 µl of leaf extract supernatant, 160 µl of a 100 nm potassium phosphate buffer (pH 6.8), and 680 µl of a 1 M KI solution. The blank consisted in TCA 0.1% in absence of extract. The reaction was incubated in darkness for one hour, and absorbance was measured at 390 nm. The presence of H_2O_2 in the medium reacts with silver iodide in an acidic environment, releasing iodine (I2), which generates a yellow coloration detectable spectrophotometrically. H_2O_2 concentration was expressed in nmol per gram of fresh weight, using a calibration curve obtained from a 1 nmol H_2O_2 standard solution.

Oxidative lipid damage, measured as concentration of thiobarbituric acid reactive substances (TBARs), was quantified following the Heath & Packer methodology. This technique allows the detection of malondialdehyde (MDA), a compound generated by lipid peroxidation that reflects the degree of cellular damage under oxidative stress conditions. For the analysis, 0.1 g of leaves were homogenized in 20% TCA (1:10 w/v), and the extract was centrifuged at 12000 g for 15 minutes; 500 µl of the supernatant were taken and mixed with 500 µl of 20% TCA containing 0.5% thiobarbituric acid (TBA). The samples were incubated in a water bath at 95 °C for 25 minutes and then quickly cooled in an ice bath, followed by centrifugation at 9000 g for 6 minutes. Absorbance was measured at 532 nm, with turbidity correction at 600 nm. Results were calculated using the MDA molar extinction coefficient (155 nM^{-1}-cm^{-1}) and expressed in nmol of MDA per gram of fresh weight.

Statistical Analysis

The obtained data were analyzed using INFOSTAT version 8.0 statistical software [59]. An analysis of variance (ANOVA) was performed, followed by Duncan's multiple comparison test, with a significance level set at $p < 0.05$ to identify significant differences between treatments. Normality and homogeneity of variances were evaluated using the Shapiro-Wilk and Levene tests, respectively, ensuring the validity of the results and the robustness of the analysis.

Results and Discussion

The analysis of photosynthetic pigments in M. alba exposed to varying urban environmental conditions revealed significant variations in chlorophyll content and reactive oxygen species (ROS) production, notably in the urban micro-town site. These results suggest that the urban environment, characterized by high atmospheric pollution and microclimatic variations, generates considerable stress in plants. This stress is reflected in the observed adaptations and damage to their photosynthetic structure and pigment composition. Such physiological responses are key to understanding how urban plants adapt or are impacted in environments exposed to multiple stress factors, such as heavy traffic and pollutant emissions, especially in cities in arid regions like Mendoza.

Chlorophyll Content and Chlorophyll a/b Ratio

Figure 2 presents data showing a marked increase in total chlorophyll content in M. alba leaves from the micro-town area compared to those from the park site. This increase in chlorophyll content suggests that urban stressors may stimulate chlorophyll synthesis as a compensatory response to heightened environmental challenges, such as high light exposure and pollution levels, which can otherwise impair photosynthetic efficiency [60]. A closer examination reveals that the chlorophyll a/b ratio is significantly reduced in the micro-town site, with values of 1.04 compared to 1.3 in the park (Figure 3). This reduction in the chlorophyll a/b ratio is a noteworthy adaptive response that has been documented in other studies on urban plants [61, 62]. It is thought to occur due to increased chlorophyll b synthesis, which can support plants under conditions of photic stress and pollution by enhancing the capture of light across a broader spectrum [63, 64].

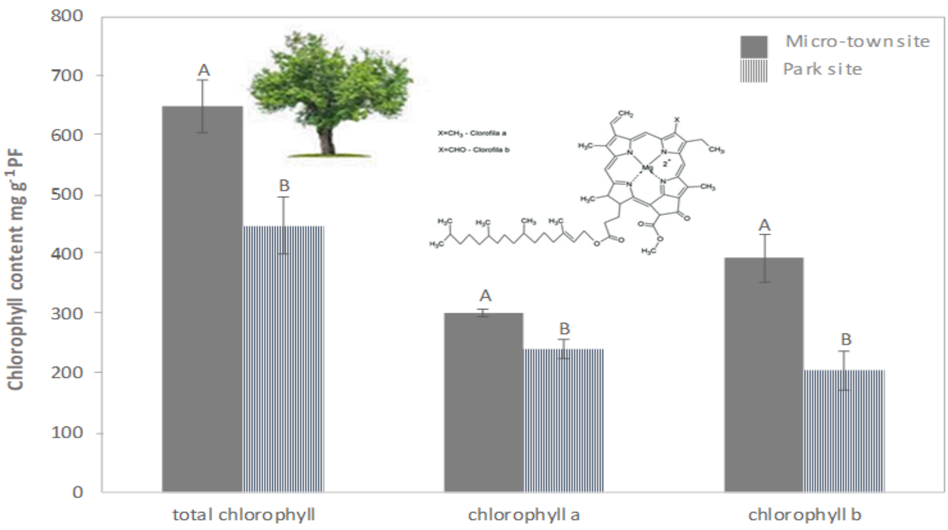

Figure 2: Chlorophyl Content in Leaves of M. Alba (n=10). Different Words Indicate Significant Differences Among Treastments (p<0.05)

In general, chlorophyll a is the primary pigment responsible for capturing light and converting it into energy during photosynthesis, while chlorophyll b functions as an accessory pigment. Chlorophyll b captures light at wavelengths that chlorophyll a does not absorb efficiently, thereby extending the spectrum of light usable for photosynthesis. The lower chlorophyll a/b ratio in the microcenter site may reflect an increase in chlorophyll b relative to chlorophyll a, suggesting an adaptive strategy in M. alba to maximize light absorption in environments with intense photic and oxidative stress (Figure 3). This strategy can enhance photosynthetic efficiency by capturing additional light energy, which is crucial in urban settings where factors such as particulate pollution can filter and scatter light.

