












Impact of dust load and lead (Pb) stress on leaf functioning of urban vegetation

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Abstract: Excessive lead (Pb) in industrial dust increases serious concerns about its potential impact on human health and environment. Urban trees, as a green and cost-effective alternative, are now considered an eco-sustainable tool used to monitor and mitigate this environmental pollution. The present study investigated the effect of dust particles and lead deposition on plant functioning and micromorphology of two common roadside plants, *Eucalyptus camaldulensis* and *Conocarpus lancifolius*, in an industrial city, Faisalabad, Pakistan. Tree leaf samples were collected from 20 sites varying in dust exposure intensity. These samples were analyzed for antioxidant enzymes such as superoxidase dismutase (SOD), peroxidases (POD), and catalases (CAT), as well as for proline and protein contents. An increased level of reactive oxygen species and proline contents in response to increasing dust load and lead tissue lead concentrations across the tested locations suggested the sensitivity of plants to urban dust. This study suggested that *C. lancifolius* leaves accumulated relatively higher dust particles and lead contents than *E. camaldulensis*. However, this excessive dust deposition and lead uptake inhibited carbon and protein synthesis in *E. camaldulensis* and *C. lancifolius*, as evidenced by relatively lower leaf photosynthesis and protein contents. We also found that the concentration of CAT (4.90 nmol min⁻¹ g⁻¹ protein), SOD (38.42 U mg⁻¹ protein), and POD (2.64 μmol min⁻¹ g⁻¹ protein) were higher in *E. camaldulensis*. The results provide experimental support for the hypothesis that genetically rich populations are better adapted to changing conditions and suggest that *C. lancifolius* is less sensitive to dust load and lead uptake in terms of growth reduction. Regarding lead uptake and dust removal, *C. lancifolius* performed better as compared to *E. camaldulensis*. The above findings are helpful in the quantification of services, assessing the diversity, and identification of eco-friendly trees in urban areas. Furthermore, this study will be helpful for the formulation of future tree-planting policies in urban areas.

Keywords: Abiotic stresses, environmental pollutants, bio-evaluation, dust particles, lead uptake, trees response

1. Introduction

Cities are growing faster around the world, and consequently, more land and resources are needed to fulfil the demand of urban dwellers. Humans have been utilizing natural resources for centuries to improve their lives, and in this effort, the degree of exploitation of resources has become a severe threat to the precise functioning of the ecosystem (Squires et al., 2002). Currently, more than half of the world's population prefers to live in towns and megacities, and this proportion will exponentially grow in the coming decades. The urban population in 2015 was 4 billion (54%) of the total global population, and it is anticipated

to increase by 4.9 billion (60%) in 2030 (WHO, 2016). By 2050, it is expected that 67% of the world's population will migrate to urban areas (WHO, 2016). Currently, land encroachment by the urban population will increase by 1.2 million km² globally by 2030. The effects of air pollutants on living organisms are not easy to determine because they are exposed to various uncontrolled variables such as pesticides, changing weather, and a complex mixture of pollutants. The air pollutants in the urban environment pose a threat to human health, causing nearly seven million premature deaths worldwide each year (WHO, 2014). Air quality in urban areas is particularly poor due

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to high emissions from human activities (Sawidis et al., 2011). Almost 54% of the world's population lives in these regions, which exacerbates its negative impact (Deguignet et al., 2014). It is estimated that in 2015 in Europe, 53% and 82% of the urban population were exposed to levels higher than the daily levels of coarse particulate matter (PM₁₀) and fine particulate matter (PM_{2.5}) recommended by WHO.

