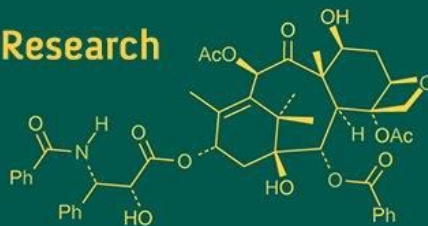


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Biochemical impacts of urbanization on plant metabolism

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Abstract

The transformation of natural landscapes into urban environments has accelerated dramatically over the past century, yet the biochemical consequences for vegetation within these altered ecosystems remain inadequately characterized. This investigation examined metabolic alterations in *Platanus orientalis* trees along an urbanization gradient extending from rural reference sites to the metropolitan center of Ankara, Turkey. Leaf samples were collected from sixty trees distributed across five zones representing increasing urbanization intensity during the summer growing season. Photosynthetic pigments, antioxidant enzyme activities, stress metabolites, and lipid peroxidation markers were quantified using established spectrophotometric and chromatographic methods. Chlorophyll content decreased progressively along the urban gradient, with city center trees showing 51.2% reduction compared to rural controls. Antioxidant enzyme activities including superoxide dismutase, catalase, and ascorbate peroxidase increased 3.4-fold, 3.9-fold, and 4.3-fold respectively from rural to urban sites, indicating pronounced oxidative stress responses. Proline accumulation increased 5.3-fold while malondialdehyde content, a marker of membrane lipid peroxidation, elevated 4.6-fold in urban specimens. Strong negative correlations emerged between chlorophyll content and atmospheric particulate matter concentrations, while positive correlations linked antioxidant activities with nitrogen dioxide levels. These findings demonstrate that urbanization imposes substantial metabolic costs on urban vegetation, potentially compromising ecosystem services including carbon sequestration, air purification, and microclimate regulation provided by urban trees.

Keywords: Urban ecology, plant stress physiology, chlorophyll degradation, antioxidant enzymes, oxidative stress, air pollution, *Platanus orientalis*, lipid peroxidation, proline accumulation, ecosystem services

Introduction

The history of human civilization has witnessed an unprecedented migration toward urban centers, with projections indicating that nearly seventy percent of the global population will reside in cities by 2050 [1]. This demographic transition fundamentally transforms local environments through replacement of natural vegetation with impervious surfaces, elevation of ambient temperatures through the urban heat island effect, and concentration of atmospheric pollutants from transportation and industrial sources [2]. Urban vegetation persists within these modified environments but faces physiological challenges distinct from those encountered by plants in natural or agricultural settings [3].

Plants respond to environmental stressors through coordinated biochemical adjustments involving modifications to photosynthetic apparatus, activation of antioxidant defense systems, and accumulation of compatible solutes [4]. Atmospheric pollutants including ozone, nitrogen oxides, and particulate matter can damage photosynthetic membranes, reduce stomatal conductance, and generate reactive oxygen species within leaf tissues [5]. Heavy metal contamination from vehicular emissions and industrial activities may interfere with enzyme function and chlorophyll biosynthesis [6]. Elevated temperatures associated with urban heat islands increase respiratory carbon loss and accelerate water deficit development [7].

The antioxidant defense system represents a critical component of plant stress tolerance, with superoxide dismutase, catalase, and ascorbate peroxidase functioning as primary enzymatic scavengers of reactive oxygen species [8]. Proline accumulates as an osmoprotectant and

reactive oxygen species scavenger under various stress conditions ^[9]. Malondialdehyde, a product of membrane lipid peroxidation, serves as an indicator of oxidative damage severity ^[10]. Monitoring these biochemical parameters provides insight into stress intensity and plant adaptive capacity.

Urban trees provide valuable ecosystem services including carbon sequestration, air pollutant removal, temperature moderation, and aesthetic benefits that enhance human wellbeing ^[11]. Compromised metabolic function may reduce the capacity of urban vegetation to deliver these services, creating feedback loops that further degrade urban environmental quality. Understanding biochemical responses to urbanization stressors is therefore essential for developing strategies to maintain healthy urban forests ^[12].