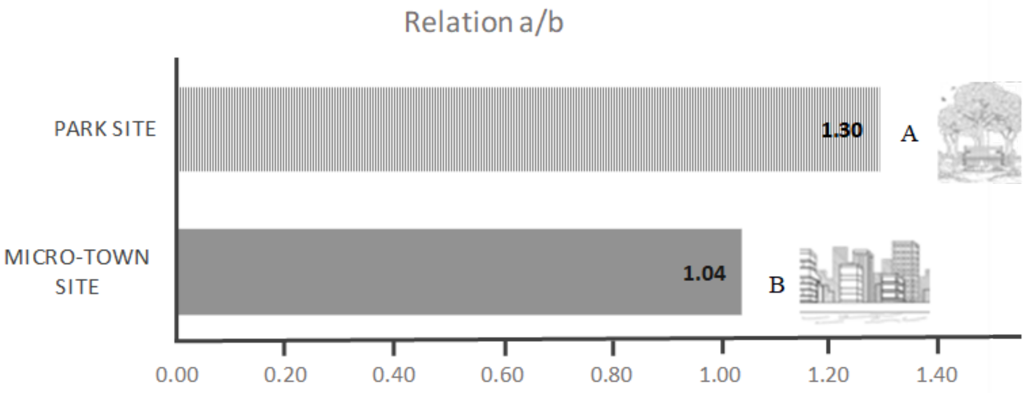

Figure 3: Relation Between Chlorophyl a and b, According Treatments. Different Words Indicate Significant Differences Among Treatments ($p<0.05$)

Moreover, this decrease in the chlorophyll a/b ratio may also signify a response to specific urban pollutants, such as ozone (O_3) and nitrogen oxides (NO_x), commonly found in areas with high vehicular traffic. These pollutants are known to impact the photosynthetic machinery directly, leading to oxidative stress and photooxidative damage that can impair chlorophyll function [65]. For instance, Demmig-Adams found that elevated ozone levels can lead to chlorophyll degradation and a corresponding rise in chlorophyll b, a change which helps mitigate damage to photosystem II under high oxidative conditions. Similarly, Moreno observed in Platanus hispanica, a tree commonly found in urban environments, that the chlorophyll a/b ratio was significantly lower in sites with high pollution, indicating that this ratio can serve as an early marker of environmental stress and pollution-induced damage.

This shift in pigment composition, with an increased proportion of chlorophyll b relative to chlorophyll a, supports the hypothesis that M. alba adjusts its photosynthetic apparatus to counteract the challenges of urban pollution and intense light exposure. The observed chlorophyll response in M. alba not only underscores its adaptability but also aligns with findings from other urban studies, where changes in chlorophyll ratios serve as indicators of environmental stress. For example, Yousafzai noted that urban plants like Ficus carica showed increased chlorophyll b levels under high pollution, suggesting that this shift is a conserved mechanism across different species subjected to urban stressors [66].

Carotenoid Content and its Relationship with Total Chlorophyll

The results indicate that, although there were no statistically significant differences in carotenoid content between the two sites, a slight increase was observed in the micro-town compared to the park (Figure 4). This increase in carotenoids, although modest, is noteworthy as it may suggest an adaptive mechanism in M. alba for dealing with urban stressors. Carotenoids are essential pigments with multifaceted roles in plant stress physiology. Not only do they assist in light capture as accessory pigments, they also serve as protective agents by dissipating excess energy and scavenging reactive oxygen species (ROS), which can accumulate under stress conditions like high light exposure and pollution [67, 68]. The ability of carotenoids to quench singlet oxygen and other ROS helps protect cell membranes from oxidative damage, preserving cellular integrity and function under adverse conditions.

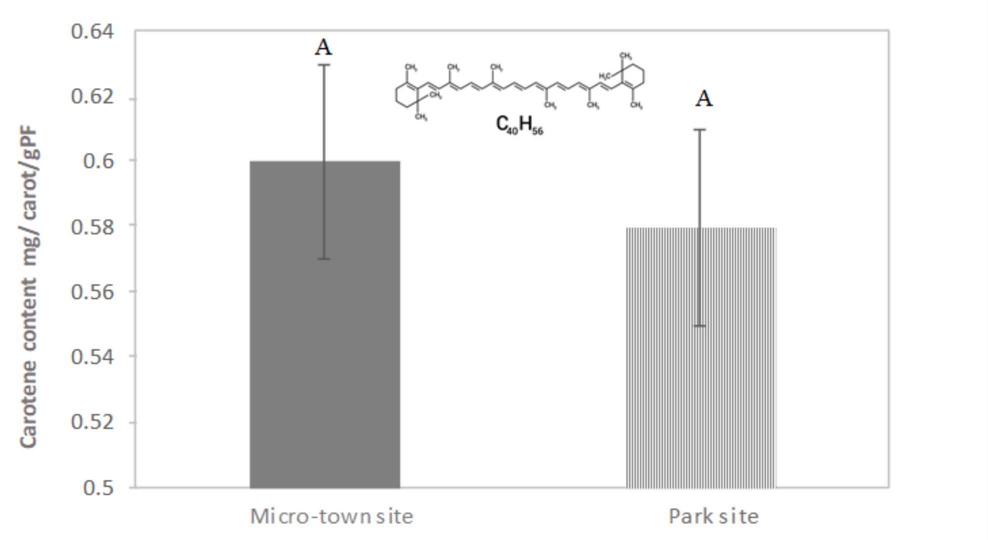

Figure 4: Carotene Content in Leaves of M. Alba L. Data Represent the Mean and the Standard Error (n=10). Different Words Indicate Significant Differences Among Treatments ($p<0.05$)