Environmental contaminants, particularly heavy metals (HMs) are considered highly toxic due to their persistent and bioaccumulative properties, posing a significant risk to biological substances and the environment (Farooq et al. 2022a,b; Ameen et al. 2023). Dust particles are the most abundant air pollutants and are a mixture of heavy metals, elements, black carbon, soil, and other substances (Ma et al., 2022a,b; Abeer et al., 2022a,b). Improving air quality by reducing atmospheric pollutants is now a widely accepted ecosystem service urban green spaces provide (Janhäll, 2015; Sæbø et al., 2011). The dry deposition of solid particles on leaf surfaces is a fundamental mechanism for the beneficial effects of plants on air quality (McDonald et al., 2007; Nowak et al., 2013). Generally, atmospheric pollution's physical and chemical properties significantly affect growth patterns (Pandey et al., 1999). The plants show some morphological and physiological variations when grown in polluted areas. These variations may include plant height, leaf color, shape, and length (Leghari and Zaidi, 2013). In plants, leaves are the most critical areal part, which is highly sensitive to air pollutants as they are directly facing harsh weather conditions. Environmental pollutants directly affect the plant by damaging its leaves and indirectly by acidifying soils (Steubing et al., 1989). Plants absorb environmental pollutants through the leaf surface, disrupting the photosynthesis process. Vegetation with more foliage is more susceptible to airborne particles (Pandey et al., 1999; Farmer, 1993). The results of exposure of plants to high air pollutants can be devastating and can reduce yields to greater extents (Samal et al., 2002). It is also reported that atmospheric pollutants have adverse effects on plant physiology regarding metabolic function, enzyme activity, discoloration of leaves, and reduced photosynthesis (Zainab et al., 2021; Zulfiqar et al. 2023; Mustafa et al. 2023). Adverse effects like stunted growth and reduction in crop yields by air pollutants (Rodriguez et al., 2008). The cuticle changes in tree leaves planted along the road found that particulate pollution can change the foliar morphology of leaves and induce stress, which can identify the impacts of pollution on trees (Kayode et al., 2007). Sondergeld et al. (2010) reported that particulate matter from vehicular emissions could cause stomata closure, changes in epidermal cells, changes in trichome, and alterations in leaf surfaces. Several experiments show that different physiological, morphological, and

anatomical changes occurred in plants due to the polluted environment (Brandão et al., 2018).

This study was conducted in Faisalabad, a megacity in Pakistan, to analyze atmospheric pollutants, particularly lead and dust deposits, on urban trees. However, it is also valuable to recognize the sources and major pollutants of air pollution and take preventive measures accordingly. Overall, ambient air quality levels in Pakistan's urban centres are frightening, with various air pollutants, particularly particulate matter and dust loads, many times higher than WHO guidelines and national standards (Colbeck et al., 2010). However, air quality is deteriorating due to a lack of air quality management capabilities. Evidence from many international agencies and government organizations indicates that air pollution is a significant risk to residents' health, environment, and quality of life (Niaz et al., 2015). Faisalabad is 2nd most polluted city in Pakistan. Air pollution in an urban environment is a great threat to urban dwellers and urban vegetation. The current study focused on determining the trends in dust load deposition and lead uptake by urban trees in Faisalabad city and the impact of dust particles and heavy metal pollution on plant function.

2. Materials and methods

2.1. Description of the study site and climatic conditions

The research was carried out in the industrial heart of Pakistan, which is known for its textiles. Faisalabad, also known as the "Manchester of Pakistan", is the third most populous city and the second largest city in Punjab. Near the selected sampling sites are different brick kilns, food processing plants, engineering complexes, soap factories, chemical factories, marble factories, flour mills, and textile factories. Heavy traffic is a major source of air pollution near the study area, so its ambient air quality is a major concern. It contributes more than 20% of Punjab's GDP, with an average annual GDP of \$20.5 billion. It has the highest recorded population growth since 1941, with only 69,930 inhabitants, which increased to 7.874 million in 2019 (Pakistan Bureau of Statistics, 2018–19). It has an area of 1300 square km (490 square miles) and is located at 31°25'7.37"N latitude and 73°4'44.79"E longitude. This area is located at an altitude of 184 m. Geographically, Faisalabad has a semiarid climate in the Cobain-Geiger classification, with hot and humid summers and dry, cold winters. Summer starts in April and lasts until October, with three months (May, June, and July) being the hottest months. Winter, on the other hand, starts in November and lasts until March. December, January, and February are the coldest months. Faisalabad has an average temperature of 24.2 °C and an average annual rainfall of 346 mm.