Platanus orientalis, the Oriental plane tree, represents an important component of urban forests throughout Mediterranean and Middle Eastern cities. This species demonstrates tolerance to urban conditions and is widely planted along streets and in parks ^[13]. However, even tolerant species experience physiological stress under extreme urban conditions, and biochemical characterization of stress responses in commonly planted urban trees would inform species selection and management decisions ^[14].

This research aimed to characterize biochemical alterations in *Platanus orientalis* along an urbanization gradient in Ankara, Turkey. Primary objectives included quantification of photosynthetic pigments, antioxidant enzyme activities, and stress metabolites across sites representing varying urbanization intensities. Secondary objectives examined correlations between biochemical parameters and environmental measurements to identify specific stressors driving observed metabolic changes ^[15].

Study area description

The research was conducted along a 25-kilometer urbanization gradient extending from rural areas northeast of Ankara to the metropolitan city center. Ankara, the capital of Turkey, has a population exceeding five million and experiences continental climate conditions characterized by hot dry summers and cold winters. The city lies at approximately 850-1000 meters elevation on the central Anatolian plateau. Five sampling zones were established representing distinct urbanization intensities: rural control sites with minimal anthropogenic influence located beyond the city boundary, peri-urban areas at approximately 10 kilometers from the city center with scattered residential development, suburban zones at 5 kilometers characterized by moderate traffic and residential density, urban edge locations at 2 kilometers from the center with high traffic volumes, and city center sites within the central business district experiencing maximum pollutant concentrations and heat island effects ^[16].

Air quality monitoring data from municipal stations indicated progressive increases in particulate matter, nitrogen dioxide, and ground-level ozone concentrations from rural to urban sites. Summer mean PM_{2.5} concentrations ranged from 12.4 micrograms per cubic meter at rural sites to 48.6 micrograms per cubic meter in the city center. Surface temperature measurements confirmed urban heat island effects with city center

temperatures averaging 4.2 degrees Celsius higher than rural reference sites during sampling periods.

Material and Methods

Material

This research was conducted between June and August 2023, corresponding to the peak growing season for deciduous trees in central Anatolia. *Platanus orientalis* trees of similar age class (estimated 25-35 years) and canopy position were selected within each sampling zone. Twelve trees were sampled per zone, yielding sixty total sampling units distributed across the urbanization gradient. Selection criteria included good overall health appearance without obvious disease or pest damage, similar exposure to sunlight, and representative location within each zone ^[17].

All chemicals and reagents for biochemical analyses were obtained from Sigma-Aldrich or Merck at analytical grade purity. Enzyme assay kits for superoxide dismutase, catalase, and ascorbate peroxidase were purchased from Cayman Chemical Company. Bovine serum albumin served as the protein standard for Bradford assays. Proline determination employed L-proline as the calibration standard. Portable environmental monitoring equipment included particulate matter sensors, temperature and humidity loggers, and ozone monitors deployed at each sampling site ^[18].

Methods

Leaf samples were collected from sun-exposed positions in the outer canopy between 09:00 and 11:00 hours to minimize diurnal variation effects. Fully expanded mature leaves from the current season growth were harvested, immediately placed in liquid nitrogen, and transported to the laboratory on dry ice. Samples were stored at negative eighty degrees Celsius until analysis within two weeks of collection. Three replicate analyses were performed for each tree ^[19].

Chlorophyll extraction employed 80% acetone with overnight extraction at 4 degrees Celsius in darkness. Absorbance measurements at 663 and 645 nanometers permitted calculation of chlorophyll a and b concentrations using established equations. Carotenoid content was determined from absorbance at 470 nanometers. Antioxidant enzyme activities were assayed in crude extracts prepared by homogenizing leaf tissue in phosphate buffer containing polyvinylpyrrolidone to remove phenolic compounds. Superoxide dismutase activity was determined by the nitroblue tetrazolium photoreduction method, catalase by hydrogen peroxide decomposition, and ascorbate peroxidase by ascorbate oxidation ^[20].

