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Presented by:

*Baghzou Dounia*

*Hassad Aya*

**Topic:**

**Long-term Effects of Atmospheric  
Pollution on a Forest Tree Species**

**Thesis publicly defended on 22/06/2025 before a jury composed of:**

Mm : AROUA Khaoula University abbes laghrou, President

Mr. BOUCHAMA Khaled University abbes laghrou, Supervisor

Mm : BERKANI Cherif University abbes laghrou, Examiner

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*We are equally grateful to the university laboratory and its entire team for providing the facilities and support necessary to carry out our research. Finally, we extend our heartfelt thanks to all those who encouraged us and wished us success and good fortune.*

# ***Dedication***

To the memory of my beloved father,  
Though you are no longer with me,  
your values, your words, and your silent strength continue to  
guide me in every step.

This work is a reflection of the path you helped shape,  
and I carry your memory with pride and love.

To my dear mother, for her love and unwavering support.

To my brothers and sisters, for always being there.

To my friends and acquaintances, for their kind words and  
encouragement.

I dedicate this humble work to all who were part of this journey.

***Dounia***

# ***Dedication***

To the most important person in my life my Mom thank you for your unwavering support, your countless sacrifices that have opened many doors for me to grow and succeed, and all of the little and big things you have taught me along the way. I hope I make you as proud as you make me.

To my dad for all your efforts, love, and support, I cannot say enough thanks to you in a lifetime.

To my siblings: Chaima and Abd Aljalil Thank you for loving me unconditionally and supporting me always

To my friends: thank you for the care, love, and support you have given me throughout my studies.

To my supervisor, without your constant support, this day could not be possible.

Thank you

To my promo it was so nice to meet you all Thank you for all the memories have made

***Aya***

## Summary

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### *Abstract*

This study investigated the long-term effects of atmospheric pollution on two Mediterranean forest tree species, *Pinus halepensis* (Aleppo pine) and *Cedrus atlantica* (Atlas cedar), by comparing trees growing in urban zones of Khenchela City where particulate matter (PM<sub>0.3</sub>, PM<sub>2.5</sub>, PM<sub>10</sub>) levels are elevated with nearby natural forest stands. Leaf samples were analyzed for chlorophyll a and b concentrations and free proline content, while spatially resolved PM measurements identified pollution gradients. In Aleppo pine, urban trees exhibited higher chlorophyll b and total chlorophyll likely a compensatory response to light-quality changes or nitrogen deposition alongside marked proline accumulation ( $\approx 32 \mu\text{g/g}$ ) indicative of osmoprotective stress responses. Forest pines showed minimal proline ( $< 10 \mu\text{g/g}$ ) and lower chlorophyll adjustments. By contrast, Atlas cedar displayed reduced total chlorophyll in urban sites (down to  $\sim 24 \mu\text{g/mL}$ ) compared to forest sites ( $\sim 36\text{--}37 \mu\text{g/mL}$ ), suggesting pollutant-induced impairment of photosynthesis; urban cedars also accumulated more proline (11–14  $\mu\text{g/g}$ ). These species-specific physiological profiles reveal that *P. halepensis* can maintain or augment pigment synthesis under moderate pollution, whereas *C. atlantica* is more sensitive. The findings highlight the importance of long-term field assessments trees integrate chronic pollutant exposures over decades and provide practical guidance for urban forestry: Aleppo pine may be better suited for polluted environments, while Atlas cedar requires protective measures.

**Key words;** Atmospheric, Pollution ,*Pinus halepensis* (Aleppo Pine) ,*Cedrus atlantica* (Atlas Cedar), Chlorophyll a and b, Proline Accumulation, Long-Term Ecophysiological Effects

## Résumé

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### *Résumé*

Cette étude a évalué les effets à long terme de la pollution atmosphérique sur deux espèces forestières méditerranéennes—*Pinus halepensis* (pin d'Alep) et *Cedrus atlantica* (cèdre de l'Atlas) en comparant des arbres situés dans des zones urbaines de la ville de Khenchela (où les concentrations de particules fines  $PM_{0.3}$ ,  $PM_{2.5}$  et  $PM_{10}$  sont élevées) à des peuplements forestiers naturels voisins (charges polluantes faibles). Des échantillons foliaires ont été analysés pour leurs concentrations en chlorophylles a et b et leur teneur en proline libre, tandis que des mesures spatialisées de particules ont défini les gradients de pollution. Chez le pin d'Alep, les arbres urbains présentaient une augmentation du taux de chlorophylle b et de la chlorophylle totale probablement un ajustement compensatoire aux modifications de la qualité lumineuse ou à l'apport azoté et une accumulation marquée de proline ( $\approx 32 \mu\text{g/g}$ ), témoin d'une réponse osmotique au stress. En forêt, le pin d'Alep montrait une proline faible ( $< 10 \mu\text{g/g}$ ) et des ajustements chlorophylliens moindres. En revanche, le cèdre de l'Atlas présentait une chlorophylle totale réduite en milieu urbain ( $\approx 24 \mu\text{g/mL}$ ) comparée à la forêt ( $\approx 36\text{--}37 \mu\text{g/mL}$ ), ce qui indique un affaiblissement de la photosynthèse lié à la pollution ; les cèdres urbains accumulaient également plus de proline ( $11\text{--}14 \mu\text{g/g}$ ). Ces profils physiologiques spécifiques soulignent que *P. halepensis* peut entretenir ou accroître la synthèse pigmentaire sous pollution modérée, alors que *C. atlantica* est plus sensible. Les résultats démontrent l'importance des évaluations de terrain à long terme les arbres intègrent la pollution sur plusieurs décennies et fournissent des recommandations pour la sylviculture urbaine : le pin d'Alep est mieux adapté aux milieux pollués, tandis que le cèdre de l'Atlas nécessite des mesures de protection.

**Mots cle :** Pollution Atmosphérique, Matière, *Pinus halepensis* (Pin d'Alep), *Cedrus atlantica* (Cèdre de l'Atlas), Chlorophylle a et b, Accumulation de Proline, Effets Écophysiologicals à Long Terme

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### ملخص

درس هذا البحث التأثيرات طويلة الأمد للتلوث الجوي على نوعين من الأشجار الغابية المتوسطة: صنوبر حليبي (*Pinus halepensis*) وأرز الأطلس (*Cedrus atlantica*)، من خلال مقارنة أشجار تنمو في مناطق سكنية حضرية بمدينة خنشلة، حيث ترتفع تركيزات الجسيمات العالقة  $PM_{0.3}$  و  $PM_{2.5}$  و  $PM_{10}$  بحثيات إلى مواقع غابية طبيعية مجاورة ذات تلوث منخفض. أُخذت عينات ورقية لقياس تراكيز الكلوروفيل a و b، ومحتوى البرولين الحر، بينما حُدثت التدرجات المكانية للجسيمات عبر أجهزة قياس مباشرة. في صنوبر حليبي المناطق الحضرية، وُجد ارتفاع ملموس في الكلوروفيل b والكلوروفيل الكلي، وهو تكيف محتمل تعويضي نتيجة تغير نوعية الضوء أو زيادة الإخصاب النيتروجيني الثقيل، إلى جانب تراكم عالي للبرولين ( $\approx 32$  ميكروجرام/غرام) دلالة على استجابات الأسمولية للضغط التأكسدي. في مواقع الغابات الطبيعية، بقي البرولين أقل من 10 ميكروجرام/غرام مع تغييرات قليلة في تراكيز الكلوروفيل. أما أرز الأطلس، فلوحظ انخفاض واضح في إجمالي الكلوروفيل في الشجرة الحضرية ( $\sim 24$  ميكروجرام/مل) مقارنة بالغابة ( $\sim 36-37$  ميكروجرام/مل)، مما يشير إلى ضعف محتمل في آلية التركيب الضوئي بفعل الملوثات؛ كما زاد تراكم البرولين إلى 11-14 ميكروجرام/غرام في المواقع الحضرية. تُبين هذه النتائج اختلافًا بين النوعين، حيث يستطيع صنوبر الحليبي الحفاظ على أو زيادة إنتاج الصبغات تحت مستوى تلوث معتدل، بينما يظل أرز الأطلس أكثر حساسية. تُبرز الدراسة أهمية تقييمات الميدان طويلة الأمد، إذ تدمج الأشجار المعرض المزمّن للتلوث عبر عقود، وتقدم إرشادات عملية للغابات الحضرية: يُعتبر صنوبر الحليبي أكثر ملاءمة للبيئات الملوثة، في حين يحتاج أرز الأطلس إلى إجراءات حماية خاصة.

الكلمات المفتاحية: التلوث الجوي، صنوبر حليبي (*Pinus halepensis*)، أرز الأطلس (*Cedrus atlantica*)، الكلوروفيل أ و ب، تراكم البرولين، التأثيرات الفيزيولوجية على المدى الطويل

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## **List of abbreviations**

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### **List of abbreviations**

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# *Introduction*

# Introduction

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## 1. General introduction

Atmospheric pollution represents one of the most pervasive environmental challenges of our time, arising from both natural processes (e.g., wildfires, volcanic eruptions, dust storms) and anthropogenic activities such as fossil-fuel combustion, industrial emissions, and vehicular exhaust (**Onivefu & Imarhiagbe, 2024; Sonwani *et al.*, 2022**). Gaseous compounds—sulfur dioxide (SO<sub>2</sub>), nitrogen oxides (NO<sub>x</sub>), ground-level ozone (O<sub>3</sub>)—alongside particulate matter (PM<sub>2.5</sub>, PM<sub>10</sub>, PM<sub>0.3</sub>), heavy metals, and emerging organic contaminants not only degrade air quality but also deposit on vegetation, soil, and water bodies, thereby affecting complex biogeochemical cycles (Agrawal & Agrawal, 2023; Nakazato *et al.*, 2021). In forested regions, long-lived tree species serve as continuous recorders of ambient air quality: their tissues accumulate pollutants over months and years, allowing researchers to detect gradual shifts in ecological conditions that shorter-lived organisms cannot capture (**Gong *et al.*, 2021; Odubo & Kosoe, 2024**).

Forest tree species fulfill fundamental ecological roles: their extensive root systems stabilize soil and reduce erosion; their canopies intercept precipitation and regulate local hydrological cycles; they provide critical habitat and trophic resources for animal and microbial communities; and—through photosynthesis and carbon sequestration—they mitigate climate change (Bytnerowicz *et al.*, 2001; Nowak *et al.*, 2006). As primary producers and structural elements of forest landscapes, trees influence microclimates, nutrient cycling, and biodiversity, creating feedbacks that maintain ecosystem function and resilience (Phillips, 2018; Mulenga *et al.*, 2020). Yet, because trees are essentially sessile and can live for decades or even centuries, they remain continually exposed to atmospheric stressors—often at sublethal levels—so subtle physiological and biochemical alterations may accumulate over time (**Michał *et al.*, 2015; Likus-Cieślik *et al.*, 2020**).

Studying the long-term effects of pollution on forest tree health is crucial for several reasons. First, trees integrate multiple pollutant exposures over extended periods—episodic high-level events and chronic low-level background loads both leave imprints in anatomical features (such as annual ring width), leaf biochemistry (chlorophyll concentrations), and stress-related metabolites (proline, malondialdehyde) (**Cape, 2008; Jyethi, 2016**). This temporal integration provides insights into cumulative damage and delayed responses that short-term experiments often overlook. Second, forest trees sustain ecosystem services directly tied to

## Introduction

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human well-being—water purification, timber and non-timber forest products, carbon storage, and recreational values—so declines in tree health can cascade into economic and societal impacts (Paoletti & Grulke, 2010; Zhang *et al.*, 2019). Third, because Mediterranean forests often span urban–rural gradients, assessing how pollution gradients affect tree physiology can inform land-use planning and forest-management strategies aimed at maximizing both biodiversity and human health (Fagorite *et al.*, 2021; Villarejo, 2022). Finally, many forest tree species exhibit species-specific sensitivities to particular pollutants; shifts in chlorophyll content, proline accumulation, or radial growth can serve as early warning signals of ecosystem stress (Madruga & Patier, 2006; Mulenga *et al.*, 2020).

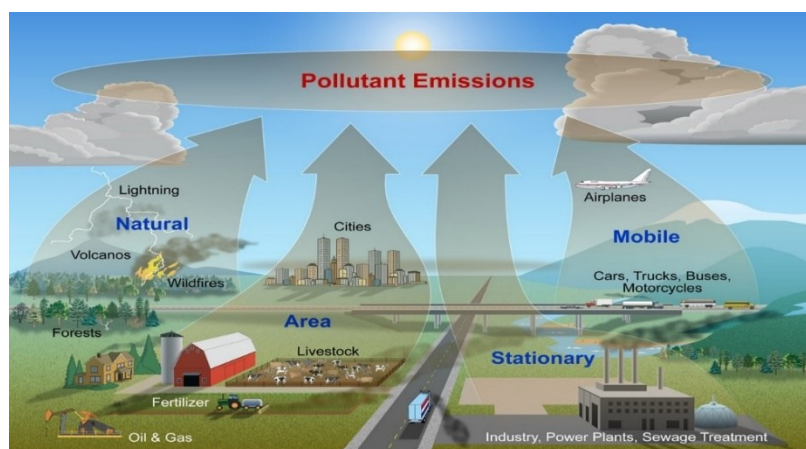
In this study, Aleppo pine (*Pinus halepensis*) and Atlas cedar (*Cedrus atlantica*)—two ecologically and economically significant Mediterranean forest species—were selected to evaluate physiological responses to differing atmospheric pollution levels. Sampling took place in urban residential zones of Khenchela City (characterized by elevated PM<sub>0.3</sub>, PM<sub>2.5</sub>, and PM<sub>10</sub> loads) and nearby natural forest stands (comparatively low pollutant burdens). By measuring leaf chlorophyll (a and b) and free proline content alongside spatially resolved particulate matter concentrations, this work aims to link chronic air-quality variation with subtle shifts in tree physiology (Monneveux & Nemmar, 1986; Felzer *et al.*, 2007). Because both species are perennial and can persist for many decades, they provide an ideal framework to observe gradual ecological changes and to understand how long-term pollutant exposure alters primary productivity, stress-response mechanisms, and potentially reproductive capacity (Mulenga *et al.*, 2020; Nakazato *et al.*, 2021).

*Chapter I : Literature*  
*Review*

### 1. Types of atmospheric pollutants

Air pollution is a significant environmental and public health issue worldwide, with diverse sources and complex effects on human health, ecosystems, and the climate (**Onivefu and Imarhiagbe, 2024**). Atmospheric pollutants adversely affecting forest tree species can be categorized into distinct classes. Traditional pollutants, such as sulfur dioxide, nitrogen oxides, ozone, and acid deposition, have long been recognized as harmful to forests and trees (**Sonwani et al., 2022**). Recently, attention has shifted to other air pollutants, especially those resulting from anthropogenic emissions. These include airborne particulates, volatile organic compounds (VOCs), heavy metals, and organic pollutants that deposit on tree foliage (**Agrawal and Agrawal, 2023**). Each pollution group varies in sources, chemical properties, and atmospheric behavior. Generally, the overall presence of air pollution can compound its effects on forest species rather than just the presence of a single pollutant. Therefore, it's crucial to define chemicals based on their atmospheric occurrence rather than their specific tree effects; highlighting the connection to atmospheric processes impacting forest health (**Nakazato et al., 2021**).