Interestingly, while carotenoid content increased, the carotenoid/total chlorophyll ratio was slightly lower in the micro-town (0.0009) than in the park (0.0012). This subtle reduction could imply that, even in a high-stress environment, M. alba maintains a balance between chlorophyll and carotenoids to sustain its photosynthetic activity. Plants in urban areas often undergo adaptive adjustments in pigment composition, enabling them to optimize light harvesting while simultaneously countering oxidative stress. A lower carotenoid/chlorophyll ratio in the micro-town may reflect a strategy to allocate resources towards chlorophyll synthesis, which supports the primary photosynthetic function, while still maintaining sufficient carotenoid levels for protective purposes (Figure 5)

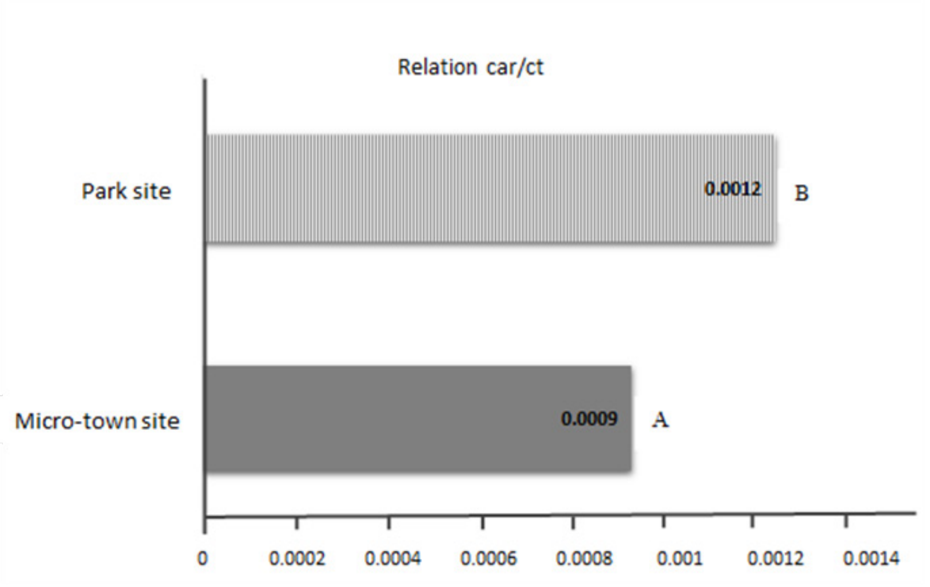

Figure 5: Carotene/Chlorophyl Total Relationship, According Treatments. Different Words Indicate Significant Differences Among Treatments ($p<0.05$)

Recent research provides further support for the protective role of carotenoids in urban environments. Talebzadeh & Valeo demonstrated that carotenoids often work in tandem with chlorophyll b to stabilize the photosynthetic apparatus under environmental stress conditions, including high levels of ozone and airborne particulate matter commonly found in urban settings. In particular, chlorophyll b and carotenoids may provide a synergistic effect that allows plants to manage light more efficiently and protect against photooxidative damage. The ability of carotenoids to act as natural antioxidants is especially crucial under elevated pollution conditions, as seen in studies of Ficus carica conducted by Yousafzai, where plants exposed to high urban pollution exhibited increased carotenoid concentrations [69]. This increase was interpreted as an adaptive response to counteract the oxidative stress induced by urban pollutants.

In the case of M. alba, the slightly lower carotenoid/chlorophyll ratio observed in the micro-town suggests that, despite the high-stress conditions, the plant prioritizes chlorophyll production to sustain photosynthetic performance while maintaining stable carotenoid levels for cellular protection. This balance highlights M. alba's adaptability in urban environments, where sustaining photosynthetic efficiency amidst oxidative stress is vital for survival.

Overall, the carotenoid response observed in M. alba underscores the critical role of these pigments in enabling plants to endure urban stressors. This adaptive trait not only points to the plant's resilience but also affirms the utility of carotenoid-to-chlorophyll ratios as a physiological marker of plant health in urban ecosystems.

Hydrogen Peroxide (H_2O_2) Production and Lipid Oxidative Damage

H_2O_2 production was significantly higher in the micro-town than in the park, suggesting a higher level of oxidative stress in the urban center (Figure 6). Hydrogen peroxide is a common ROS that, under stress conditions, can accumulate to harmful levels in plant cells, causing lipid peroxidation and, ultimately, compromising the integrity of cell membranes. The accumulation of H_2O_2 in the micro-town supports the hypothesis that the plant's antioxidant defenses are insufficient to counter ROS production in a high-pollution environment as confirmed by the elevated malondialdehyde (MDA) levels observed in the same site.