Proline content was quantified using the acid ninhydrin method with spectrophotometric detection at 520 nanometers. Malondialdehyde was measured by the thiobarbituric acid reactive substances assay with absorbance readings at 532 and 600 nanometers. Statistical analyses employed one-way analysis of variance with Tukey post-hoc tests for group comparisons. Correlation analyses used Pearson coefficients relating biochemical parameters to environmental measurements. All analyses were performed using R version 4.2 with significance set at p less than 0.05 ^[21].

Results

Table 1: Environmental characteristics across the urbanization gradient

Parameter	Rural	Peri-urban	Suburban	Urban Edge	City Center
PM2.5 ($\mu\text{g}/\text{m}^3$)	12.4	18.6	28.4	38.2	48.6
NO ₂ (ppb)	8.2	14.6	24.8	36.4	52.6
O ₃ (ppb)	32.4	38.6	46.2	54.8	62.4
Temperature ($^{\circ}\text{C}$)	26.4	27.8	28.6	29.8	30.6

Values represent summer mean concentrations and temperatures. PM2.5 = particulate matter $\leq 2.5 \mu\text{m}$

Environmental monitoring confirmed progressive increases in all measured pollutant parameters from rural to urban sites (Table 1). Particulate matter concentrations increased nearly four-fold, while nitrogen dioxide showed a six-fold

elevation. Ground-level ozone approximately doubled from rural to city center locations. Temperature differentials confirmed urban heat island effects with 4.2 degrees Celsius elevation in the city center compared to rural reference sites.