The criteria for 'air pollution' are based on the biophysical characteristics of air quality, rather than on the specific physiological responses of trees to individual **pollutants** (**Gong et al., 2021; Sonwani et al., 2022**). This definition also encompasses secondary pollutants formed in the troposphere, as well as the major photochemical processes that generate atmospheric oxidants. The vulnerability of essential, long-lived plant structures is taken into account, since trees due to their longevity can exhibit delayed and cumulative responses to environmental changes (**Cape, 2008; Nakazato et al., 2021**).



**Figure 1.** Types of pollution sources (<https://www.nps.gov/subjects/air/sources.htm>)

### 1.1. Air pollutants

Air pollution is commonly defined as the presence of a wide range of impurities and pollutants in the atmosphere, in quantities sufficient to cause harmful effects on human health and damage to materials and vegetation. It is also understood that air pollutants do not respect political borders, with different regions being affected indirectly by emissions originating elsewhere (**Odubo and Kosoe, 2024**). Among airborne pollutants, various substances of natural or anthropogenic origin harm human health and natural ecosystems, particularly by impacting vegetation. Key air pollutants harmful to forests include sulphur dioxide, nitrogen oxides, ammonia, and particulate matter. Also elevated ozone concentrations in the lower atmosphere raise concerns due to their adverse effects on plant physiology (**Fagorite et al., 2021**). Atmospheric pollutants are classified by state into gaseous and particulate forms. Gaseous pollutants include CO<sub>2</sub>, NO<sub>x</sub>, SO<sub>2</sub>, O<sub>3</sub>, and VOCs, mainly from combustion and industrial sources. Particulate pollutants, such as dust, soot, aerosols, PM 2.5, and PM 10, originate from both natural and human activities and pose serious health risks (**Phillips, 2018; Daramola et Omokpariola, 2025**).

#### 1.1.1. Nitrogen oxides (NO<sub>x</sub>)

Nitrogen oxides (NO<sub>x</sub>) are significant atmospheric pollutants with crucial implications for air quality and human health (Villarejo, 2022). NO<sub>x</sub> chemistry varies between day and night, influencing their impact on air quality, particularly in urban areas (Madruga et Patier, 2006). Nitrogen oxides are significant atmospheric pollutants affecting forest ecosystems, with impacts varying based on concentration and form (Bytnerowicz et al., 2001). In urban areas, NO<sub>x</sub> contributes to photochemical smog formation, with nitric acid (HNO<sub>3</sub>) being particularly harmful due to its high deposition velocity and direct assimilation by plants. NO<sub>x</sub> affects trees both directly, through stomatal uptake, and indirectly, via soil acidification and eutrophication (**Michal et al., 2015**).

#### 1.1.2. Sulphur oxides (SO<sub>x</sub>)

Sulphur oxides (SO<sub>x</sub>) are a significant air pollutant that adversely affects plants and trees. It inhibits photosynthesis, promotes excessive water loss, and reduces plant growth. SO<sub>2</sub> can cause both acute and chronic damage, depending on exposure duration and intensity (Jyethi, 2016). Trees are particularly vulnerable to SO<sub>2</sub> effects, even before visible symptoms appear.

SO<sub>2</sub> exposure can lead to reduced growth, increased susceptibility to biotic and abiotic stressors, and metabolic changes in trees (Mulenga et al., 2020). Conifers are especially sensitive to SO<sub>2</sub>, which causes chlorophyll reduction. SO<sub>2</sub> pollution also contributes to acid rain, which leaches nutrients from plant canopies and soil. At the ecosystem level, SO<sub>x</sub> can alter species composition by eliminating sensitive species, affecting primary productivity and trophic relationships (Likus-Cieřlik et al., 2020).

### **1.1.3. Ozone (O<sub>3</sub>)**

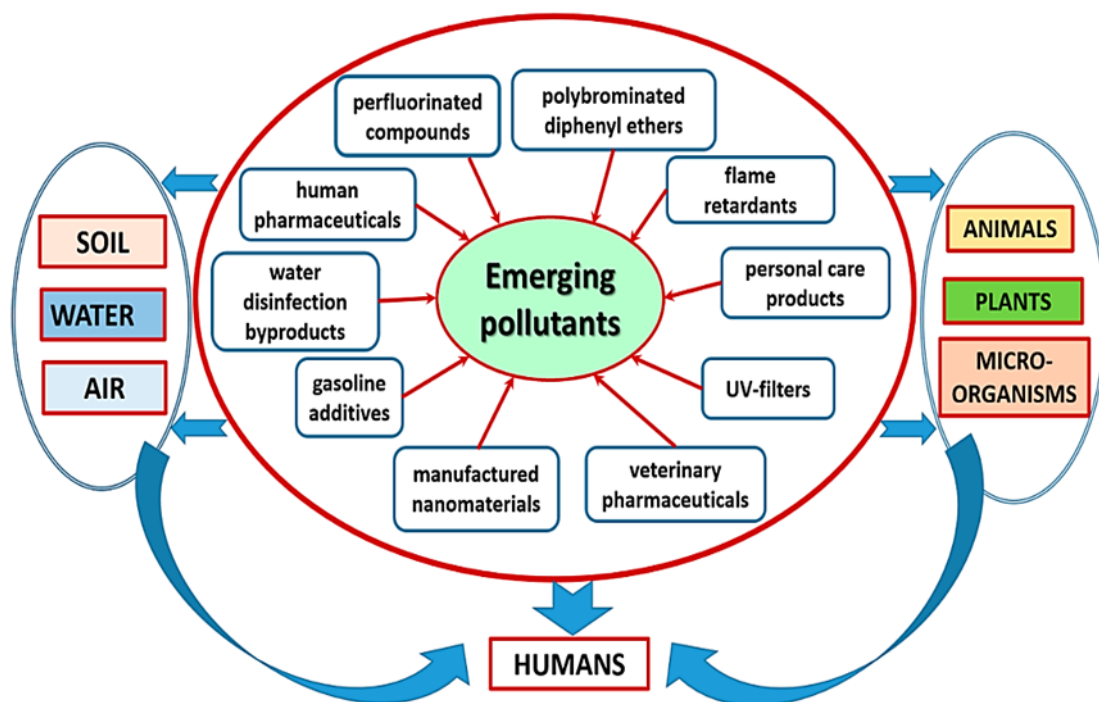
Ozone is an air pollutant formed through photochemical reactions, is considered one of the most significant threats to forest ecosystems and plant health (Nowak et al., 2006). Ozone formation in the troposphere results from chemical reactions between primary pollutants, including nitrogen oxides (NO<sub>x</sub>) and volatile organic compounds (VOCs), in the presence of sunlight (Zhang et al., 2019). Ozone exposure negatively impacts physiological processes such as photosynthesis, respiration, and stomatal conductance, resulting in reduced growth and wood. Studies have shown that even moderate levels of ozone can significantly reduce net photosynthesis in both tree and crop species (Felzer et al., 2007). Species with higher stomatal conductance tend to be more susceptible to ozone damage (Paoletti et Grulke, 2010).

### **1.1.4. Particulate matter (PM)**

PM consists of extremely small particles and liquid droplets from various natural and human sources, classified by size into coarse (2.5-10 μm) and fine (<2.5 μm) particles (Thompson, 2018). Particulate matter (PM) pollution significantly impacts plants, affecting their morphology, physiology, and biochemistry. Studies show that plants in polluted urban areas accumulate more PM on their foliage, leading to reduced photosynthetic efficiency and altered stomatal behavior (Popek et al., 2018). PM can cover leaf surfaces, impeding light penetration and stomatal function, thereby decreasing photosynthesis rates (Gheorghe et Ion, 2011). Research indicates that PM pollution can affect leaf area, stomatal structure, growth, and reproduction in plants. Specific tree species exhibit varying responses to PM exposure, with some showing significant changes in stomatal density and width (Rai, 2016). Vegetation plays a crucial role in atmospheric purification and carbon sequestration; the negative impacts of PM on plants may compromise their ability to provide these ecosystem services (Rai, 2016; Thompson, 2018).

## 1.2. Emerging pollutants (EPs)

Rapid urbanization and industrial growth have introduced a wide array of new and diverse pollutants, which are challenging to detect and characterize due to their volatile nature and complex sources. These pollutants are categorized as emerging pollutants (EPs), and their list continues to expand. They include emerging volatile organic compounds (VOCs) such as chloroform, dichloromethane, ethylene oxides, formaldehyde, toluene and trichloroethylene, as well as heavy metals like arsenic, manganese, and vanadium. Additionally, ultrafine particles, micro- and nano-plastics, engineered nanoparticles, diesel/black carbon, and bioaerosols contribute to this growing list (Enyoh *et al.*, 2020).



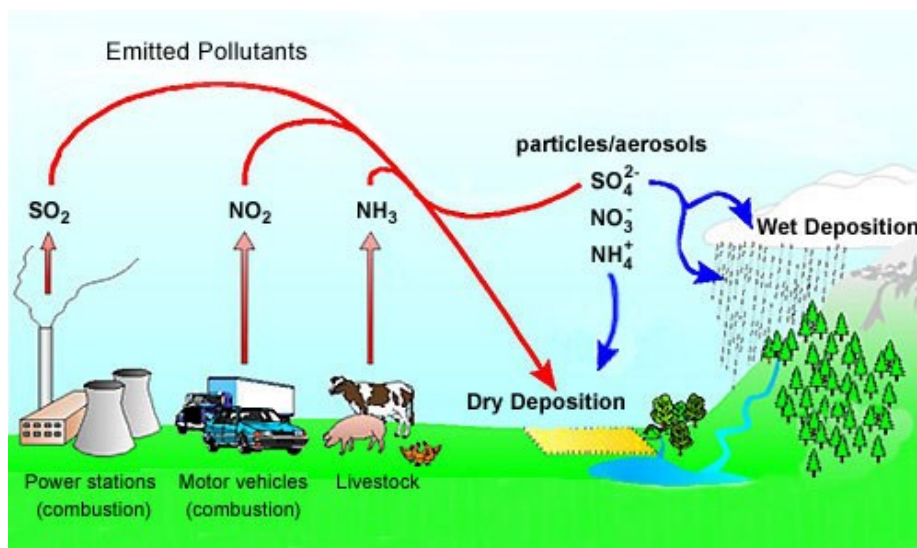
**Figure 2.** Categories of emerging pollutants that impact on soil, air, water, animals, plants, microorganisms and human (Vasilachi *et al.*, 2021).

## 1.3. Sources of atmospheric pollution

Atmospheric pollutants come from different sources, some are natural, such as forest fires, volcanic eruptions, and biotic volatile organic compounds (Seaward and Richardson, 2024). The release of pollutants and pollutants' precursors due to human activities is referred to as anthropogenic pollution. These activities were initially limited to a limited number of substances and sources but, beginning in industrialization, the amount of pollution has increased and diversified in terms of pollutants and impact (de Vries, 2021).

## Chapter I : Literature Review

The main human sources of atmospheric pollution that affect European forests today include the following: emissions from fuel combustion and small-scale industrial processes; vehicle tires and brakes abrasion and the dispersion of exhaust gas; off-road vehicles; forest and urban fires; and long-distance airborne transport of pollutants (**Brodny and Tutak, 2021**). Recent results have emphasized that localized effects of pollution on tree species can vary in intensity and even differ in sign (some species being positively affected) due to the extension and structure of forests and the nature of specific pollution sources. Hence, a more demanding air pollution scenario for forest conservation and management emerges (**Sonwani et al.,2022**). At the same time, the potential of forests for cleaning the atmosphere strengthens the need to adapt pollution policies at different scales and possibly across boundaries (**De et al.,2022**). Such policies should be placed into a larger debate encompassing current city growth, land use changes, and increasing pollution levels (**Skiba et al.,2021**).



**Figure 3.** Air Pollution and Pollution Sources (<https://www.apis.ac.uk/starters-guide-air-pollution-and-pollution-sources>)

Urban air pollution, a complex mixture of gaseous and particulate components, poses significant challenges to public health in cities worldwide. Major sources include industrial activities, vehicular emissions, and domestic energy consumption, which contribute to the release of pollutants such as  $\text{PM}_{2.5}$ ,  $\text{NO}_2$ , and  $\text{O}_3$  (**Akomolafe et al., 2024**). The urban pollution is primarily linked to demographics and is likely to increase with population growth. Mitigation strategies include implementing stringent emission standards, adopting

cleaner technologies, promoting renewable energy, and improving urban planning with efficient public transportation and green spaces (Sarzynski, 2012).

### 2. Physiological and ecological impact on forest tree species

Modern commercial forestry has transformed traditional low-intensity agriculture and forest management. Airborne pollution now adds a new dimension, demanding further knowledge and research (Gong *et al.*, 2021). Globally, forest health is impacted by rising greenhouse gases and ozone levels, while many remote forests suffer from pollutants like heavy metals and organic compounds. Atmosphere-harbored fine combustion particles may also harm these ecosystems (Cape, 2008).

Forest tree species face significant physiological and ecological challenges due to climate change and human activities. Extreme weather events like droughts, floods, and heat waves can lead to massive forest dieback and biodiversity loss (Menezes-Silva *et al.*, 2019). These stressors affect various plant processes, including photosynthesis, respiration, and mineral nutrition (Kozłowski, 2002).

#### 2.1. Effects on Tree Growth and Productivity

Atmospheric pollutants significantly affect the growth and productivity of forest trees by disrupting physiological processes like photosynthesis and respiration. These disruptions can lead to long-term carbon balance issues necessary for growth (Zhang *et al.*, 2022). Growth reductions linked to pollutants have been observed in controlled experiments and natural settings (Hou *et al.*, 2022). A key question is whether these reductions stem from irreversible physiological damage or reversible effects that decrease survival and potential yield. Chronic exposure to elevated ground-level ozone has been shown to reduce biomass increments in young trees due to physiological damage and lower CO<sub>2</sub> assimilation rates (Zhang *et al.*, 2022). Over time, reduced growth rates in mature trees may slow canopy development, with evidence indicating decreased biomass in Scot's pine compared to beech (Zhang *et al.*, 2025). Such growth reductions could alter forest canopy functioning, affecting nutrient cycling, solar radiation interception, and broader ecosystem services. Conifers may also suffer from pollutants that result in leaf discoloration or loss, particularly in low insolation areas (Lukasová *et al.*, 2025). If defoliation occurs over multiple seasons, trees may struggle to recover, putting them at a competitive disadvantage. Species responses to pollution are variable; for instance, chestnut oak showed acute reactions to ozone exposure

in the past, which were less significant in other species (Wang *et al.*,2024).