2.2. Sampling site

Through the field survey of Faisalabad, 20 sites were selected, representing parks, institutional hospitals,

commercial areas, vehicle areas, industrial areas, and residential areas, and were sampled as shown in Table and Figure 1, and the ambient air quality of the respective sites was measured.

2.3. Analyzing pollutant effect on plant functioning

Faisalabad is an industrial city; here, the atmospheric quality is poor compared to its neighboring cities. Several atmospheric pollutants were polluting the urban environment. It is important to check the pollutant load, concentration in their surroundings, and trees' health. So, the leaves of two abundant urban tree species *E. camaldulensis* and *C. lancifolius* were collected from the sides of the upper canopy of these sites. These 20 sites have been categorized based on dust load and lead concentration i.e. not-polluted, partially polluted, polluted, and highly polluted. The clipped leaves were kept in paper bags, and tagging was done and brought to the laboratory to store in cold conditions lead uptake, dust deposition, protein and proline contents, and enzymatic activity was measured.

2.4. Estimation of lead (Pb) uptake

The dried ground material (5 g) was incubated in a 5 mL of nitric acid (HNO₃) indigestion flask for 12 h at room temperature (Wolf, 1982). The flasks were transferred to a hot plate and heated up to 350 °C until fumes appeared and continued to heat for 30 min. The above-mentioned steps were repeated until the digestion material was converted to clear and colorless. The transparent extract was filtered with Whatman paper and made the volume up to 50 mL by adding distilled water. An Atomic Absorption Spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan) was used to determine the Pb concentration in the prepared samples. For the determination of Pb by AAS the wavelength was adjusted at 283.3 nm, lamp current (7.5 mA), slit width (1.3 nm), oxidant gas pressure (160 kpa) burner head (standard type), flame (Air-C₂H₂), burner height (7.5 mm), and fuel gas pressure (7 kpa). In all the experimental steps, highly purified deionized water was used to prepare working standards. All the glass apparatus used during the process of analytical work was

Table. Sampling site for calculating dust load in Faisalabad City.

Sr. No.	Sampling Site	Major Occupation	Dust Concentration	Category
1	Jinnah Park	Vegetation	0.42	N.P
2	D-Ground	Commercial area	0.84	P
3	Tariqabad	Residential area	0.97	H.P
4	Millat Town	Residential area	0.91	P
5	Peoples Colony	Residential area	0.88	P
6	Samanabad	Residential area	0.92	P
7	Machli Farm	Commercial area	0.71	PP
8	Gulfishan Colony	Residential area	0.74	PP
9	Chenab Chowk	Commercial area	0.92	P
10	Kaleem Shaheed Park (KSP)	Vegetation	0.47	N.P
11	Nazimabad	Residential area	0.89	P
12	Gulberg	Residential area	0.77	PP
13	Allied Chowk	Residential area	0.93	P
14	Wapda Town	Residential area	0.81	PP
15	Ghulam Muhammad Abad (GMA)	Industrial area	1.02	H.P
16	Razaabad	Industrial area	1.04	H.P
17	Muhammad Pura	Residential area	0.90	P
18	Nishatabad	Industrial area	0.91	P
19	Madina Town	Residential area	0.79	PP
20	University of Agriculture Faisalabad (UAF)	Vegetation	0.54	N.P