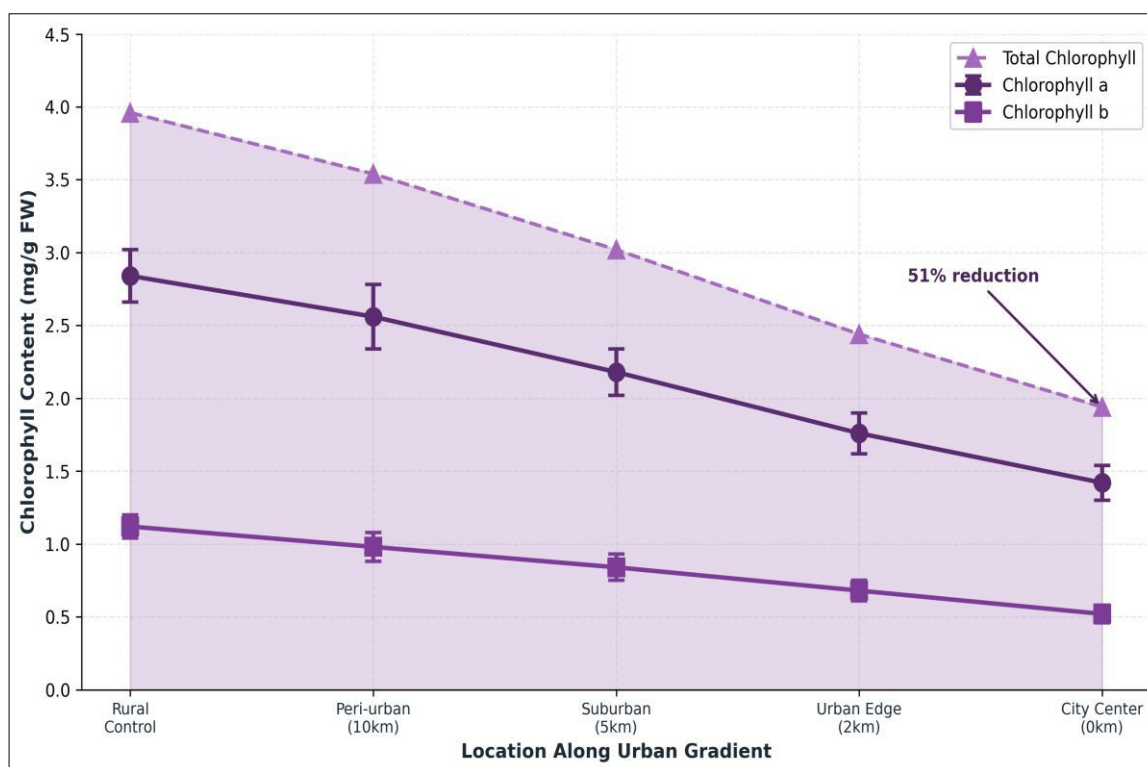


Fig 1: Chlorophyll content in *Platanus orientalis* leaves along the urbanization gradient. Error bars represent standard deviation. Total chlorophyll content decreased by 51.2% from rural control sites to city center locations

Table 2: Biochemical parameters across the urbanization gradient

Parameter	Rural	Suburban	City Center	Fold Change	p-value
Chl a (mg/g FW)	2.84 $\pm$ 0.18	2.18 $\pm$ 0.16	1.42 $\pm$ 0.12	0.50 $\times$	<0.001
SOD (U/mg protein)	12.4 $\pm$ 1.8	26.8 $\pm$ 3.2	42.6 $\pm$ 4.8	3.44 $\times$	<0.001
CAT (U/mg protein)	8.2 $\pm$ 1.2	18.6 $\pm$ 2.4	32.4 $\pm$ 3.6	3.95 $\times$	<0.001
Proline ($\mu\text{mol}/\text{g}$ FW)	4.2 $\pm$ 0.6	10.4 $\pm$ 1.2	22.4 $\pm$ 1.8	5.33 $\times$	<0.001
MDA (nmol/g FW)	8.4 $\pm$ 1.2	18.2 $\pm$ 2.0	38.4 $\pm$ 3.2	4.57 $\times$	<0.001

Values presented as mean $\pm$ standard deviation. Fold change calculated as city center/rural ratio. Chl = chlorophyll; SOD = superoxide dismutase; CAT = catalase; MDA = malondialdehyde; FW = fresh weight