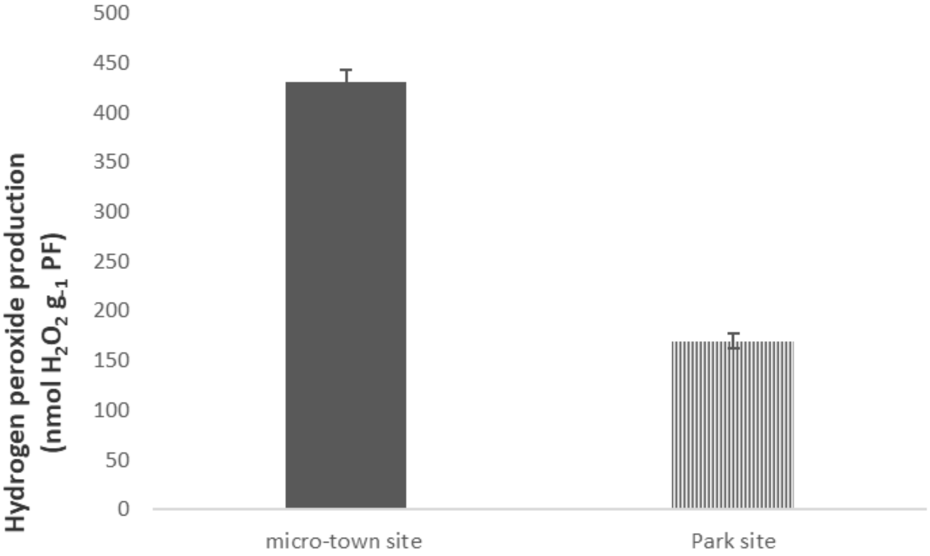

Figure 6: Hydrogen Peroxide Production (H2O2) in M. Alba Leaves. Data Indicates the Media and Standard Deviation (n=10). Different Words Indicate Significant Differences Among Treatments (p<0.05)

The highest oxidative damage in leaves was found in the micro-town site. This was significantly different from the park site, which had less damage (Figure 7).

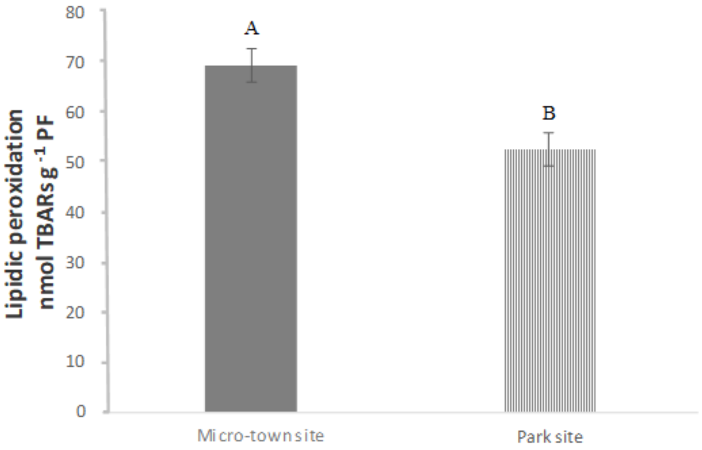

Figure 7: Lipidic Peroxidation in Leaves. Different Words Indicate Significant Differences Among Treatments (p<0.05)

MDA is a direct indicator of lipid oxidative damage and a widely used marker for lipid peroxidation [70]. Elevated MDA levels in the microcenter suggest that M. alba in this site is experiencing significant structural damage to its cell membranes due to exposure to atmospheric pollutants. This interpretation is consistent with studies conducted in urban areas with high vehicular traffic levels, where ROS generated by pollutants such as NO_2 and O_3 have been observed to cause considerable oxidative stress in plants highlighted that MDA accumulation is a reliable indicator of chronic oxidative stress in plants exposed to polluted environments, supporting the use of this measure to assess the health of urban vegetation [71-73].

In a recent study by it was found that exposure of M. alba and Ligustrum lucidum to urban environments with high particulate matter and sulfur compound loads led to an increase in H_2O_2 production and a rise in MDA levels, affecting their photosynthesis and cellular integrity. These findings align with the results of our study and reinforce the importance of using oxidative stress indicators such as H_2O_2 and MDA to assess the impact of pollution on urban vegetation [74].

The results obtained in this study have important implications for managing urban vegetation in highly polluted environments. M. alba exhibited a series of physiological adaptations to urban stress, such as adjustments in the chlorophyll a/b ratio and a slight increase in carotenoids, which suggests an adaptive capacity to moderate pollution conditions. However, the elevated levels of H_2O_2 and MDA indicate that, although the species can withstand some degree of pollution, the chronic accumulation of pollutants overwhelms the plant's antioxidant defenses, causing oxidative damage and limiting its longevity and photosynthetic effectiveness in densely polluted urban environments [75].

The choice of M. alba as a bioindicator in this study is particularly relevant in arid cities where urban vegetation not only fulfills an aesthetic function but also an ecological one, helping to mitigate the effects of climate change and improve air quality [76]. The chlorophyll a/b ratio, carotenoid content, and H_2O_2 and MDA levels in this species allow for an accurate assessment of the impact of atmospheric pollution on its physiology and offer a practical and effective methodology for monitoring urban tree health.

Conclusion

This study provides an in-depth view of the physiological responses of M. alba to urban environmental stress in the metropolitan area of Mendoza, highlighting its potential as a bioindicator of environmental quality in cities with high atmospheric pollution. The analyses of photosynthetic pigments and oxidative stress markers in M. alba leaves revealed significant adaptations in chlorophyll and carotenoid content, as well as an increase in reactive oxygen species (ROS) production and lipid damage in the urban microcenter [77]. These findings suggest that while M. alba exhibits physiological mechanisms to adapt to the adverse urban environment, its antioxidant defense capacity may be overwhelmed in areas with high pollution levels. The reduction in the chlorophyll a/b ratio and the tendency to increase chlorophyll b synthesis in the microcenter reflect an effort to optimize light capture and mitigate photooxidative damage, a response that has been documented in other species subjected to environmental stress in densely populated cities.