According (Mehmood *et al.*, 2024), many morphological effects, like chlorosis, necrosis, leaf area, stomatal clogging, plant productivity, leaf falling, and reduction in flower yield, are observed due to the influence of air pollution. Air pollutants also damage the DNA and affect the biochemicals of the plants, as well as pH, relative water content (RWC), simple sugar, total chlorophyll content, proline, and polyamines (Figure 4).

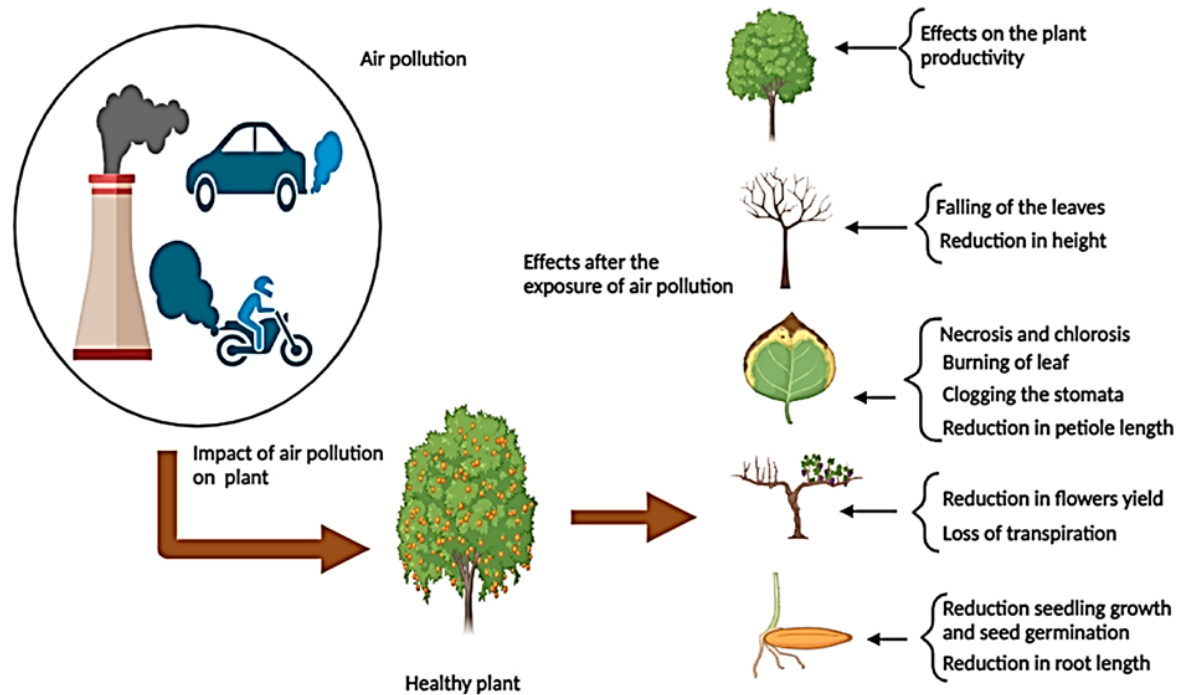


Figure 4. Effects of air pollution on plant morphology (Mehmood *et al.*, 2024).

## 2.2. Altered photosynthetic processes

Photosynthesis is essential for tree health, directly influencing growth and productivity (Mandal *et al.*, 2023). However, atmospheric pollutants can disrupt this process by impairing chlorophyll-a activity and damaging the oxygen-evolving complex through oxidative stress, ultimately reducing overall photosynthetic efficiency (Khan *et al.*, 2022). Certain pollutants specifically interfere with carbon fixation and the conversion of light energy (Figure 6) (Mandal *et al.*, 2023). Gaseous pollutants may cause necrotic spots on leaves, limiting internal CO<sub>2</sub> concentrations, while acidic gases and mist can block the pores of conifer needles, further restricting photosynthesis. Additionally, smog reduces photosynthetic activity by obstructing light penetration, increasing foliar leaching, raising

stomatal resistance to gas exchange, and hindering electron transport processes (Soheili et al., 2023; Mehmood et al., 2024).

Nitrate compounds can undergo photochemical hydrolysis in the atmosphere, and subsequent reactions within leaves may generate nitric acid (HNO<sub>3</sub>), leading to tissue damage. Under high oxidant conditions, deciduous species often outcompete conifers due to the latter's greater sensitivity (Sadhna et al., 2023).

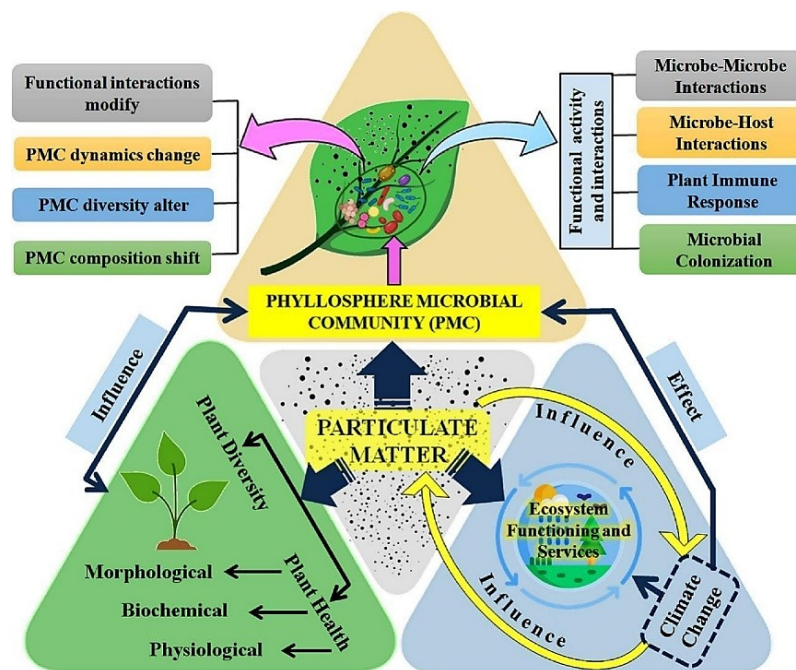


Figure 5. Interactive relations between plants, the phyllosphere microbial community, and particulate matter pollution (Mandal et al.,2023)

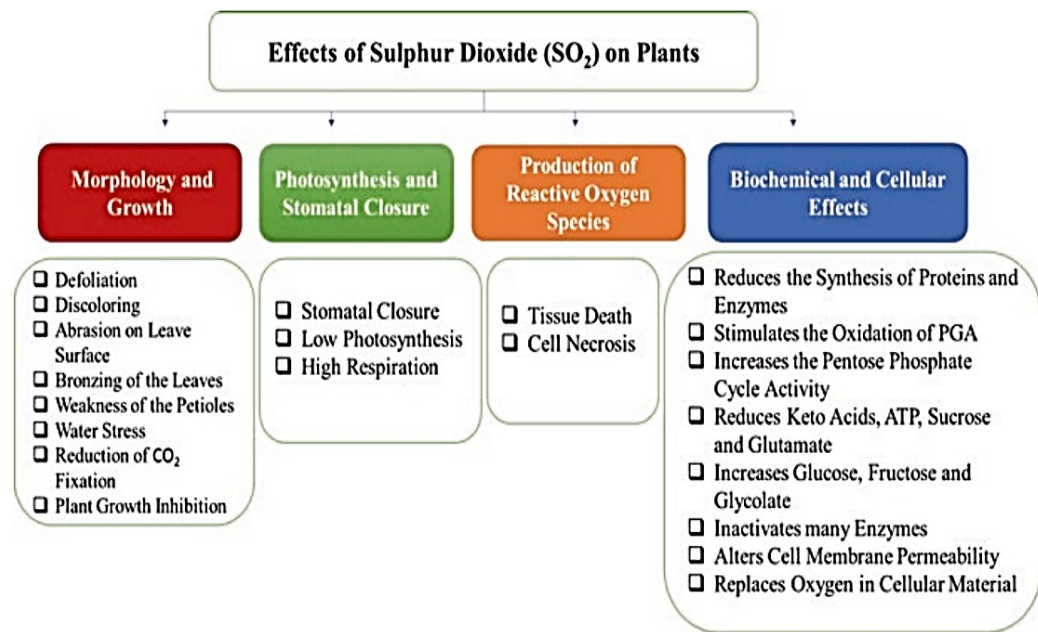


Figure 6. Effect of Sulphur Dioxide (SO<sub>2</sub>) on plants (Khan *et al.*,2022)

### 2.3.Impact on water and nutrient uptake

Anthropogenic air pollution, particularly ozone and nitrogen oxides, affects soil chemistry, impacting tree growth in both rural and urban areas. This pollution leads to problematic soil conditions, potentially altering trees' root-absorption capabilities. Understanding nutrient acquisition processes remains fragmented, revealing complex soil-root relationships in tree species. Soil pollutants, including heavy metals and organic contaminants, further hinder nutrient absorption (De *et al.*,2022).

This text discusses the effects of atmospheric pollutants on water and nutrient uptake in deciduous trees in polluted environments like forests (Pereira *et al.*,2021). Deciduous trees rely heavily on mycorrhizal partnerships for nutrient uptake and require suitable soil for seedling growth, compromising natural soil health. Recent studies indicate that root damage from pollution can stifle growth, comparing two investigations on trees in open settings versus those in organic cultures. (Rather *et al.*,2022) (Giltrap *et al.*,2021) Findings suggest pollutants may impair water availability at the roots, raising broader ecological concerns for forests, including effects on biodiversity and resilience to stresses. Leaf damage from pollution could worsen during drought, complicating ecological relationships as it affects the tree's water exchange properties. (N. Cape, 2008;Sonwani *et al.*,2022)

### 2.4. Tree reproductive health

The impact of atmospheric pollutants on forest ecosystems has typically been evaluated through static structures like tree growth and foliar function. However, the survival and functional status of trees depend on their ability to maintain healthy populations through various stochastic processes, with reproduction being crucial due to its unique genetic and demographic outcomes. (Singh *et al.*,2023) Consequently, tree reproductive health is assessed concerning atmospheric pollutant effects. Natural tree health encompasses variables such as the quantity of sexual organs and the success of key reproductive processes. (Sawarkar *et al.*,2023; Richard *et al.*,2024)

Numerous examples show that atmospheric pollutants can significantly impact these aspects, decreasing trees' capacity to regenerate and causing effects that manifest more quickly than traditionally evaluated. Reduced fertility leads to decreased maternal stocks for forest dynamics and more vulnerable population structures, resulting in higher edge effects and negative population feedbacks among conspecifics (Plutino *et al.*,2022) This is often linked to early phytopathological infections, which can reduce crown complexity and aeration variability of leaves, critical for ecosystem resilience. (Goswami *et al.*,2022) This suggests that species with specialized reproductive strategies, such as masting or disturbance-triggered flowering, may be particularly vulnerable to atmospheric pollutants. Thus, pollution can exacerbate factors like biotic invasions and climate change, leading to a decline in forest diversity and functionality and highlighting the necessity of researching air pollution's impact on biodiversity conservation. (Agrawal and Agrawal,2023)

### 2.5. Short-term and Long-term Effects

Air pollution has become a source of international concern as a result of the rapid industrial development and the large-scale burning of fossil fuels during the second half of the 20th century. Acute effects of pollution exposure, in general studied by monitoring programs, include immediate physiological responses and visible damage to leaves, needles or bark (Mei *et al.*, 2021 ; Brumberg *et al.*,2021). When damage becomes conspicuous, a large number of studies have already found chronic and detrimental effects on tree species health. These long-term effects have been observed in a variety of forms, from substantial reductions in tree growth, vigour or yield to significant shifts in plant community

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composition. Ultimately, chronic exposure to harmful levels of air pollutants may increase tree mortality rates. (**Joshi *et al.*, 2022 ; Hartmann *et al.*,2022**)

Concerning tree species, such an interaction depends, among other factors, on the pollutant concentration and time of exposure. To begin with, many of these compounds affect trees only when they exceed a given dose threshold. (**Hartmann *et al.*,2022**) A sublethal or moderate concentration of a given air pollutant stimulates the biosynthetic machinery of the exposed organisms, which in turn increases the productivity and growth of the trees or the photosynthates. For instance, trees exposed to low to medium levels of O<sub>3</sub> have been shown to release monoterpenes, which in turn enhance tree resilience to O<sub>3</sub>. (**Chojnacka-Ozga and Ozga, 2021**)

If exposure to harmful substances is above 3 different moderate levels, forests may be partly resilient to further increases of exposure, although decay starts to be apparent. This overall view substantial experimental evidence strongly suggests that high levels of pollutants with low or moderate toxicity will become harmful for exposed forest tree species over time. Clean air acts have been proved to maintain or improve forest health. Nevertheless, and unexpectedly, evidence has also shown that short-term air pollution episodes can be greatly amplified in time and can increase their negative impacts on forest ecosystems in the following 2–3 years if the compounded upon a subsequent similar event. Modern and complex air quality management requires long-term research to follow-up and understand the ecology of atmospheric pollution in order to control it. (**De *et al.*,2022 ; Joshi *et al.*, 2022**)

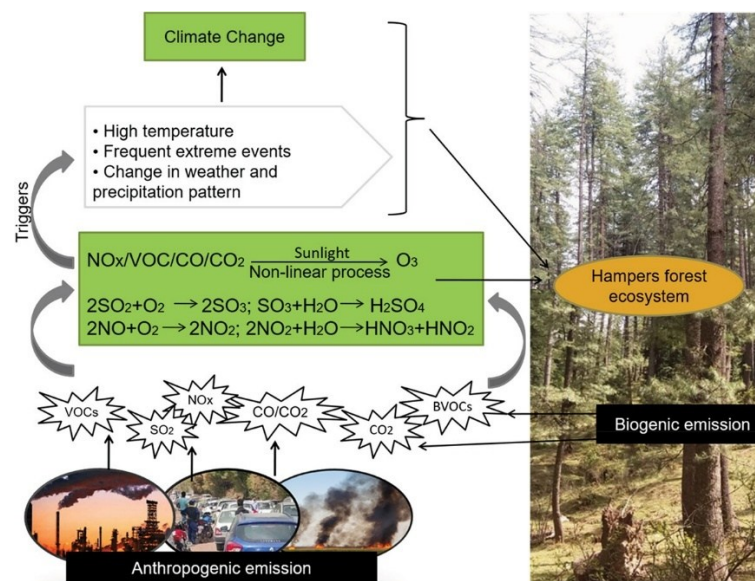
### **2.6.Interaction with climate change**