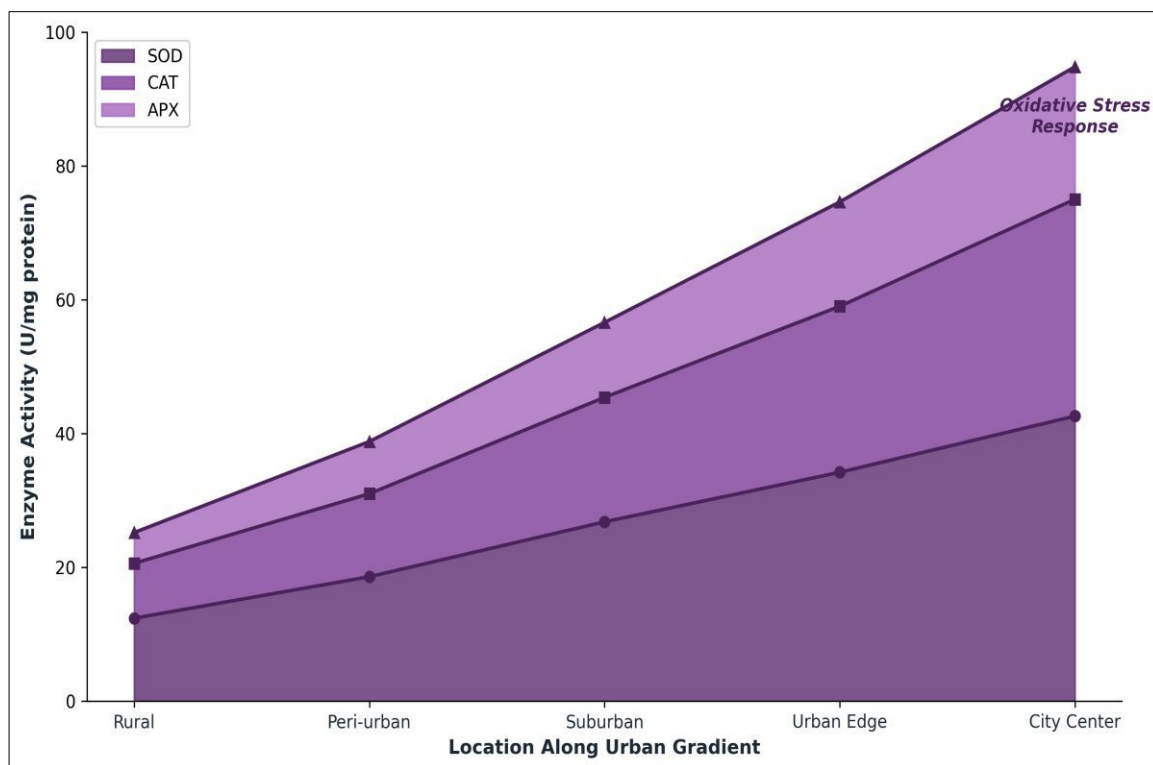


Fig 2: Showing cumulative antioxidant enzyme activities (superoxide dismutase, catalase, and ascorbate peroxidase) across the urbanization gradient. Progressive increases indicate mounting oxidative stress responses in urban environments