The elevated production of hydrogen peroxide (H_2O_2) and significant levels of malondialdehyde (MDA) in the microcenter reinforce the hypothesis that M. alba's antioxidant defenses are insufficient to counteract oxidative damage in high-exposure environments. These markers provide direct evidence that the urban setting imposes chronic stress, potentially compromising the structural and functional integrity of plants over the long term. This study underscores the importance of utilizing M. alba as a bioindicator in urban green space planning, as its sensitivity to pollution and adaptive responses can yield valuable insights for managing and mitigating the effects of air pollution on urban vegetation.

Our results underscore the potential of M. alba as a bioindicator for monitoring urban environmental quality. The plant's physiological adaptations and vulnerabilities make it an effective measure of air pollution impact. In particular, the chlorophyll a/b ratio, carotenoid content, H_2O_2 production, and MDA levels in M. alba leaves offer practical and accessible indicators for assessing urban plant health. The use of M. alba in urban green planning can contribute to more informed decisions that enhance urban resilience and ecological balance. However, it is clear that chronic pollution levels may exceed the adaptive capacity of species, resulting in oxidative damage that could affect their long-term survival and the functionality of their ecosystems in densely populated urban areas [78-80].

Future studies should explore how different urban species respond to similar environmental stressors, with a focus on identifying species with higher resilience to urban pollutants. Additionally, examining seasonal variations and extended exposure effects on M. alba could offer further insights into its adaptability and provide a stronger basis for sustainable urban forestry practices. Overall, our study reinforces the relevance of bioindicators like M. alba for urban environmental health monitoring and supports the implementation of evidence-based management strategies to mitigate the ecological impacts of urbanization.

Acknowledgement

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References

1. Alexieva, V., Sergiev, I., Mapelli, S., & Karanov, E. (2001). The effect of drought and ultraviolet radiation on grouwth and stress markers in pea and wheat. Plant Cell Environ., 24, 1337-1344. https://doi.org/10.1046/j.1365-3040.2001.00778.x
2. Alamilla-Martínez, D. G., Tenorio-Sánchez, S. A., & Gómez-Ramírez, M. (2025). Review: Implications of air pollution on trees located in urban areas. Earth, 6(2), 38. https://doi.org/10.3390/earth6020038