Atmospheric pollutants and climate change are inextricably linked, but the intricate relationships are not well understood within the context of forest ecosystems. The diverse effects of pollutants on tree health and productivity are likely to interact with the changing climatic conditions that are already becoming apparent. (**Gong *et al.*,2021**) These effects could include some that also exacerbate the impacts of pollutants, such as greater vulnerability to other environmental stresses, and other more complex, indirect effects that alter the feedback loops between forests and climate. (**Mikhail *et al.*,2021; Noel *et al.*, 2021**) For example, stressed trees might influence feedbacks by altering vegetation dynamics – by

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increasing mortality rates, for example – in ways that are different to the response from undamaged systems. (Pacheco *et al.*,2021; Santos *et al.*, 2022)

Current attention on forests and climate is largely focused on the ability of forests to mitigate climate change, with the emphasis on the capacity of vegetation to act as a sink for CO<sub>2</sub> and to thereby reduce net atmospheric CO<sub>2</sub> levels. While the broader environmental impacts of forests and their responses to changing climate are also considered, atmospheric pollutants are rarely part of this discourse. (De *et al.*,2022) This is despite the several potential commonalities with climate change that may make forests affected by pollutants more susceptible to some of the anticipated effects of climate change thus increased the heterogeneity and shifting of both forest and pollutant impacts worldwide. (Diener & Mudu, 2021; Noel *et al.*, 2021) However it is now recognized that for a meaningful understanding and development of policies to counter problems of air pollution and climate change simultaneously; these combined environmental problems will need to be tackled in a highly integrated way. (Artaxo *et al.*,2022 ; Mueller *et al.*,2022)



**Figure 7.** Schematic representation of interlink between air pollution and climate change. (Sonwani *et al.*,2022)

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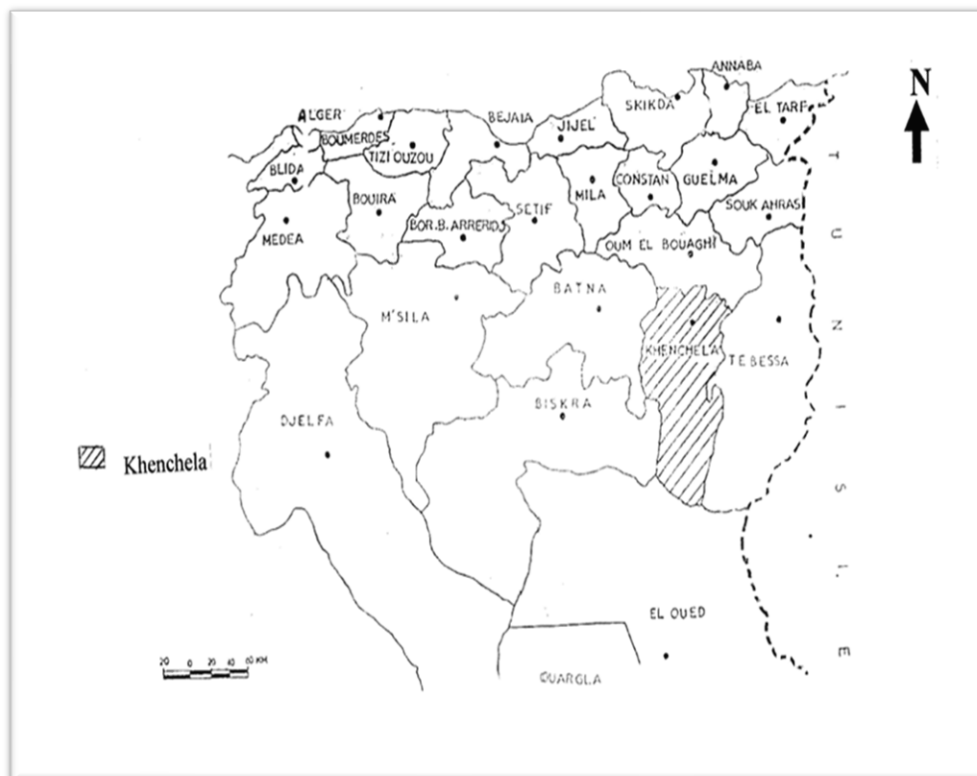
## Chapter II : Methodology

### 1. Study Area Overview

Located in the eastern part of the country, at the foothills of the Aurès Mountains, within the geographical area between 6° 32' and 7° 34' East longitude and between 35° 7' and 35° 38' North latitude, the province of Khenchela is bordered (figure 8):

- To the north, by the province of Oum El Bouaghi
- To the northwest, by the province of Batna
- To the southwest, by the province of Biskra
- To the south, by the province of El Oue
- To the east, by the province of Tébessa

Its territorial extent covers a total area of 9,715 km<sup>2</sup> (OESRP, 2014), it has benefited from a diverse but hostile physical environment and holds one of the highest altitudes in northern Algeria, at 2,326 meters (Chelia) (DPAT, 2012 in Ghedir and Kattoum, 2023).

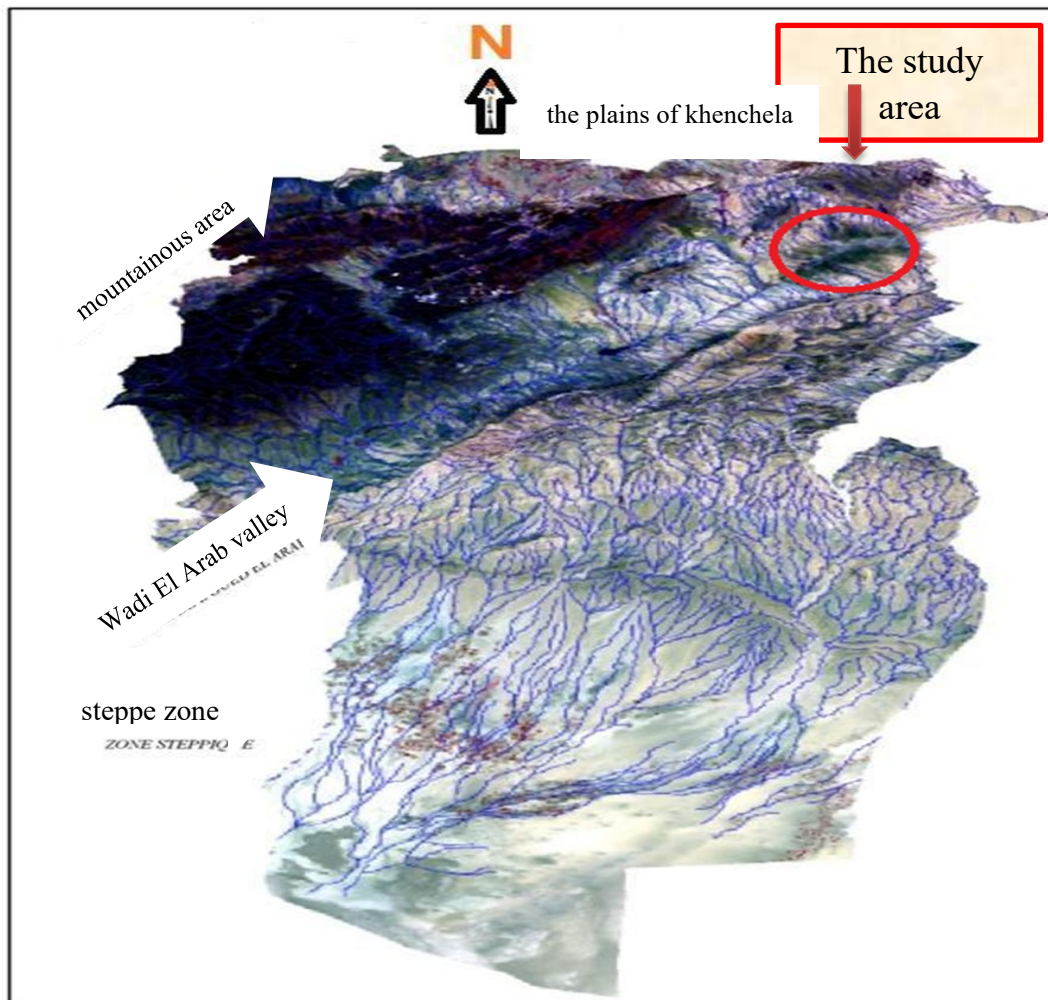


**Figure 8.**Geographical location of wilaya of Khenchela (OESRP,2014)

### 2. Geomorphology and Relief of the Region

The terrain is shaped by altitude and slope, with the latter posing significant challenges for land development, especially in agriculture. Khenchela province features three distinct areas:

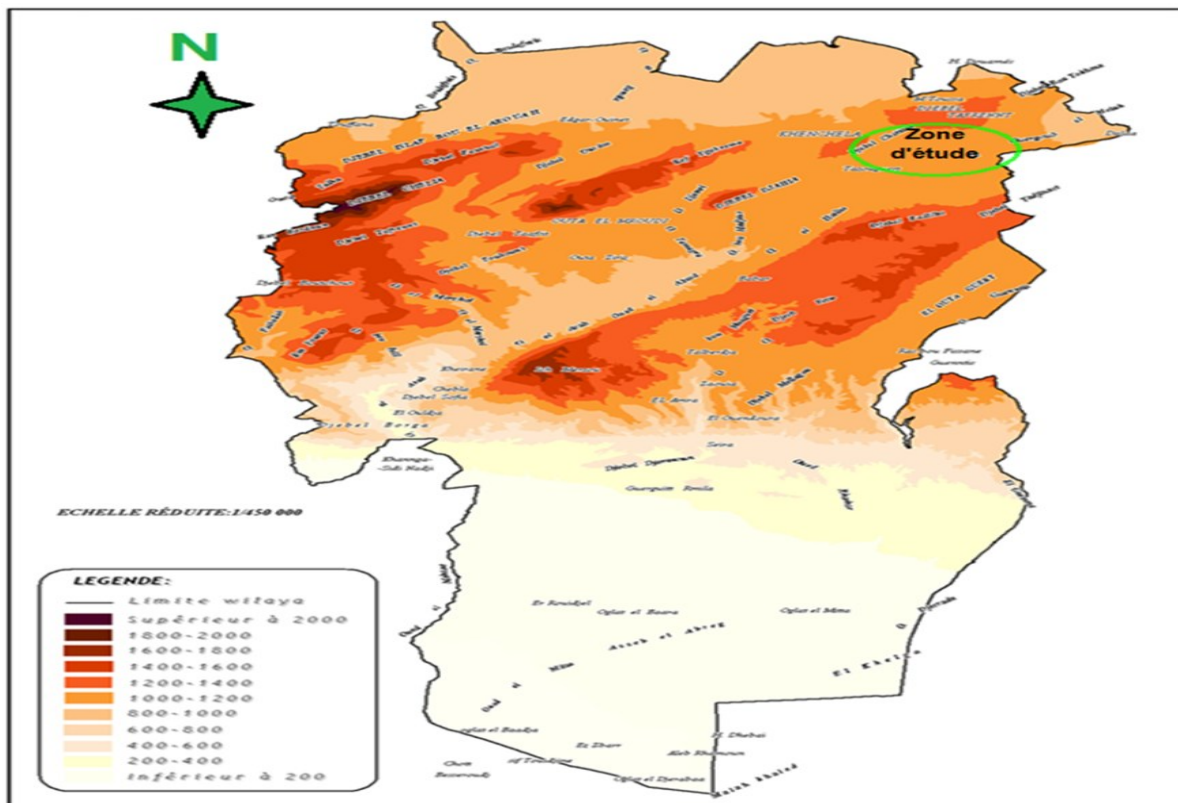
- **Northern High Plains:** Characterized by altitudes between 850 and 900 meters and gentle slopes (less than 3%).
- **Central Mountain Zone:** Includes the Aurès and Néménchas massifs, with Djebel Chélia reaching 2,169 meters, one of the highest peaks in the Algerian Atlas. Altitudes range from 1,000 to 2,169 meters, interspersed with narrow, northeast-trending valleys.
- **Southern Steppe and Presaharian Plains:** Includes areas below sea level (e.g., Oglat El Barra at -26 meters) and features relatively flat topography within the Melghir basin and the Grand Erg Oriental.



**Figure 9.** Digital relief model of Khenchela region (source CENEAP P.A.D.D of the wilaya of Khenchela, 2009).

### 2.1. Elevation

The wilaya of Khenchela is characterized by a very steep drop in altitude. Indeed, the altitude oscillates between minus 26 meters below sea level (Chott Melghir in the south-east of the wilaya) and 2169 meters above sea level (Djebel Chélia) on the Aurès mountains in the north west of the wilaya (**Figure 10**).



**Figure 10.**The altitude map of the Khenchela region (Source CENEAP P.A.D.D of the wilaya of Khenchela, 2009).

### 2.2. Lithological overview

Lithologically, the wilaya of Khenchela presents a multitude of facies, the most represented of which are listed below:

- Alluvial deposits and sands.
- Marls.
- Hard limestones and dolomites.
- Friable limestones.
- Triassic (gypsum).

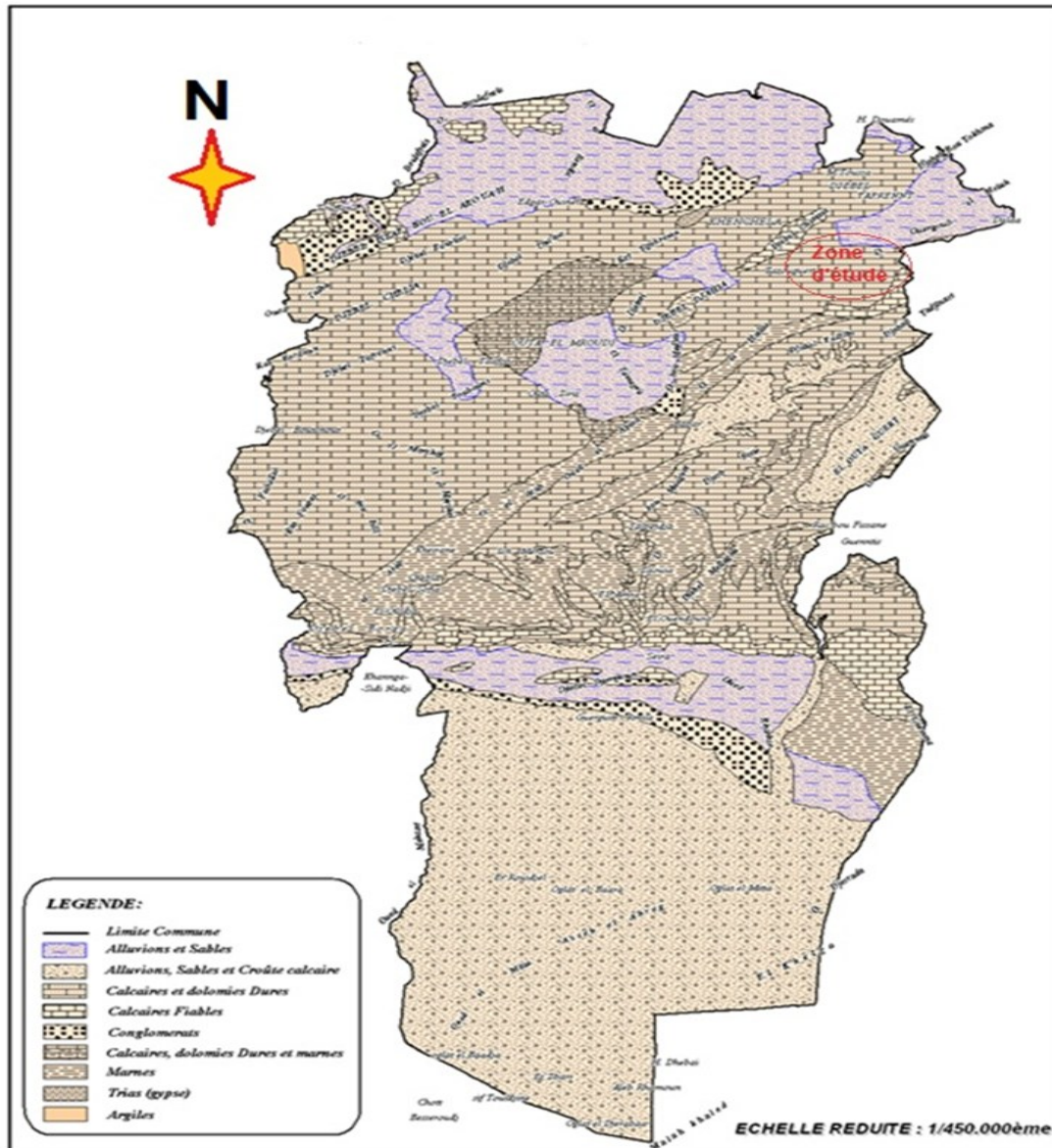


Figure 11. The lithological map of the Khenchela region (OESRP,2014)

### 2.3. Soil overview of the main soils of the wilaya.

Given the diverse morphological, lithological, and climatic characteristics of the territory of the province, a wide range of soils is formed, influenced by vegetation cover. The soil map of Algeria, established by JH DURAND and supplemented by more recent localized studies, provides a general overview of the main soil types encountered in the province. According to the soil map produced by DURAND J. H. in 1954 at a scale of 1:500,000, six soil classes were identified, in addition to the class of raw mineral soils (rock outcrops) and halomorphic soils (saline soils) (Figure 12) (OESRP,2014).