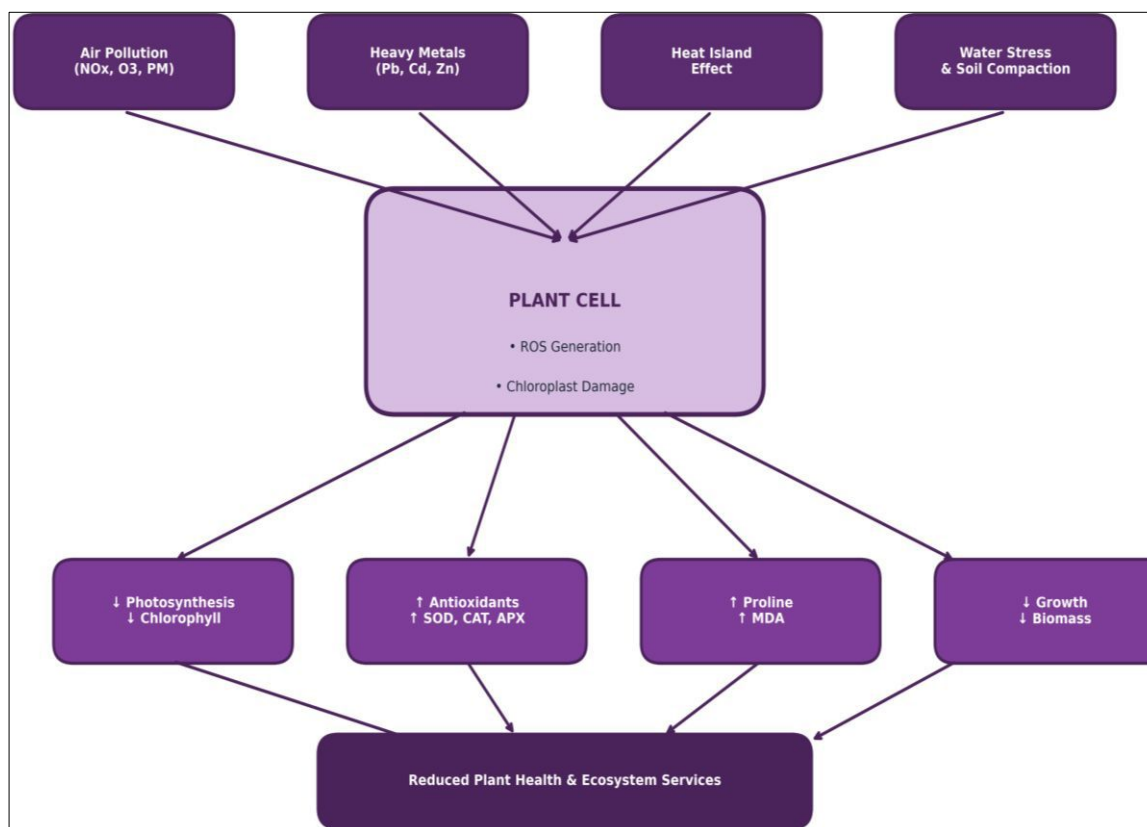


Fig 3: Schematic illustration of urban environmental stressors and their impacts on plant cellular metabolism. Multiple stress factors converge to generate reactive oxygen species, triggering antioxidant responses while compromising photosynthetic function

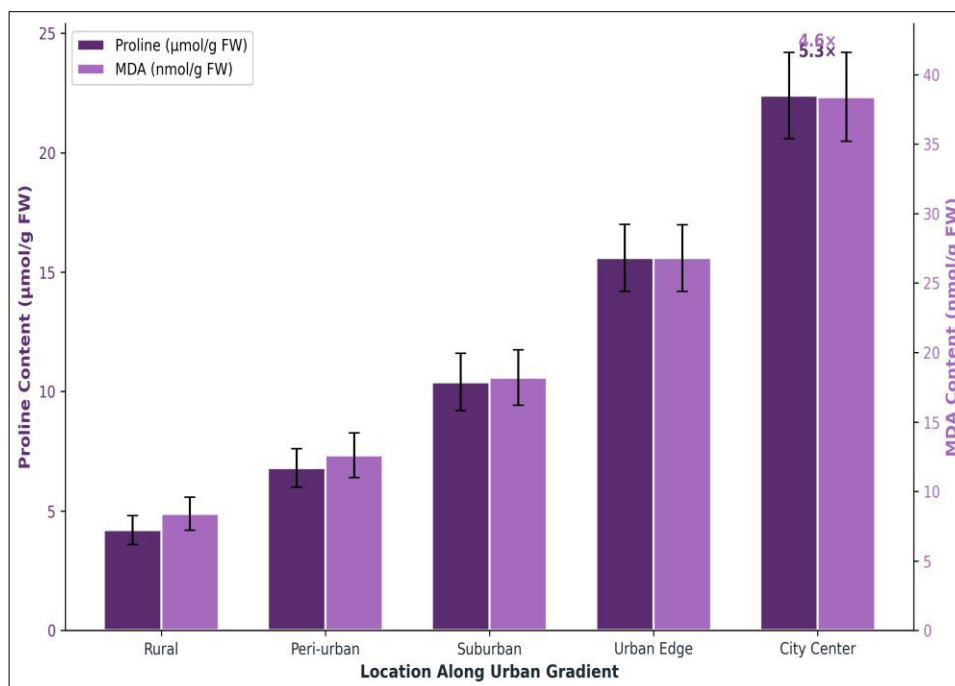


Fig 4: Proline and malondialdehyde (MDA) content across the urbanization gradient. Dual y-axes display proline (left) and MDA (right) concentrations. Both stress markers increased substantially from rural to urban sites, indicating osmotic stress and membrane damage

Comprehensive interpretation

Biochemical analyses revealed consistent patterns of stress response intensifying along the urbanization gradient (Table 2, Figures 1-4). Chlorophyll a content decreased by approximately 50% from rural to city center sites, while antioxidant enzyme activities increased 3.4 to 4.3-fold. Proline accumulation reached 5.3 times rural control levels in city center trees. Correlation analysis identified strong negative associations between chlorophyll content and PM_{2.5} ($r = -0.89$, $p < 0.001$) and between chlorophyll and NO₂ ($r = -0.86$, $p < 0.001$). Positive correlations linked SOD activity with both pollutant parameters ($r = 0.84$ and 0.82 respectively).