3. Antenozio, M. L., Caissutti, C., Caporusso, F. M., Marzi, D., & Brunetti, P. (2024). Urban air pollution and plant tolerance: Omics responses to ozone, nitrogen oxides, and particulate matter. Plants, 13(15), 2027. https://doi.org/10.3390/plants13152027
4. Apel, K. & Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol., 55, 373-399. https://doi.org/10.1146/annurev.arplant.55.031903.141701
5. Arnon, D.I. (1949). Copper Enzymes in Isolated Chloroplasts. Polyphenoloxidase in Beta Vulgaris. Plant Physiol., 24, 1-15.
6. Barik, A., et al. (2016). Biomonitoring urban air quality using tree leaves: approaches and limitations. Atmos. Environ., 132, 123–134. https://doi.org/10.1016/j.atmosenv.2016.02.012
7. Blokhina, O., Virolainen, E., & Fagerstedt, K.V. (2003). Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann. Bot., 91(2), 179-194. https://doi.org/10.1093/aob/mcf118
8. Bruno, G., Stiefkens, L., Hadid, M., Liscovsky, I., Cosa, M.T., & Dottori, N. (2007). Efecto de la contaminación ambiental en la anatomía de la hoja de Ligustrum lucidum (Oleaceae). Bol. Soc. Arg. Bot., 42(3-4), 231-236.
9. Cabrera, C.N., Gelsi, G.A., Albornoz, P.L., & Arias, M. (2009). Anatomia foliar de Ficus maroma (Moraceae) y análisis de hojas expuestas a la polución atmosférica en la provincia de Tucumán (Argentina). Lilloa, 46 (1-2), 34-42.
10. Calatayud, V., et al. (2019). Air pollution effects on urban trees: physiological and biochemical responses. Urban For. Urban Green., 43, 126–136. https://doi.org/10.1016/j.ufug.2019.04.009
11. Calfapietra, C., Peñuelas, J., & Niinemets, Ü. (2015). Urban plant physiology: adaptation-mitigation strategies under permanent stress. Trends Plant Sci., 20(2), 72-75. https://doi.org/10.1016/j.tplants.2014.11.001
12. Callejas, R., Kania, E., Contreras, A., Peppi, C., & Morales, L. (2013). Evaluación de un método no destructivo para estimar las concentraciones de clorofila en hojas de variedades de uva de mesa. Idesia (Arica), 31(4), 19-26. http://dx.doi.org/10.4067/S0718-34292013000400003
13. Cambrón-Sandoval, V.H., España-Boquera, M.L., Sánchez-Vargas, N.M., Sáenz-Romero, C., Vargas-Hernández, J.J., & Herrerías-Diego, Y. (2011). Producción de clorofila en Pinus pseudostrobus en etapas juveniles bajo diferentes ambientes de desarrollo. Rev. Chapingo Ser. Cienc. For. Amb., 17(2), 253-260. https://doi.org/10.5154/r.rchscfa.2010.09.077
14. Сарсацкая, А., & Sarsatskaya, A.S. (2017). Content of photosynthetic pigments in urban wood species. Vestn. Kemerov. Gos. Univ., 4, 9-14. https://doi.org/10.21603/2542-2448-2017-4-9-14
15. Carter, G.A. & Knapp, A.K. (2001). Leaf optical properties in higher plants: linking spectral characteristics to stress and chlorophyll concentration. Am. J. Bot., 88(4), 677-684. https://doi.org/10.2307/2657068
16. Casierra-Posada, F., Ávila-León, O., & Riascos-Ortíz, D. (2012). Cambios diarios del contenido de pigmentos fotosintéticos en hojas de caléndula bajo sol y sombra. Temas Agrar., 17(1), 60-71. https://doi.org/10.21897/rta.v17i1.697
17. Choppala, G., Saifullah, N. Bolan, Bibi, S., Iqbal, M., Rengel, Z., Kunhikrishnan, A., Nanjappa, A., & Yong, S.O. (2014). Cellular Mechanisms in Higher Plants Governing Tolerance to Cadmium Toxicity. Crit. Rev. Plant Sci., 33(5), 374–391.
18. Curieses Andrés, C. M., Pérez de la Lastra, J. M., Juan, C. A., Plou, F. J., & Pérez-Lebeña, E. (2023). Superoxide Anion Chemistry — Its role at the core of the innate immunity. Int. J. Mol. Sci., 24(3), 1841.
19. Dadkhah-Aghdash, H., Rasouli, M., Rasouli, K., & Salimi, A. (2022). Detection of urban trees sensitivity to air pollution using physiological and biochemical leaf traits in Tehran, Iran. Sci. Rep., 12(1), 15398.
20. Das, D., Bhattacharyya, S., Bhattacharyya, M., Sashankar, P., Ghosh, A., & Mandal, P. (2022). Transcriptome analysis of mulberry (Morus alba L.) leaves to identify differentially expressed genes associated with post-harvest shelf-life elongation. Sci Rep, 12(1), 18195. https://doi.org/10.1038/s41598-022-21828-7
21. Demmig-Adams, B. & Adams, W.W. (1992) Photoprotection and Other Responses of Plants to High Light Stress. Annu. Rev. Plant Physiol. Plant Mol. Biol., 43, 599-626. https://doi.org/10.1146/annurev.pp.43.060192.003123
22. Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M., & Robledo, Y.C. (2011). InfoStat versión 2011. (Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.). Disponible en: http://www.infostat.com.ar
23. Feng, Z., et al. (2017). Urbanization effects on plant physiology and pollutant exposure. Ecol. Indic., 78, 58–66. https://doi.org/10.1016/j.ecolind.2017.02.050
24. Gechev, T.S., Van Breusegem, F., Stone, J.M., Denev, I., & Laloi, C. (2006). Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. BioEssays, 28, 1091-1101. https://doi.org/10.1002/bies.20493
25. Gerhardt, K.E., Huang, X.D., Glick, B.R., & Greenberg, B.M. (2009). Phytoremediation and rhizoremediation of organic soil contaminants: potential and challenges. Plant Sci., 176, 20- 30. https://doi.org/10.1016/j.plantsci.2008.09.014
26. Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem., 48(12), 909–930.
27. Goel, N., Harron, J., Hu, B., Miller, J., Mohammed, G., Noland, T., Sampson, P., & Zarco, P. (2004). Needle chlorophyll content estimation through modelling version using hyperspectral data from boreal conifer forest canopies. Remote Sens. Environ., 89(2), 189-199.
28. Gull, A., Lone, A. A., & Wani, N. U. I. (2019). Biotic and abiotic stresses in plants. (In Bosco de Oliveira, A. (Ed.) Abiotic and biotic stress in plants (pp. 1-19).
29. Hasanuzzaman, M., Bhuyan, M. B., Parvin, K., Bhuiyan, T. F., Anee, T. I., Nahar, K., ... & Fujita, M. (2020). Regulation of ROS metabolism in plants under environmental stress: A review of recent experimental evidence. Int. J. Mol. Sci., 21(22), 8695. https://doi.org/10.3390/ijms21228695
30. wHeath, R.L. & Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid

peroxidation. Arch. Biochem. Biophys., 125(1), 189-198. https://doi.org/10.1016/0003-9861(68)90654-1