N.P, nonpolluted; HP, highly polluted; PP, partially polluted; P, polluted

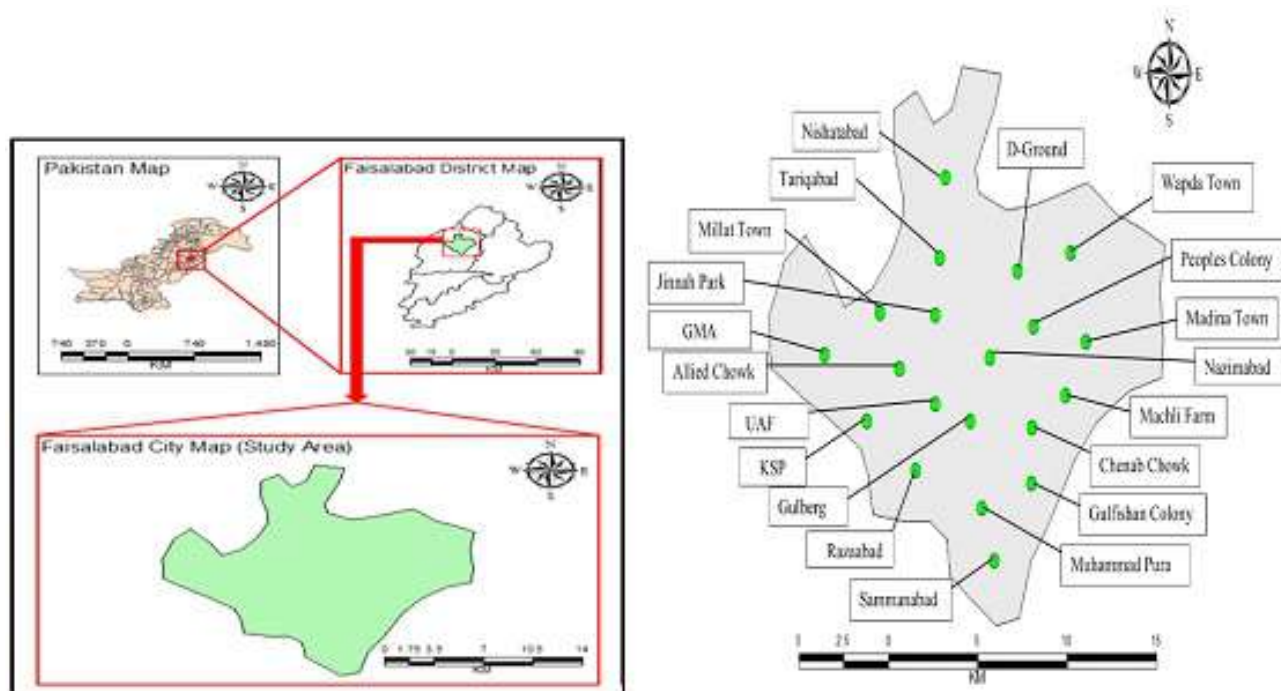


Figure 1. Mapping of the study area and twenty selected sites used in this study.

immersed in 8 N HNO₃ overnight and then washed with several dilutions of deionized water before use.

2.5. Estimation of dust deposition through the leaf surface

Fully matured leaves at the top of the selected tree species were taken randomly from these twenty sites. The leaf area index of collected leaf samples was measured using a leaf area meter. Later, samples were washed and filtered through filter paper. The wet filter paper was first weighed, and then it was dried and weighed again to measure the dust. Top pan electronic balance (Model- CAC-34, Contech Instruments Limited, Bengaluru, India) was used to weigh the amount of dust on the leaves and calculated by the following equation:

$$W = W_2 - W_1 / A$$

Here, W = Dust content, W₁ = Initial weight of filter paper, W₂ = Final weight of filter paper, A = Total leaf area.

2.6. Extract preparation for biochemical analysis

For analyzing soluble protein and antioxidant enzymes (Superoxide dismutase (SOD), Catalase (CAT), and Peroxidase (POD)), extraction was made as defined by Naqvi et al. (2011). About 0.5 g of the sample was grounded in mortar and pestle, and the extract was prepared with 2 mL of 50 mM phosphate buffer. The extracts were filtered and centrifuged at 8000 rpm for 10 min. Extracts were separated from residues in Eppendorf tubes for analysis. Due to its perishable nature, the extract was stored at -800 °C until analysis (Figure 2).