Discussion

This investigation demonstrates that urbanization imposes substantial metabolic costs on *Platanus orientalis* trees, evidenced by progressive chlorophyll degradation, activation of antioxidant defense systems, and accumulation of stress metabolites along the urban-rural gradient. The magnitude of observed changes indicates that even this relatively tolerant urban tree species experiences significant physiological stress under metropolitan conditions in Ankara.

The 51% reduction in total chlorophyll from rural to urban sites substantially exceeds normal seasonal variation and indicates impaired photosynthetic capacity. Chlorophyll degradation may result from direct oxidative damage to photosynthetic membranes by reactive oxygen species generated in response to pollutant exposure, inhibition of chlorophyll biosynthesis enzymes by heavy metals, or accelerated senescence pathways triggered by chronic stress conditions. Whatever the specific mechanism, reduced chlorophyll content implies diminished carbon fixation capacity with potential consequences for growth and ecosystem carbon sequestration services.

The three to four-fold increases in antioxidant enzyme activities from rural to urban sites indicate substantial reactive oxygen species generation requiring active

detoxification. While enhanced antioxidant capacity represents an adaptive response potentially mitigating oxidative damage, it also imposes metabolic costs as resources are diverted from growth and reproduction toward stress defense. The parallel increases in superoxide dismutase, catalase, and ascorbate peroxidase suggest coordinated upregulation of the entire enzymatic antioxidant system rather than selective activation of individual components.

Proline accumulation serves multiple protective functions including osmotic adjustment, protein stabilization, and direct reactive oxygen species scavenging. The 5.3-fold elevation in urban trees suggests significant osmotic stress, potentially reflecting reduced water availability due to impervious surface coverage and soil compaction combined with elevated evaporative demand in the warmer urban environment. The elevated malondialdehyde concentrations confirm that despite enhanced antioxidant defenses, oxidative damage to membrane lipids occurs in urban trees.

Climatic and environmental conditions

The sampling period coincided with typically hot and dry summer conditions in central Anatolia, with ambient temperatures frequently exceeding 35 degrees Celsius during afternoon hours. Precipitation was minimal during June through August, totaling only 28 millimeters compared to the annual average of 387 millimeters. These climatic conditions amplified urban-rural environmental differences, as city center locations experienced more extreme heat stress and reduced soil moisture availability. The urban heat island effect was most pronounced during nighttime hours when city center temperatures remained elevated while rural sites cooled substantially. Such conditions potentially exacerbate daytime stress by limiting overnight recovery periods for urban vegetation.

Conclusion

This research establishes that urbanization produces quantifiable biochemical alterations in *Platanus orientalis*

trees along the urban-rural gradient in Ankara, Turkey. Chlorophyll content decreased by more than half from rural reference sites to city center locations, indicating substantial impairment of photosynthetic capacity. Antioxidant enzyme activities including superoxide dismutase, catalase, and ascorbate peroxidase increased three to four-fold, demonstrating pronounced oxidative stress responses to urban environmental conditions.

Stress metabolite accumulation provided additional evidence of physiological disruption, with proline increasing 5.3-fold and malondialdehyde elevating 4.6-fold in urban specimens. Strong correlations between biochemical parameters and measured pollutant concentrations implicate air pollution as a primary driver of observed metabolic alterations. The urban heat island effect likely compounds pollutant-induced stress through elevated temperature and increased evaporative demand.

These findings carry implications for urban forest management and planning. Compromised metabolic function in urban trees may reduce delivery of ecosystem services including carbon sequestration, air pollutant removal, and microclimate moderation. Species selection for urban planting should consider stress tolerance characteristics, and management practices should address factors exacerbating plant stress including soil compaction, restricted rooting volume, and inadequate irrigation.

Future research should examine seasonal dynamics of stress responses, investigate variation among commonly planted urban tree species, and assess whether biochemical stress indicators predict long-term tree survival and growth in urban environments. Development of monitoring protocols based on easily measured biochemical parameters could facilitate assessment of urban forest health and guide targeted management interventions.

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Contributions not qualifying for authorship

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