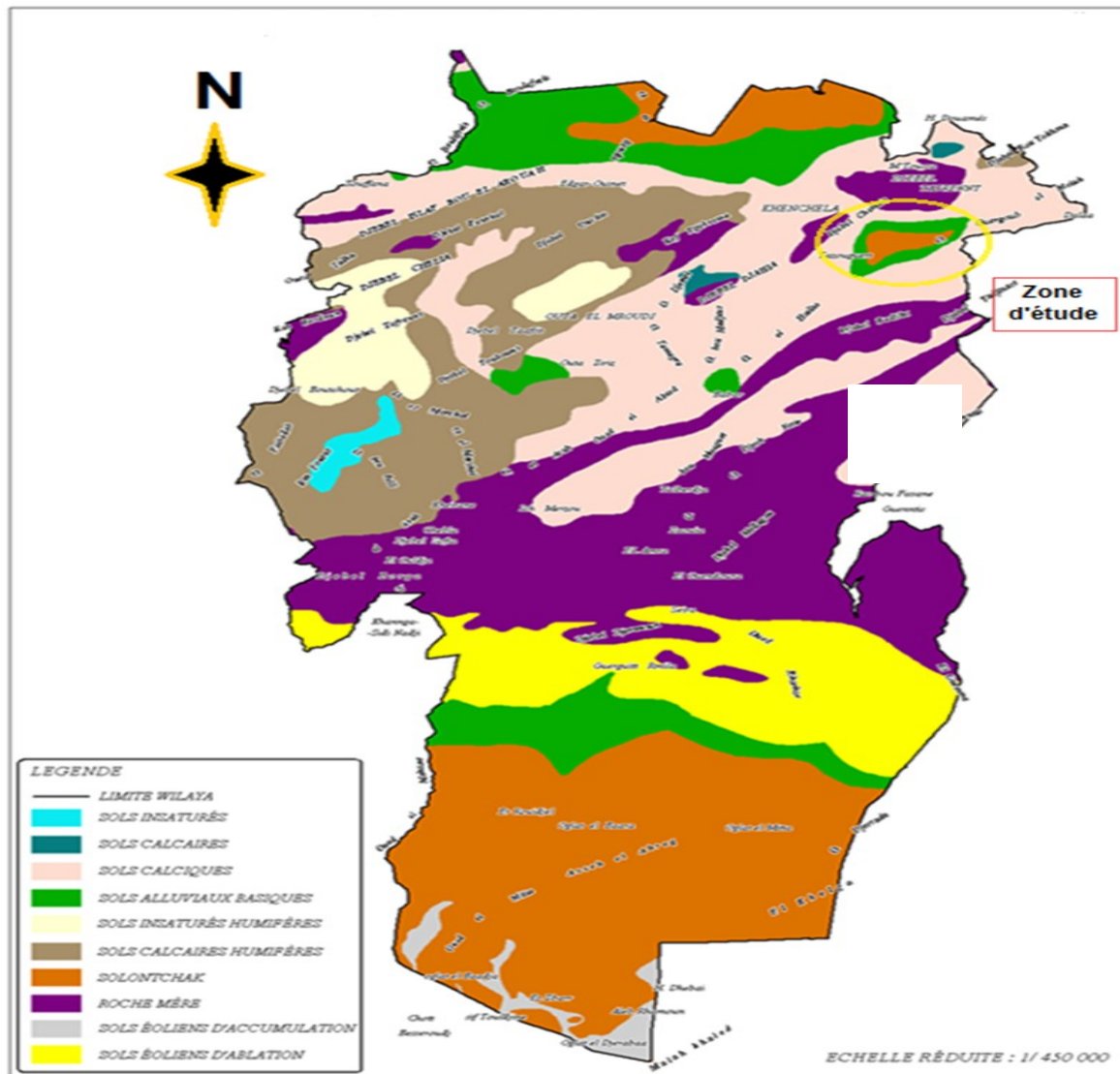


Figure 12. Soil class map of the Khenchela region (OESRP,2014)

### 3. Climate

#### 3.1. The Climate

The Wilaya of Khenchela is distinguished by 3 climates:

- A very harsh winter climate, moderate in summer in the central mountain regions.
- A moderate climate in winter, hot and dry in summer in the southern Sahrawi steppes.
- Very cold climate in winter and dry in summer in the high steppes of the north.

This climatic diversity has given the Wilaya a multiple natural inclination giving significant tourist specificities (Ghedir and Kattoum, 2023).

### 3.2. Temperatures

- The average of all minimums: - 2 C°
- Average maximum: +21.4 C°
- The absolute maximum observed: +42 C°

The absolute maxima observed during the summer season are very high, which causes a high evaporation during this season (**Ghedir and Kattoum,2023**).

### 3.3. Precipitation

It should be noted that data on precipitation, torrential rain, snow, storms, hail, hoar frost and fog are those of SELTZER; for 25 years observation. Outside the North West Mountain region (DJ. Chelia and DJ. Aidel) which receives between 700 and 1200 mm of rain per year and from the south (the Saharan rangelands) which receives less than 200 mm of rain per year (Oued EL Meita) (**Allague et Nedjuo, 2013**).

## 4. Plant cover

### 5. Sampling

The sampling process began on **April 16, 2025**, during which leaves were collected from two tree species: **Aleppo pine (*Pinus halepensis*)** and **Atlas cedar (*Cedrus atlantica*)**. The aim was to evaluate the physiological responses of these trees to atmospheric pollution.

Two ecologically distinct zones were selected:

- An urban residential area, located in the city center of Khenchela.
- A natural forest area, which included the sites of Aïn Mimoun and Bouhmama.

#### 5.1. Urban Area (Khenchela)

Three different urban sites were selected within the city, identified as sites **6, 12, and 14** according to **figure 13**

At each site, leaves ere collected from Aleppo pine trees, with three replicates per site to ensure statistical accuracy and the reliability of the results.

As for Atlas cedar, samples could only be collected from Site 1, due to the absence of this species in the other urban locations (**As indicated in Map figure 6**).

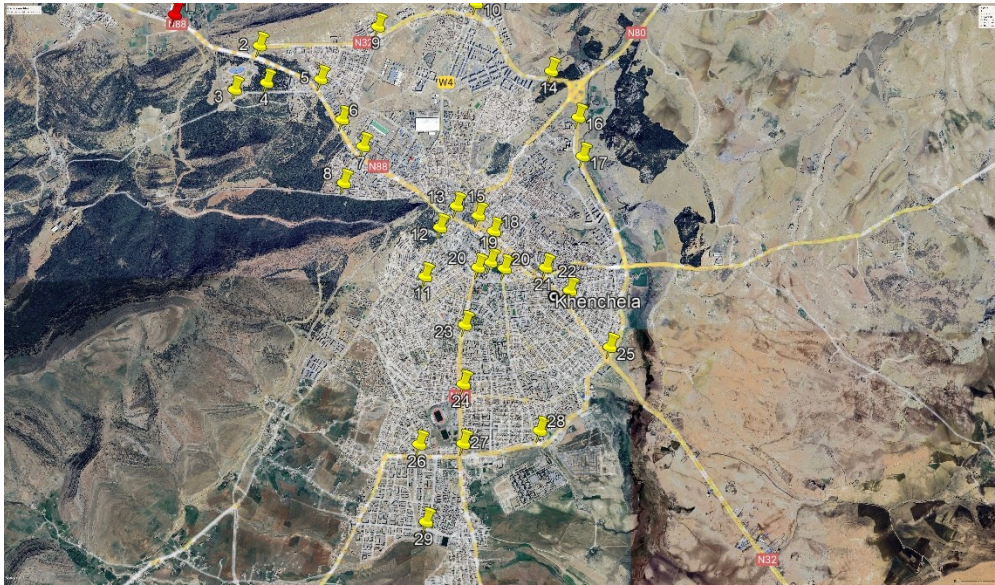


Figure 13. Urban residential area in city of Khenchela

## 5.2. Forest Area

In the forest zone, two major natural sites were selected:

- **Aïn Mimoun** (labeled A on Map 2)
- **Bouhmama** (labeled D on Map 2)

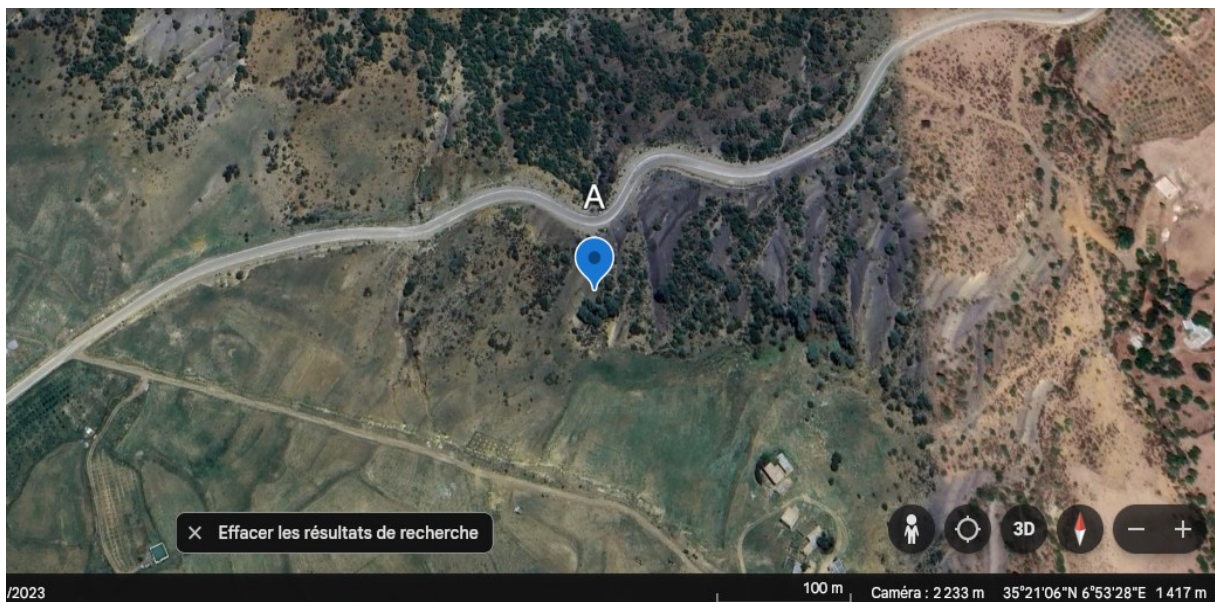
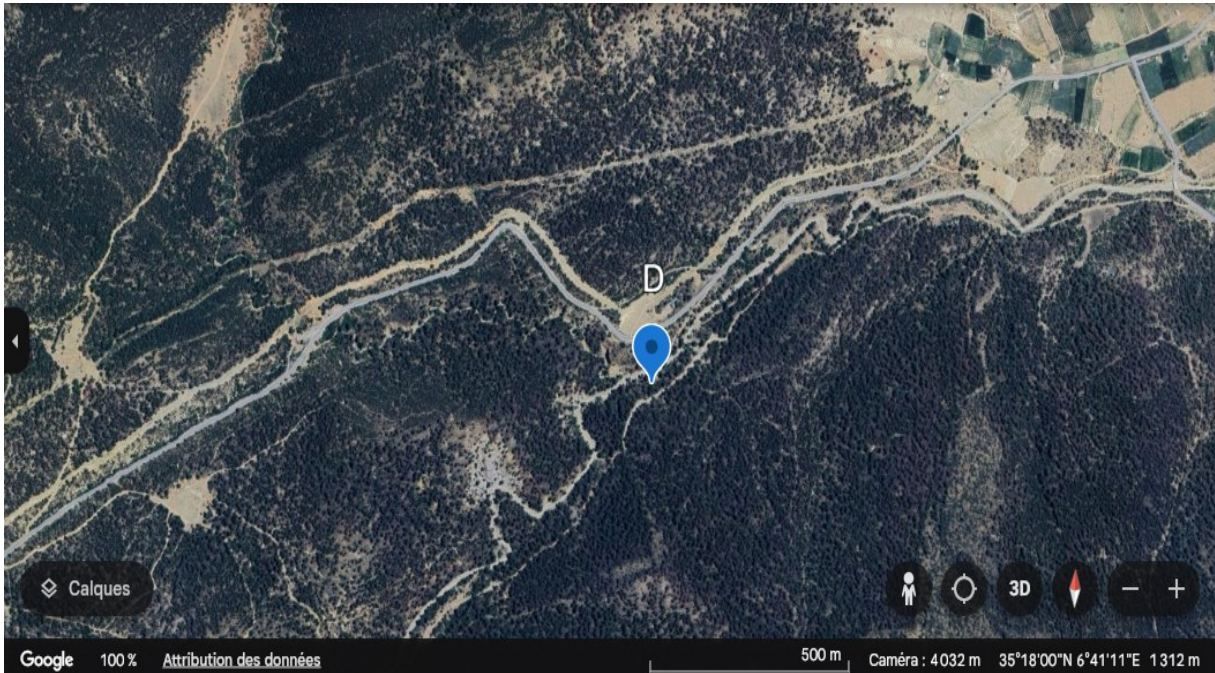


Figure 14. Forest Area of Aïn Mimoun



**Figure 15.** Forest Area of Bouhmama

At each of these sites, two sets of samples were collected for both Aleppo pine and Atlas cedar, with three replicates for each species to ensure adequate statistical representation.