2.6.1. Determination of superoxide dismutase

The activity of SOD was measured by determining its ability to inhibit the photoreduction of Nitroblue tetrazolium (NBT) using the previously described method (Giannopolitis et al., 1977). The reaction solution for SOD determination contained 0.015 g of NBT in 17.5 mL of distal water, 0.222 g methionine in 15 mL of distilled water, 0.0132 g of riboflavin in 17.5 mL of distilled water, 0.0375 g of Triton-X in 17.5 mL distilled water, and 0.2 M buffer. The tube containing the reaction solution was placed under a UV lamp before adding riboflavin for 15 min. The absorbance of the solution at 560 nm was measured with a spectrophotometer. One unit of SOD activity was defined as “the amount of enzyme that inhibited 50% of NBT photoreduction”.

2.6.2. Determination of peroxidase

Peroxidase activity was measured using the method of Liu and Huang, (2000). The POD reaction solution contained 50 mM phosphate buffer (pH 5), 20 mM guaiacol, 40 mM H₂O₂, and 0.1 mL enzyme extract. Changes in the absorbance of the reaction solution were determined by using a spectrophotometer with a frequency of 470 nm. One unit of POD activity was defined as “An absorbance change of 0.01 units per min”. The activity of each enzyme was expressed on a protein basis.



Figure 2. Extract preparation for biochemical analysis.

2.6.3. Determination of catalase

The specific activity of CAT was determined by using the method described by Naqvi et al. (2011). The reaction solution for CAT activity contained 1.9 mL phosphate buffer (pH 7), 1 mL 5.9 mM, and 0.1 mL enzyme extract. About 200 μ L of the above solution was placed in 96 well plates, and absorbance was taken at 240 nm after every 20 s. One unit of CAT activity was defined as "An absorbance change of 0.01 units per min". The activity of each enzyme was expressed on a protein basis. Bradford measured the crude extract's protein concentration (Bradford, 1976).

2.6.4. Determination of soluble protein

The soluble protein contents were counted by the Bradford method (Bradford, 1976). Fifty μ L of the sample was taken in a microcentrifuge tube, and 2 mL of Bradford reagent was added in the same tube. While blank only contained Bradford reagent. Absorbance was taken at a frequency of 595 nm. Protein content was quantified by a standard curve prepared with different concentrations of bovine serum albumin (BSA).

2.6.5. Determination of proline

Firstly, 3 g sulphosalicylic acid was dissolved into 50 mM PBS (pH 7.8) to make the final volume up to 100 mL. Later, 40.933 mL of 85% H_3PO_4 was then taken, and its volume was raised to 100 mL. After this, 60 mL pure acetic acid is mixed with 40 mL (6 M H_3PO_4), then add 2.5 g Ninhydrin and give water bath at 700 $^{\circ}C$, avoid the solution from light, and store at 40 $^{\circ}C$. The fresh samples were cut into small pieces, 0.1 g in tubes with a stopper, adding 5 mL of 3% sulphosalicylic acid solution, and incubated in boiling water at 1000 $^{\circ}C$ for 10 min. The samples were cooled down

using tap water, and then 1 mL supernatant was taken in clean tubes with a stopper. Next, 1 mL of pure acetic acid and 1.5 mL of ninhydrin solution were then added, and samples were again shaken in a water bath (1000 $^{\circ}C$) for 40 min. The samples were cooled down in tap water, added 2.5 mL of pure toluene was shaken intensively. The samples were then allowed to settle down for several minutes. Finally, read the supernatant spectrophotometrically at OD520. Pure toluene was used to set the zero value.

Formula for proline content determination =

$$(\mu\text{g/g FW} = C \times V / a) / W$$

Here, C proline content is calculated from the standard curve, V = Volume of solution used to extract proline i.e. 5 mL, a = volume of supernatant used i.e. 1 mL and W = sample weight

2.6.6 .Statistical analysis

The means of collected data were separated by using MS Excel software. The experiment was established under a randomized complete block design with factorial arrangements. Statistical analyses were performed using statistics 8.1 software. The graphical presentation was done using MS excel.