31. Iqbal, M., et al. (2019). Biochemical markers for oxidative stress in urban plants. Ecotoxicol., 28(4), 480–492. https://doi.org/10.1007/s10646-019-01957-3
32. Itziou, A., et al. (2024). Environmental pollution and oxidative stress: health and ecosystem consequences. Appl. Sci., 14(21), 9884.
33. Joshi, P. C., & Swami, A. (2009). Air pollution induced changes in the photosynthetic pigments of selected plant species. J. Environ. Biol., 30(2), 295-298. https://pubmed.ncbi.nlm.nih.gov/20121034
34. Judd, W.S., Campbell, C.S., Kellogg, E.A., Stevens, P.F., & Donoghue, M.J. (1999). Plant systematics: a phylogenetic approach. Ecol. Mediterr., 25(2), 215.
35. Kafash, Z. H., Ghanati, F., Payez, A., & Behmanesh, M. (2022). Traffic noise induces oxidative stress and phytohormone imbalance in urban plants. Environ. Pollut., 294, 118659. https://doi.org/10.1016/j.envpol.2021.118659
36. Larcher, W. (2003). Physiological plant ecology: ecophysiology and stress physiology of functional groups. (New York: Springer Science & Business Media).
37. Levitt, J. (1980). Responses of Plants to Environmental Stress, Volume 1: Chilling, Freezing, and High Temperature Stresses. (California: Academic Press).
38. Lichtenthaler, H. K., & Babani, F. (2021). Contents of photosynthetic pigments and ratios of chlorophyll a/s and chlorophylls to carotenoids (a+b)/(x/c) in C4 plants as compared to C3 plants. Photosynthetica, 60(1), 3–9.
39. Linden, J., Gustafsson, M., Uddling, J., Watne, Å., & Pleijel, H. (2023). Air pollution removal through deposition on urban vegetation: The importance of vegetation characteristics. Urban For. Urban Green., 81, 127843.
40. Lovreškov, L., Potočić, N., Seletković, A., Klarić, M. Š., Glavaš, M., & Jazbec, A. (2022). Are foliar nutrition status and indicators of oxidative stress useful for tree health assessment? Forests, 13(3), 405. https://doi.org/10.3390/f13030405
41. Mackinney, G. (1938). Some Absorption Spectra of Leaf Extracts. Plant Physiol., 13(1), 123–140. https://doi.org/10.1104/pp.13.1.123
42. Maheswari, M., et al. (2018). Chlorophyll and carotenoid adjustments under abiotic stress: a review. J. Plant Res., 131(3), 389–399. https://doi.org/10.1007/s10265-018-1032-0
43. Martí, M.C., Camejo, D., Fernández-García, N., Rellán-Álvarez, R., Marques, S., Sevilla, F., & Jiménez, A. (2009). Effect of oil refinery sludges on the growth and antioxidant system of alfalfa plants. J. Hazard. Mater., 171(1-3), 879-885. https://doi.org/10.1016/j.jhazmat.2009.06.083
44. Martínez Carretero, E., Moreno, G., Vento, B., & Ganci, C. (2016). Ecología Urbana. Estudios en una ciudad oasis. (In Martínez Carretero, E., & García, A. (Eds) San Juan Ambiental (pp. 363-378).
45. Mehmood, Z., et al. (2024). Effects of air pollution on morphological, biochemical, DNA damage and antioxidant responses in plants. Sustainability, 16(8), 3427.
46. Meyer, A.J. (2008). The integration of glutathione homeostasis and redox signaling. J. Plant Physiol., 165(13), 1390-1403. https://doi.org/10.1016/j.jplph.2007.10.015
47. Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci., 7(9), 405–410.
48. Moreno-Echeverry, D.A.R.W.I.N., Quiroga, I.A., Balaguera-López, H.E., & Magnitskiy, S. (2016). El estrés por boro afecta la fotosíntesis y el metabolismo de pigmentos en plantas. Una revisión. Rev. Colomb. Cienc. Hortíc., 10(1), 137-148. http://dx.doi.org/10.17584/rcch.2016v10i1.4189
49. Moreno, G., Martínez Carretero, E., Faggi, A. M., & Vento, B. (2008). Sedimentación atmosférica seca en hojas de Morus alba L: En la ciudad de San Juan, Argentina. Interciencia, 33(11), 844–849.
50. Moreno, G., Martínez Carretero, E., & Duplancic, A. (2013). El papel de los árboles urbanos en la remoción de partículas atmosféricas en Mendoza. (In Puliafito, S.E. (Ed) Contaminación atmosférica e hídrica en Argentina. Tomo II PROIMCA- PRODECA. UTN. Facultad Regional Mendoza (pp. 411-424). ISBN 978-950-42-0150-2.
51. Moreno, G., Martínez Carretero, E., Duplancic, A., & Alcalá, J. (2016). Frecuencia de diferentes tamaños de material particulado en hojas de Morus alba en el arbolado urbano de Mendoza (Argentina). Multequina, 25, 5-12.
52. Moreno, G., Martínez Carretero, E., & Alcalá, J. (2022). Evaluación de la sedimentación atmosférica seca en Morus alba en la ciudad de Mendoza, Argentina. Multequina, 31(1), 28-36.
53. Noctor, G., Reichheld, J. P., & Foyer, C. H. (2018). Modulation of plant redox metabolism in response to stress. New Phytol., 221(3), 1178–1205. https://doi.org/10.1111/nph.15461
54. Norte, F. (2000). Mapa climático de Mendoza. (In Abraham, E. and Martínez, F. (Eds) Inventario de recursos para la planificación y gestión de la región andina argentina. Universidad de Granada, Andalucía, España.)
55. Peralta-Pérez, M.D.R., & Volke-Sepúlveda, T.L. (2012). La defensa antioxidante en las plantas: una herramienta clave para la fitorremediación. Rev. Mex. Ing. Quím., 11(1), 75-88.
56. Perelman, P., Martínez Carretero, E., Moreno, G., Castro, M.A., & Faggi, A. (2007). El uso de corteza de Mora (Morus alba L.) como biomonitor para detectar contaminación en la ciudad de Mendoza. Hologramática, 4(7), 205-214.
57. Petrova, S., Todorova, K., Dakova, M, Mehmed, E., Nikolov, B., Denev, I., & Velcheva, I. (2017). Photosynthetic Pigments as Parameters/Indicators of Tree Tolerance to Urban Environment (Plovdiv, Bulgaria). Ecologia Balkanica, 9(1), 53-62.
58. Prado, F. E., Rosa, M., & Hilal, M. (2010). Las especies C4 y el estrés ambiental. In: C4 y CAM: características generales y uso en programas de desarrollo de tierras áridas y semiáridas, Fundación Ramón Areces (pp. 31–42).
59. Programa Regional de Meteorología (IANIGLA). (2016). Consultado diciembre 2016. Disponible en: http://www.prmarg.org/upload/20131.pdf
60. Puliafito, E., Guevara, M., & Puliafito, C. (2003). Characterization of urban air quality using GIS as management system. Environ. Pollut., 122, 105-117. https://doi.org/10.1016/S0269-7491(02)00278-6
61. Romero-Puertas, M.C., Palma, J.M., Gómez, M., Del Río,

L.A., & Sandalio, L.M. (2002). Cadmium causes the oxidative modification of proteins in pea plants. Plant Cell Environ., 25, 677-686.