All samples were collected using sterile tools to prevent contamination.

The leaves were placed in numbered plastic bags and stored in a field cooler until they were transported to the laboratory for physiological analyses, particularly the determination of chlorophyll and proline contents

### **6. Measurement of Particulate Matter**

During the months of April and May, we measured the concentrations of fine airborne particulate matter (PM) in the city of Khenchela at 29 different locations distributed across various neighborhoods and areas, as shown in the attached map. The measurements covered three particle sizes: PM<sub>0.3</sub>, PM<sub>2.5</sub>, and PM<sub>10</sub>, using a portable measuring device. The measurements were conducted during three different time intervals throughout the day to capture daily variations in pollutant concentrations: the first in the morning from 07:30 to 08:30, the second from 11:30 to 13:30, and the third in the late afternoon from 16:30 to 17:30. The device was placed horizontally at a height ranging between 1 meter and 1.5 meters above ground level to ensure accurate representation of the atmospheric conditions surrounding plants, as well as to reflect human exposure levels. The collected data will later be used to

compare pollution levels across the studied locations and to analyze their potential impact on local vegetation.



Figure 16. Particulate Matter device

### 7. Determination of Proline Content

Proline content was measured using the method described by Monneveux and Nemmar (1986). A sample of 100 mg was taken from the leaf, cut into small pieces, and placed in a test tube.

1 ml of 80% methanol was added, and the mixture was heated in a water bath at 85°C for one hour (tubes were covered with aluminum foil to prevent alcohol evaporation).

After cooling, 1 ml of the extract was taken and mixed with the following reagents:

- 1 ml glacial acetic acid
- 1 ml of a reaction mixture consisting of: 12 ml distilled water, 30 ml acetic acid, and 8 ml orthophosphoric acid
- 2.4 ml of ninhydrin solution

The mixture was heated in a boiling water bath at 100°C for 30 minutes until the violet color developed.

After cooling, 5 ml of toluene was added, and the solution was vigorously mixed using a Vortex to separate the phases:

- Upper phase: contains the proline

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- Lower phase: does not contain proline
- The upper organic phase was collected, and absorbance was measured at 528 nm.



Figure 17. Some photos of the proline assay steps

### 3. Preparation of the Calibration Curve

A stock solution was first prepared containing 500 mg of proline per 100 ml of distilled water.

Then, serial dilutions were performed as follows:

- Solution 1: 50 ml of stock solution + 50 ml of water → 250 mg/ml
- Solution 2: 50 ml of Solution 1 + 50 ml of water → 125 mg/ml
- Solution 3: 50 ml of Solution 2 + 50 ml of water → 72.5 mg/ml

Each solution was labeled according to its concentration, and absorbance was measured at 528 nm.

- A standard calibration curve was constructed using Excel, where:
- X-axis = Proline concentration (mg/ml)
- Y-axis = Absorbance (DO)



Spectrophotometer



Balance



water bath



Vortex

**Figure 18.** Some devices used in our study

### 8. Determination of Chlorophyll Content

We start with one gram of pre-cut leaves was manually ground in a mortar in the presence of **20 ml of 80% acetone**.

Once the leaves were completely ground, the extract was filtered and kept in the dark in opaque containers to prevent degradation by oxidation. The spectrophotometric assay of chlorophyll. Absorbance readings were taken at two specific wavelengths: **646.6 nm and 663.6 nm**, after calibrating the device with an 80% acetone solution.

When the absorbance value exceeded 0.8, the extracts were diluted with 1 ml of 80% acetone before a new reading.

Chlorophyll concentrations were determined using the following equations:

- **Chlorophyll a ( $\mu\text{g/ml}$ )** =  $12.25 \times \text{DO}_{663.6} - 2.55 \times \text{DO}_{646.6}$
- **Chlorophyll b ( $\mu\text{g/ml}$ )** =  $20.31 \times \text{DO}_{646.6} - 4.91 \times \text{DO}_{663.6}$
- **Total Chlorophyll ( $\mu\text{g/ml}$ )** =  $7.34 \times \text{DO}_{663.6} + 17.76 \times \text{DO}_{646.6}$  .

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**Figure 19.** Some photos of the Chlorophyll Content

***Chapter III: Results and  
discussion***

## **1. Results**

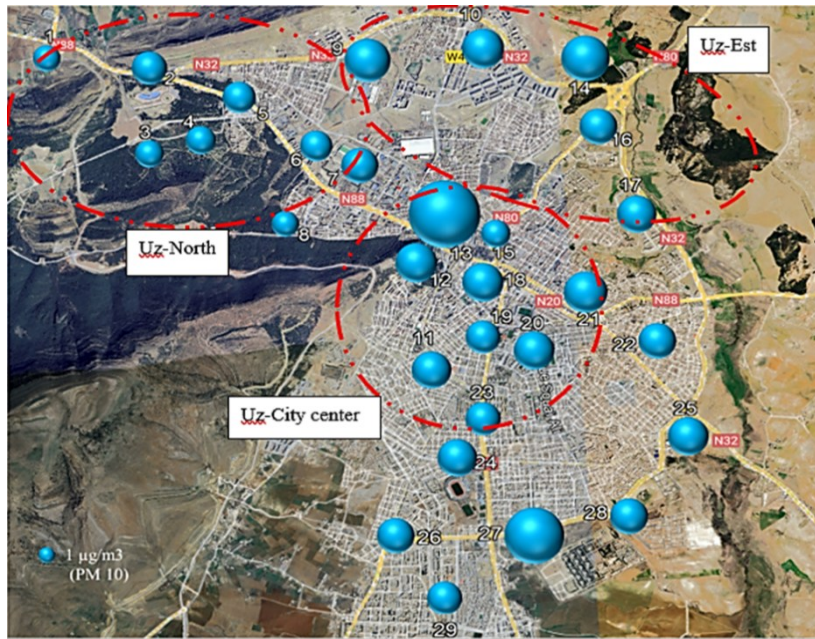
### **1.1. Atmospheric Particulate Matter (PM) Measurements**

#### **1.1.1. Spatial Distribution of PM10**

After measuring particulate matter (PM10, PM2.5, and PM0.3) in the city of Khenchela, we obtained the following results.

The Figure 20 represent the spatial distribution map of PM10 concentrations across Khenchela city's urban zones. The results reveal significant variations in particulate matter pollution levels throughout the metropolitan area. The monitoring network, demonstrates that PM10 concentrations are heterogeneously distributed across the three main urban sectors, reflecting the complex interaction between emission sources, urban morphology, and local meteorological conditions. The city center zone of Khenchela exhibits moderate to high PM10 concentrations, characteristic of dense urban environments where multiple pollution sources converge.

The eastern zone similarly shows heterogeneous pollution patterns, with several monitoring points indicating elevated concentrations but appearing less severe than those observed in the city center. The northern urban zone displays variable PM10 concentrations across multiple monitoring locations but appears less critical than both the city center and eastern zones.



**Figure 20.** Spatial Distribution of PM10 in the Urban Zones of Khenchela City

### 1.1.2. Spatial Distribution of PM2.5

The Figure 21 represents the spatial distribution of particulate matter (PM2.5) concentrations across the urban areas of Khenchela city. The results reveal a heterogeneous and uneven distribution across the three sectors. It appears that the city center of Khenchela has very high PM2.5 concentrations, which may be due to elevated air pollution caused by vehicle emissions and industrial activities. The eastern area also shows high PM2.5 levels, but they seem to be less severe compared to those in the city center. In contrast, the northern area displays low PM2.5 concentrations, which is likely due to fewer pollution sources compared to the eastern and central areas

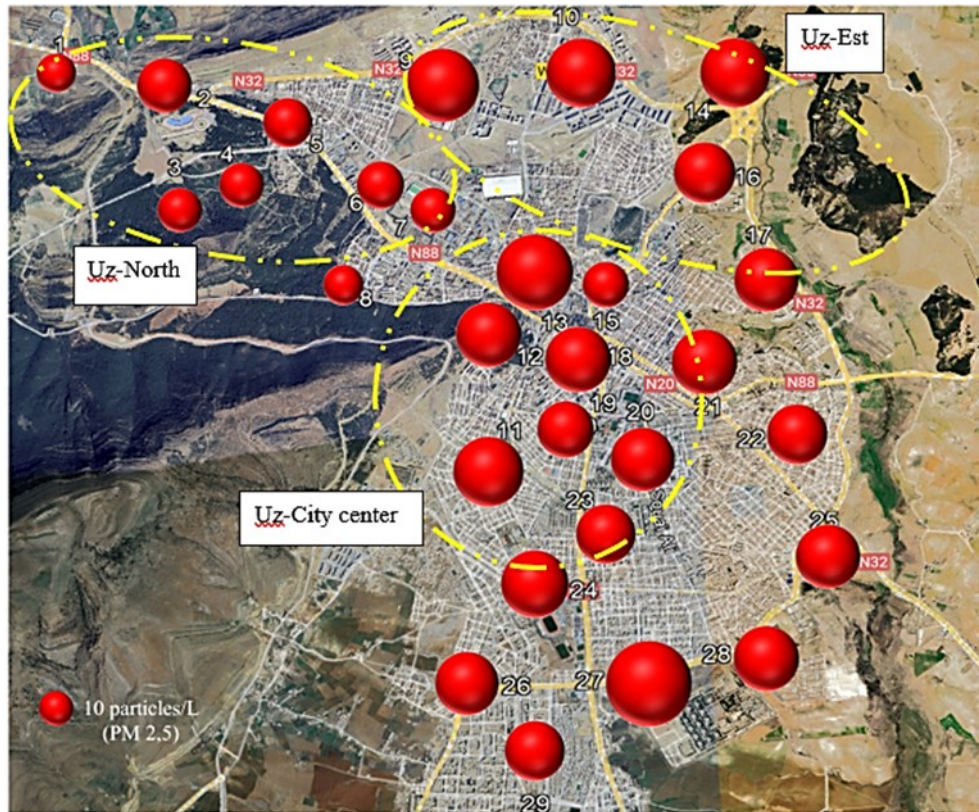


Figure 21. Spatial Distribution of PM<sub>2.5</sub> in the Urban Zones of Khenchela City

### 1.1.3. Spatial Distribution of PM<sub>0.3</sub>

The figure 22 represents the spatial distribution map of PM<sub>0.3</sub> concentrations across the urban areas of Khenchela city. The monitoring network shows that PM<sub>0.3</sub> concentrations are unevenly distributed across the three sectors. The central area of Khenchela displays high to moderate concentrations of PM<sub>0.3</sub>, which is likely due to air pollution caused by vehicle exhaust and traditional heating methods. Similarly, the eastern area shows moderate concentrations of PM<sub>0.3</sub>, though less intense than those observed in the city center. In contrast, the northern urban area records lower concentrations compared to the central and eastern sectors, which may indicate better air quality and healthier atmospheric conditions in that region.