3. Results

3.1. Catalase activity

The results showed that selected two tree species, *E. camaldulensis* and *C. lancifolius* depicted significant variation for CAT activity under different types of environments, as indicated in Figure 3. The interactive effect of two tree species and different types of environments also showed significant variation for CAT activity. The

results showed that *C. lancifolius* had significantly less activity in comparison with *E. camaldulensis* under all types of environments. Catalase activity kept increasing with the concentration of pollution i.e. these were the least in the pollution-free environment (control) and highest in the most polluted environment. Interaction value revealed that significantly more CAT activity ($4.90 \text{ nmol min}^{-1} \text{ g}^{-1} \text{ protein}$) was recorded in *E. camaldulensis* under a highly polluted environment, whereas the least catalase activity ($1.56 \text{ nmol min}^{-1} \text{ g}^{-1} \text{ protein}$) was recorded in *C. lancifolius* under nonpolluted environment. The overall range of CAT activity in both tree species under different types of environments was from $1.56\text{--}4.90 \text{ nmol min}^{-1} \text{ g}^{-1} \text{ protein}$. The descending order of species response under different types of environments for the catalase activity was *E. camaldulensis* > *C. lancifolius*, while for the different environments was highly polluted > polluted > partially polluted > not polluted.

3.2. Peroxidase activity

The results revealed that there were significant variations among tree species, different types of environments, and their interaction regarding POD activity (Figure 4). Among tree species, significantly more POD activity was recorded for *E. camaldulensis* in comparison to *C. lancifolius* under all types of environments. Likewise, a significant increase in POD activity was recorded with the progressive increase in pollution concentration in the environment. Thus, the maximum POD activity was recorded in a “highly

polluted” environment. The overall POD activity of two tree species under different types of the environment was in the range from $1.69\text{--}2.64 \text{ } \mu\text{mol min}^{-1} \text{ g}^{-1} \text{ protein}$. Interactive influence of tree species \times type of environment revealed that significantly higher POD activity ($2.64 \text{ } \mu\text{mol min}^{-1} \text{ g}^{-1} \text{ protein}$) was recorded in *E. camaldulensis* under a highly polluted environment. These were followed by the POD activity in *C. lancifolius* and *E. camaldulensis* under highly polluted environments. The interaction further revealed that the least POD activity was recorded in *C. lancifolius* under a “not polluted” environment. The order of species response under different types of environments for the POD activity was *E. camaldulensis* > *C. lancifolius*, while for the different environments was highly polluted > polluted > partially polluted > not polluted.

3.3. Superoxide dismutase activity

Superoxide dismutase (SOD) is yet another important enzyme of the plant’s defense system. Data reveals that SOD activity showed significant variations among tree species, different types of environments, and the interaction of both (Figure 5). A similar trend was recorded for SOD activity as was recorded for the catalase and peroxidase contents i.e. among tree species, significantly more SOD activity was recorded in *E. camaldulensis* in comparison to *C. lancifolius* under all types of environments. SOD activity showed a gradual increase with an increasing concentration of pollution in the environment. The superoxide dismutase activity of two tree species under