62. Salazar, M.T.J., Giraldo, Y.M., & Serna, D.M.O. (2018). Efectos en el nivel fotosintético en tres especies de plantas acuáticas sometidas a un tratamiento con agua residual de origen minero. Bol. Cient. Cent. Mus., 22(1), 43-57. https://doi.org/10.17151/bccm.2018.22.1.3
63. Sandalio, L.M., Dalurzo, H.C., Gomez, M., Romero-Puertas, M.C., & Del Río, L.A. (2001). Cadmium-induced changes in the growth and oxidative metabolism of pea plants. J. Exp. Bot., 52(364), 2115-2126. https://doi.org/10.1093/jexbot/52.364.2115
64. Sarely, V. J., Domínguez Gómez, T. G., González-Rodríguez, H., Solíz-González, S., & Graciano, J. (2021). Contenido de pigmentos fotosintéticos en cuatro especies arbustivas el noreste de México. E-CUCBA, 9(19), 165–172.
65. Sharma, P., Jha, A. B., Dubey, R. S., & Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J. Bot., Article ID 217037. https://doi.org/10.1155/2012/217037
66. Sobrino-Plata, J., Ortega-Villasante, C., Flores-Cáceres, M.L., Escobar, C., Del Campo, F.F., & Hernández, L.E. (2009). Differential alterations of antioxidant defenses as bioindicators of mercury and cadmium toxicity in alfalfa. Chemosphere, 77(7), 946-954. https://doi.org/10.1016/j.chemosphere.2009.08.007
67. Song, X., & Liu, H. (2016). Effects of building shade on photosynthesis and chlorophyll fluorescence of Euonymus fortune. Acta Ecol. Sin., 36(5), 350–355.
68. Souri, Z., et al. (2017). Antioxidant system responses in plants to various abiotic stresses. Biol. Plant., 61(1), 1–12. https://doi.org/10.1007/s10535-016-0662-8
69. Srivastava, A., et al. (2015). Antioxidant enzyme activities in plants exposed to urban pollution. Environ. Monit. Assess., 187, 1–12. https://doi.org/10.1007/s10661-015-4682-4
70. Singh, S., Roy, S., Choudhury, S., Chakraborty, S., & Bhattacharya, S. (2017). Use of plants as biomonitors of air pollution: review. Environ. Sci. Pollut. Res., 24(12), 11193–11206. https://doi.org/10.1007/s11356-017-8575-9
71. Thakur, K., & Garg, N. (2022). Oxidative Stress and Antioxidant Enzymes in Cereals Under Abiotic Stress. (In Sustainable Remedies for Abiotic Stress in Cereals (pp. 51-82). Singapore: Springer Nature Singapore.)
72. Talebzadeh, F., & Valeo, C. (2022). Evaluating the effects of environmental stress on leaf chlorophyll content as an index for tree health. (In IOP conference series: earth and environmental science (1006(1),012007). IOP Publishing).
73. Tausz, M., Grill, D., Jiménez, M.S., & Morales, D. (1997). A physiological comparison between environmental effects on Canary pine in Tenerife and Norway spruce in Austria. (In Mohren, F., Kramer, K., & Sabaté, S. (Eds.), Impacts of global change on tree physiology and forest ecosystems (pp. 103–110). Amsterdam, The Netherlands, Kluwer Publishers.)
74. Vaccarella, E., Lorenzo, M., & Canepari, S. (2025). Assessment of oxidative stress induced by atmospheric pollution in plants. Sci. Total Environ., 965, 178651.
75. Vento, B., Martínez Carretero, E., Moreno, G. & Duplancic, A. (2017). Mineral contents in leaves of Morus alba L. (Moraceae) and Platanus hispanica Miller (Platanaceae) from the urban forests of central-western area of Argentina. Bot. Lett., 164(2), 183–192. https://doi.org/10.1080/23818107.2017.1314226
76. Vernon, L.P. (1960). Spectrophotometric determination of chlorophylls and pheophytins in plant extracts. Anal. Chem., 32(9), 1144-1150. https://doi.org/10.1021/ac60165a029
77. 77. Wang, S., et al. (2016). Response of chlorophyll and carotenoids to heavy metal stress in plants. J. Plant Physiol., 198, 76–83. https://doi.org/10.1016/j.jplph.2016.02.009
78. 78. Wolfenden, J., Robinson, D.C., Cape, J.N., Paterson, I.S., Francis, B.J., Mehlhorn. H. & Wellburn, A.R. (1988). Use of carotenoid ratios, ethylene emissions and buffer capacities for the early diagnosis of forest decline. New Phytol., 109(1), 85-95. https://doi.org/10.1111/j.1469-8137.1988.tb00222.x
79. 79. Yousafzai, A., Durani, A., Hameedi, A., Mohammadi, M.H., Durrani, H., & Safiullah, K. (2018). Effects of air pollution on chlorophyll content of urban trees leaves. Int. J. Biol. Res., 3(1), 287-291.
80. 80. Zornoza, P., Vázquez, Esteban, E., Fernández-Pascual, M., & Carpena, R. (2002). Cadmium stress in nodulated white lupin: strategies to avoid toxicity. Plant Physiol. Biochem., 40, 1003-1009. https://doi.org/10.1016/