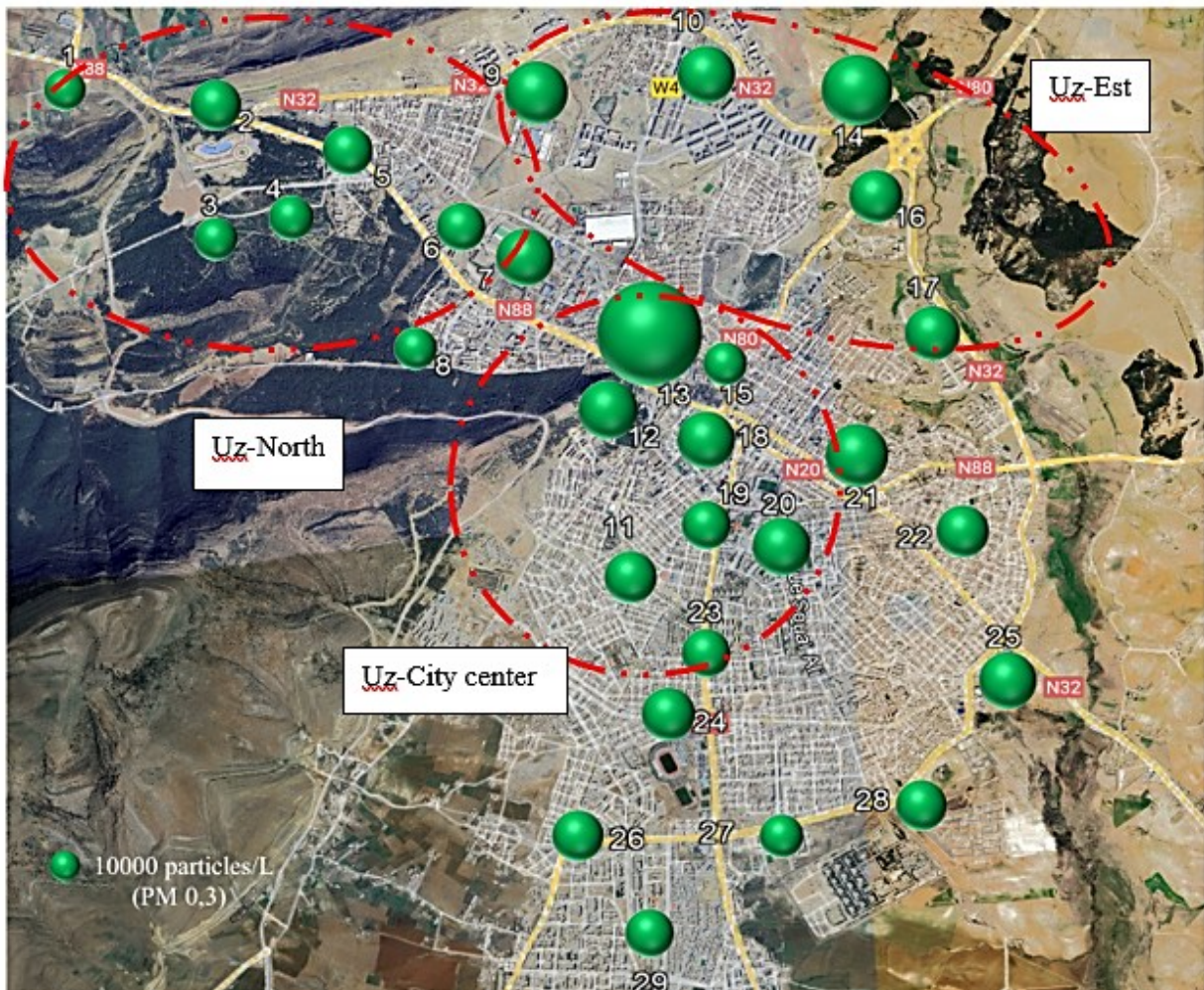


Figure 22. Spatial Distribution of PM 0.3 in the Urban Zones of Khenchela City

## 1.2. Proline Content

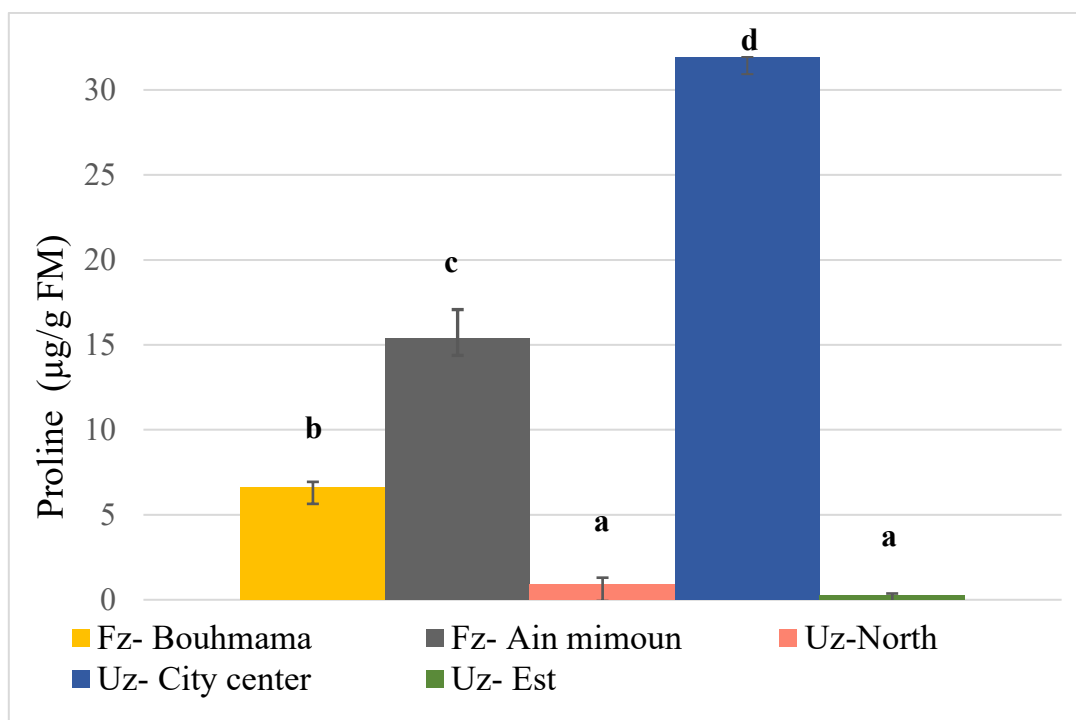
After applying the Monneveux and Nemmar (1986) method to measure proline concentration in Aleppo pine and Atlas cedar leaves across the two zones, we obtained the following results:

### 1.2.1. Measurement of proline in Pin d'alep and cedar in both zones

#### 1.2.1.1. Pin d'alep proline

The figure 23 illustrates the proline concentration in Aleppo pine (*Pinus halepensis*) leaves across two zones: urban and forested, represented by the letters a, b, c, and d. The proline level at point b (Bouhmama) is low ( $6.5 \mu\text{g/g}$ ), indicating a less stressful environment. At point c (Ain Mimoun), the proline concentration is moderate ( $15.1 \mu\text{g/g}$ ), suggesting the presence of moderate environmental stress. In contrast, the urban areas represented by point a exhibit very

low proline levels, with the Uz\_Est zone showing nearly undetectable concentrations, and the Uz\_North zone displaying low levels (1  $\mu\text{g/g}$ ), implying minimal stress conditions and indicating that both urban areas experience low or negligible pollution. The city center (point d) exhibits the highest proline accumulation, reaching 32  $\mu\text{g/g}$ , which indicates significant environmental stress, likely due to urban air pollution.



**Figure 23.** Proline content in Pin d'alep trees across urban and forest zones (Fz: Forest zone; Uz: Urban zone).

#### 1.2.1.2. Cedar proline

The figure 24 illustrates the proline concentration in the leaves of Atlas cedar (*Cedrus atlantica*) in both urban and forested zones. In the forested areas, represented by points a and ab (Ain Mimoun and Bouhmama, respectively), the proline levels are low (7.5  $\mu\text{g/g}$  and 9  $\mu\text{g/g}$ ), indicating that these environments are likely subjected to minimal environmental stress. Conversely, in the urban areas represented by points bc and c, the proline concentrations are higher (11.5  $\mu\text{g/g}$  in Uz-City Centre and 14  $\mu\text{g/g}$  in Uz-North), suggesting the presence of urban pollution that may have led to increased proline accumulation.

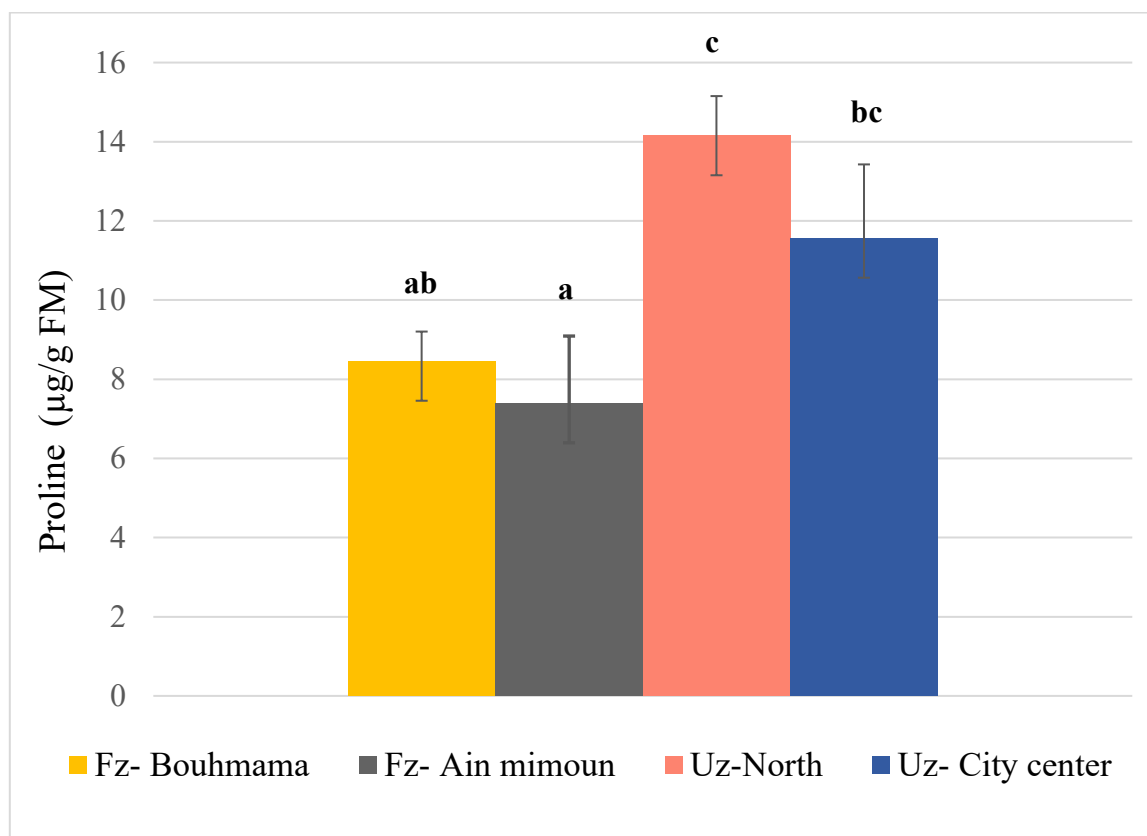


Figure 24. Proline content in Cedar trees across urban and forest zones (Fz: Forest zone; Uz: Urban zone).

### 1.3.Chlorophyll Content

After measuring the chlorophyll concentration in the leaves of Aleppo pine and Atlas cedar in both the forested and urban zones, we obtained the following results:

#### 1.3.1. Measurement of Chlorophyll in Pin d'alep and cedar in both zones

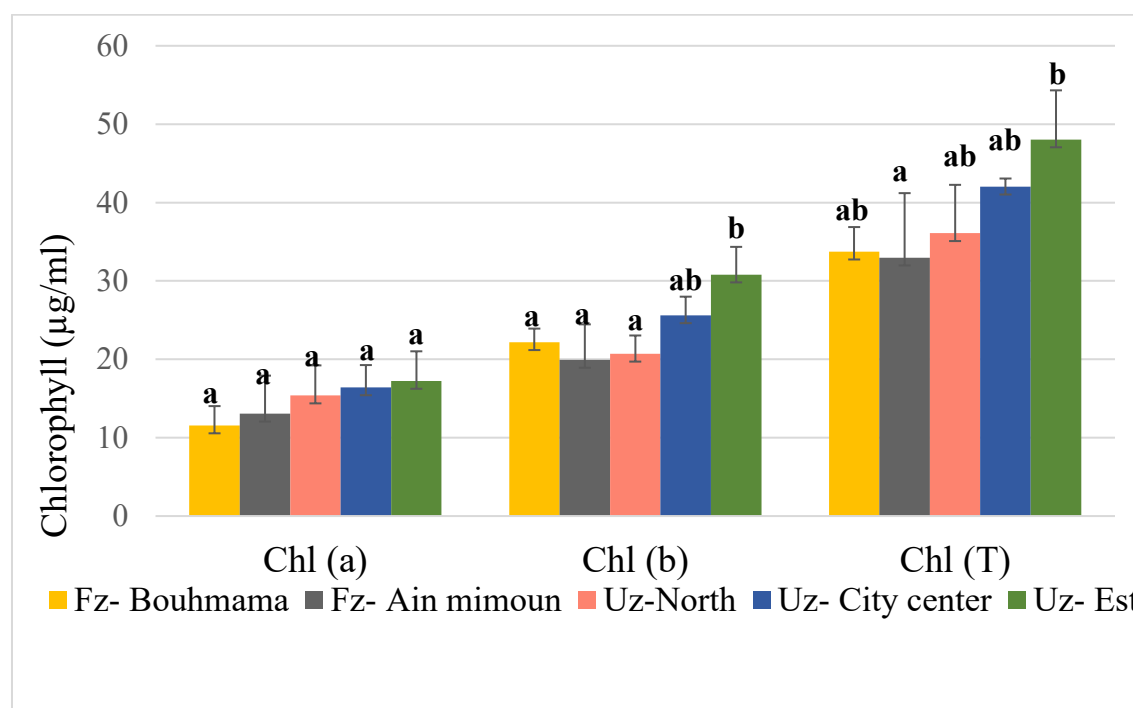
##### 1.3.1.1.Pin d'Alep Chlorophyll

The figure 25 represents the chlorophyll content in *Pinus halepensis* (Aleppo pine) trees. The results reveal distinct patterns between forest and urban zones, with statistical significance indicated by letter groupings where similar letters represent non-significant differences and different letters indicate significant variation. Chlorophyll (a) level remained relatively stable and low across all zones (11-17 µg/ml), showing no significant differences between forest and urban environments.

However, chlorophyll (b) results demonstrated statistically significant differences between the first three zones (Fz-Bouhmama, Fz-Ain mimoun, and Uz-North) and the other

two urban zones (Uz-City center and Uz-Est). The forest areas (Fz-Bouhmama, Fz-Ain mimoun ) and Uz-North maintained significantly lower levels (19,92- 20,71- 22,18  $\mu\text{g/ml}$ , respectively), while the remaining urban zones showed progressively higher concentrations, with 25,61  $\mu\text{g/ml}$  in Uz-City center trees and 30,81  $\mu\text{g/ml}$  in the tress of Uz-Est .

This pattern was most pronounced in total chlorophyll content, where forest zones consistently displayed the lowest levels at approximately 32,9-33,7  $\mu\text{g/ml}$ , while urban zones showed significantly elevated concentrations reaching up to 48  $\mu\text{g/ml}$  in Uz-Est.



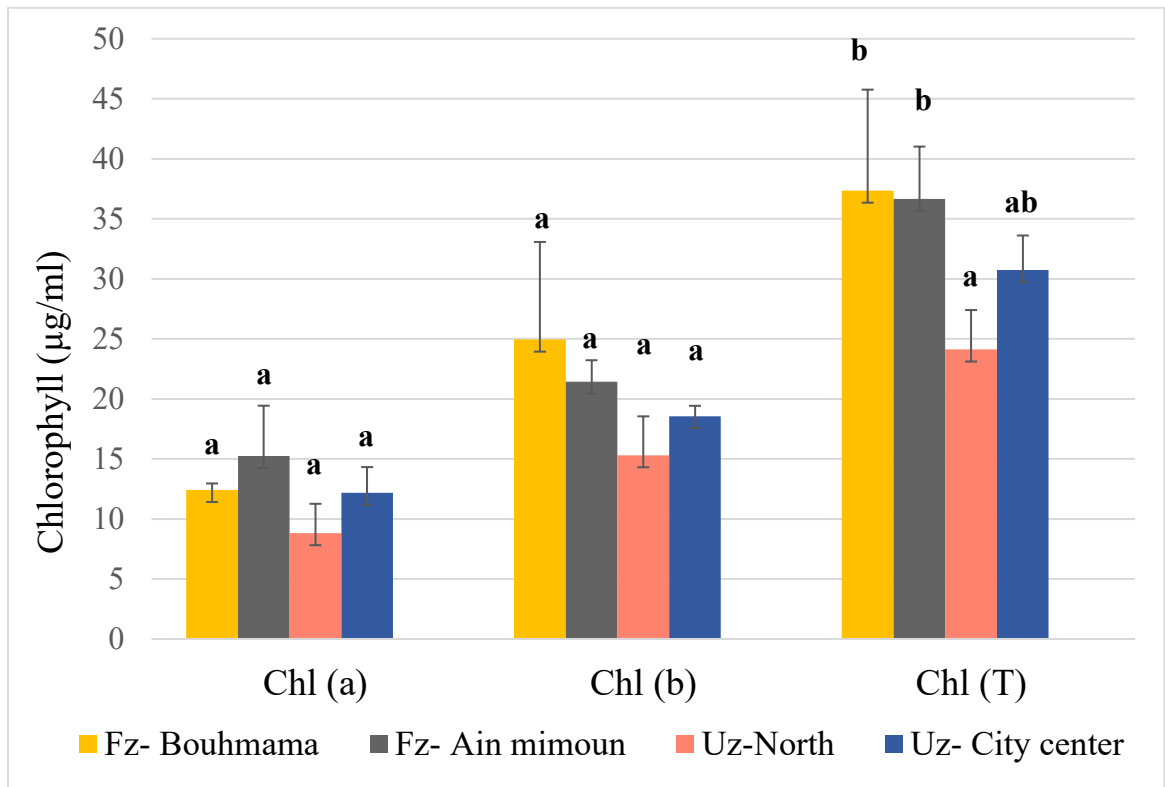
**Figure 25.** Chlorophyll content in pin d'alep trees across urban and forest zones (Fz: Forest zone; Uz: Urban zone).

### 1.3.1.2.Cedar chlorophyll

The figure 26 shows the chlorophyll concentration in the leaves of Atlas cedar (*Cedrus atlantica*) in both urban and forested areas. Similar letters indicate non-significant differences, while different letters reflect significant variations. Chlorophyll a level remained stable and relatively low across all areas (ranging from 9 to 15  $\mu\text{g/mL}$ ), indicating no statistically significant differences between forest and urban environments. The same trend was observed for chlorophyll B, which showed stable and higher levels compared to chlorophyll A (ranging from 25 -15  $\mu\text{g/mL}$ ), but without any significant differences.

### Chapter III: Results and discussion

The results revealed that the total chlorophyll content was highest in the forested zones, reaching approximately 37  $\mu\text{g}/\text{mL}$  in Bouhmama and 36.4  $\mu\text{g}/\text{mL}$  in Ain Mimoun. In contrast, the urban areas showed noticeably lower chlorophyll concentrations, with a minimum of 24  $\mu\text{g}/\text{mL}$  recorded in Uz North.



**Figure 26.** Chlorophyll content in Cedar trees across urban and forest zones (Fz: Forest zone; Uz: Urban zone).

# *Discussion*

### 1. Discussion

#### 1.1. Particulate matter

The results of the study, following the measurement of fine particulate matter in the air, revealed that their concentration in the city of Khenchela was high, especially in the city center. This is attributed to heavy traffic, concentrated commercial activities, and high building density, which all contribute to the accumulation of pollutants in the area. In contrast, the eastern and northern zones, characterized by lower urban density and reduced human activity, showed lower levels of fine particles. This increase in particulate matter is a clear indicator of urban air pollution caused by vehicle emissions, waste burning, and industrial outputs. Human health is negatively impacted by air pollutants such as particulate matter (PM), vehicle exhaust, and industrial emissions. They also disrupt plant ecosystems and have an impact on the entire planet by altering the atmosphere (**Raabe *et al.*,1999**) Recent years have seen an increase in environmental concern due to air pollution which are to blame for crop yield losses and vegetation damage (**Joshi and Swami, 2007**). The primary cause of this significant issue is industrialization. There are two categories for pollutants: main and secondary Pollutants that are released into the atmosphere and cause direct air pollution are referred to as primary pollutants, whereas secondary pollutants are those that are created in the air as a result of interactions or reactions between primary pollutants (**Agbaire, 2009**).

According to observations, a variety of contaminants, including hydrocarbons, ozone, particulate matter, hydrogen fluoride, peroxyacyl nitrates (PAN), and oxides of sulphur and nitrogen, have a significant negative impact on plants, especially those growing in metropolitan areas (**Jahan and Igbal, 1992**)

As a result of the ongoing growth in the human population, road traffic, automobile traffic, and industry, the concentration of gaseous and particle pollutants has continued to rise (**Jahan and Igbal, 1992**)

Given that plants have long been used as bioindicators of air pollution since they are primary receptors of airborne pollutants, we conducted our study on two tree species: *Pinus halepensis* (Aleppo pine) and *Cedrus atlantica* (Atlas cedar) to assess the impact of air pollution on them. After measuring chlorophyll and proline concentrations, we recorded

### 1.2. Chlorophyll content in aleppo pine tree

The Chlorophyll a remained relatively stable and low across all study sites (11–17  $\mu\text{g}/\text{mL}$ ), and no significant differences were observed between urban and forest zones.

However, chlorophyll b concentrations exhibited statistically significant variation between forest and urban zones. The forest sites (Bouhmama and Ain Mimoun) and the peri-urban Uz-North zone maintained moderately low levels of chlorophyll b (19.9–22.2  $\mu\text{g}/\text{mL}$ ), while the more polluted or urbanized sites (Uz-City Center and Uz-Est) recorded marked increases, with values reaching up to 30.8  $\mu\text{g}/\text{mL}$ . This differential pattern reflects the influence of urban environmental stressors, particularly air pollution, on pigment accumulation in *P. halepensis*. The elevated chlorophyll b and total chlorophyll in the more urbanized areas could represent a compensatory or adaptive response to reduced light quality, elevated nitrogen deposition, or pollution-induced oxidative stress. As chlorophyll b is associated with light-harvesting complex II, its upregulation may optimize light capture under altered light spectra caused by urban haze or partial shading (**Baek & Woo., 2010**).

A number of studies have demonstrated a rise in the amount of chlorophyll under air pollution. For example, **Tripathi and Gautam (2007)** found that *Mangifera indica* leaves exposed to air pollution had a 12.8% increase in chlorophyll content. In a 2009 study, Agbaire and Esiefarienrhe showed that plants from the experimental site had higher chlorophyll contents than those from the control.

Furthermore, increased nitrogen oxides ( $\text{NO}_x$ ) in urban air can act as fertilizing agents, enhancing pigment synthesis. Most strikingly, total chlorophyll content clearly distinguished urban from forest environments. While forest zones consistently showed values around 33  $\mu\text{g}/\text{mL}$ , total chlorophyll in urban sites rose significantly, peaking at 48  $\mu\text{g}/\text{mL}$  in Uz-Est. These results may appear counterintuitive since pollution is often associated with chlorophyll degradation. However, numerous studies have documented adaptive chlorophyll overproduction in hardy species like *P. halepensis* as a means to maintain photosynthetic efficiency under moderate, chronic urban stress (**Sæbø *et al.*, 2012**; **Mikkelsen *et al.*, 2015**).

This trend suggests that *Pinus halepensis*, a Mediterranean evergreen with strong xerophytic traits, possesses physiological plasticity that allows it to thrive in urban settings,

likely by adjusting pigment profiles to cope with altered microclimates, photic conditions, and pollutant loads.

### **1.3. Chlorophyll cedar**

The findings indicate that chlorophyll a level remained stable and relatively low in all zones, ranging between 9 and 15  $\mu\text{g/mL}$ , without significant statistical differences. Chlorophyll b also showed no significant spatial variation, although it consistently exhibited higher concentrations than chlorophyll a, within the range of 15 to 25  $\mu\text{g/mL}$ .

However, when examining total chlorophyll content, a clear pattern emerged: forested sites (Bouhmama and Ain Mimoun) recorded the highest total chlorophyll values (~37 and 36.4  $\mu\text{g/mL}$ ), while urban zones, particularly Uz North, showed a marked decrease, with the lowest value of 24  $\mu\text{g/mL}$ . Although individual chlorophyll types did not vary significantly.

The exposure to pollutants like  $\text{SO}_2$ ,  $\text{NO}_x$ , and particulate matter tends to degrade chlorophyll pigments, reducing photosynthetic capacity and plant vitality over time. Therefore, any reported increases could be attributed to specific adaptive responses or experimental variations but are generally overshadowed by the negative effects of pollution on chlorophyll content (**Certain, 2023**)

The study of **Joshi PC, et.al (2009); Pavlovic D et.al (2014)** confirm that degradation of photosynthetic pigments has been widely used as an indicator of air pollution, and a decrease in chlorophyll content shows that plants are more sensitive to air pollution, (Woo SY et al.,2006).

Air pollutants like sulphur dioxide, nitrogen dioxide, carbon dioxide and suspended particulate matter may lower the chlorophyll concentration, **Joshi PC, et.al (2009)** as the pollutants enter into tissues through stomata and cause partial denaturation of chloroplasts.

A significant decrease in total chlorophyll at the main location where air pollutants like  $\text{SO}_2$  and  $\text{NO}_x$  attack it. Through the stomata, air pollutants enter the tissues, partially denaturing the chloroplast and reducing the pigment content of the cells in contaminated leaves.

Chlorophyll is destroyed by high concentrations of gaseous  $\text{SO}_2$  (**Tripathi and Gautam, 2007**). Numerous studies have found that exposure to air pollution reduces the

## *Discussion*

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amount of chlorophyll (**Tiwari *et al.*, 2006; Tripathi and Gautam**), chlorophyll content varies with the tolerance as well as sensitivity of the plant species i.e. higher the sensitive nature of the plant species lower the chlorophyll content. High levels of automobile pollution decrease chlorophyll content in higher plants near roadsides (**Chandawat *et al.*, 2011; Adamsab *et al.*, 2011**). The results of present study are supported by the work of (**Chauhan *et al.*, 2011; Joshi and swami .2009**)

### **1.4.Pin alep proline**

The urban peripheries (Uz\_Est and Uz\_North) showed very low to negligible proline levels (1 µg/g or undetectable), indicating that despite being in an urban context, these areas may not be exposed to intense pollutants or stress-inducing factors. This could be explained by the presence of green infrastructure, lower vehicular traffic, or topographical shielding, all of which can mitigate air pollution (**Nowak *et al.*, 2006**).

In contrast, point d (city center) showed a significantly higher level of proline (32 µg/g), indicating high physiological stress, likely due to chronic exposure to urban air pollutants such as NO<sub>x</sub>, SO<sub>2</sub>, and particulate matter. Numerous studies have established that exposure to urban pollution can trigger oxidative stress responses in trees, leading to the accumulation of proline and other antioxidant compounds (**Bassi, R., & Sharma.,1993; Sharma & Dietz .,2006**).

This proline elevation is likely a defensive metabolic adaptation, as proline protects cellular structures, stabilizes proteins, and scavenges reactive oxygen species (ROS) under pollution-induced oxidative conditions (**Hayat *et al.*, 2012**).

### **1.5.Cedar proline**

In the forested sites of Ain Mimoun (point a) and Bouhmama (point ab), proline levels were relatively low 7.5 µg/g and 9 µg/g, respectively. These concentrations are consistent with unstressed or minimally stressed conditions, which are typical of natural forest ecosystems with good air quality, stable microclimates, and reduced anthropogenic interference (**Nowak *et al.*, 2006**).

In contrast, the urban zones show elevated proline concentrations, with 11.5 µg/g recorded at Uz-City Centre (point bc) and 14 µg/g at Uz-North (point c). This moderate to high accumulation of proline is a clear indication of stress response, likely due to chronic

## *Discussion*

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exposure to urban air pollutants, such as nitrogen oxides (NO<sub>x</sub>), sulfur dioxide (SO<sub>2</sub>), and particulate matter (PM). Urban trees often face oxidative stress, triggering the biosynthesis of proline as a protective mechanism to stabilize proteins, maintain osmotic balance, and scavenge reactive oxygen species (ROS) (Hayat *et al.*, 2012).

The proline act as a free radical scavenger to protect plants away from damage by oxidative stress. Obviously, proline has main role in protection in different kinds of stress. Accumulation of proline in plants is a physiological response to osmotic stress (Székely, 2004)

# *Conclusion*

## Conclusion

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### General conclusion

This study has demonstrated that chronic atmospheric pollution exerts measurable, species-specific effects on two Mediterranean forest trees *Pinus halepensis* and *Cedrus atlantica*—when comparing urban and forested environments. Particulate matter measurements in Khenchela City (PM<sub>0.3</sub>, PM<sub>2.5</sub>, PM<sub>10</sub>) revealed that the city center consistently exhibited the highest fine-particle concentrations, while peripheral and forested stands showed progressively lower levels (Mulenga *et al.*, 2020; Odubo & Kosoe, 2024). In response, *P. halepensis* individuals in heavily polluted urban sites displayed a significant increase in chlorophyll b and total chlorophyll relative to those in natural forest zones a possible compensatory adjustment under altered light conditions and elevated nitrogen deposition (Nakazato *et al.*, 2021; Sonwani *et al.*, 2022). Concurrently, proline accumulation in urban *P. halepensis* reached its peak in the city center ( $\approx 32$   $\mu\text{g/g}$ ), indicating activation of osmoprotective pathways under oxidative stress (Monneveux & Nemmar, 1986; Bytnerowicz *et al.*, 2001). In contrast, proline remained low ( $< 10$   $\mu\text{g/g}$ ) in forested areas such as Bouhmama and Ain Mimoun, where pollutant loads were minimal (Lamprecht *et al.*, 2015).

*Cedrus atlantica* exhibited a different pattern: total chlorophyll content was highest in forested stands ( $\approx 36$ – $37$   $\mu\text{g/mL}$ ) and declined in urban sites, dropping to as low as  $24$   $\mu\text{g/mL}$  in the northern urban zones suggesting pollutant-induced impairment of photosynthetic machinery (Zhang *et al.*, 2019; Villarejo, 2022). Although individual chlorophyll a and b levels did not vary significantly on their own, the overall reduction of total pigment in urban cedars implies a higher sensitivity in this species compared to *P. halepensis* (Phillips, 2018). Proline levels in cedar likewise increased under urban conditions ( $11.5$ – $14$   $\mu\text{g/g}$ ), evidencing oxidative stress responses, while remaining below  $10$   $\mu\text{g/g}$  in forested trees (Felzer *et al.*, 2007).

By linking spatially explicit PM measurements with leaf-level biomarkers, this research provides a robust framework to detect subtle yet ecologically significant shifts in tree health under chronic pollution. These findings underscore the importance of long-term field assessments: whereas short-term experiments might miss delayed-onset damage, examining perennial species over extended periods reveals cumulative physiological adjustments (Paoletti & Grulke, 2010; Nakazato *et al.*, 2021). Practically, recognizing that *P. halepensis* can maintain or even augment chlorophyll content under moderate urban stress suggests its suitability for urban forestry and air-quality mitigation efforts. Conversely, the sensitivity of *C. atlantica*—as evidenced by reduced pigment concentrations indicates the need for protective

## Conclusion

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measures if this species is to be integrated into polluted settings (**Zhang *et al.*, 2019; Likus-Cieřlik *et al.*, 2020**).

Beyond species-specific management, these results have broader implications for forest conservation and urban planning. Chronic air pollution can reshape physiological baselines in long-lived trees, potentially affecting growth rates, carbon sequestration capacity, and resilience to secondary stressors such as drought or pest outbreaks (**Bytnerowicz *et al.*, 2001; Mulenga *et al.*, 2020**). As global urbanization intensifies, integrating air-quality considerations into afforestation and reforestation programs becomes increasingly urgent. For policymakers, this study provides empirical evidence that maintaining or improving urban air quality directly influences ecosystem function and the long-term sustainability of urban and peri-urban forests (**Nakazato *et al.*, 2021; Sonwani *et al.*, 2022**).

In summary, by elucidating the physiological trajectories of two keystone tree species under varying pollution regimes, this research advances our understanding of how long-term atmospheric stressors alter forest vitality and emphasizes the need for integrated strategies to preserve forest ecosystem services in an era of intensifying air-quality challenges.

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