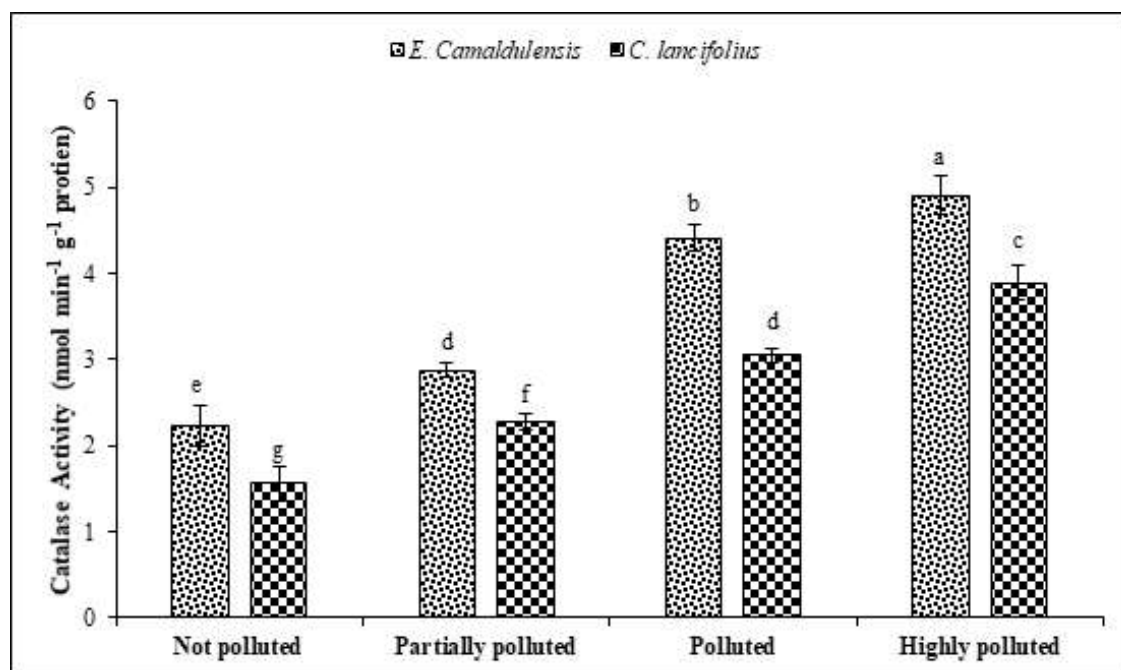


Figure 3. Catalase activity in *E. camaldulensis* and *C. lancifolius* under different environments. Different lowercase letters show significant differences among various environments and species.

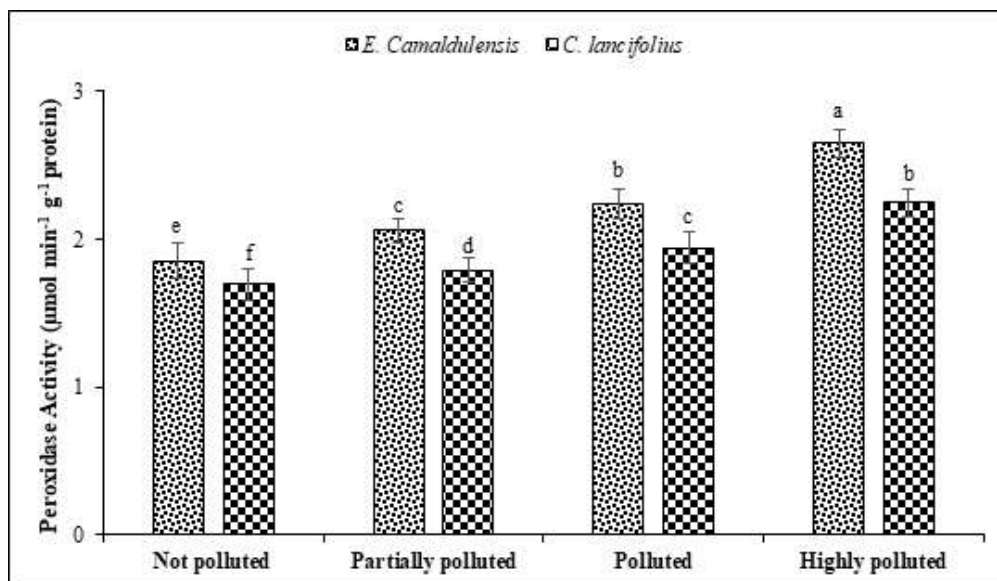


Figure 4. Peroxidase activity in *E. camaldulensis* and *C. lancifolius* under different environments. Different lowercase letters show significant differences among various environments and species.

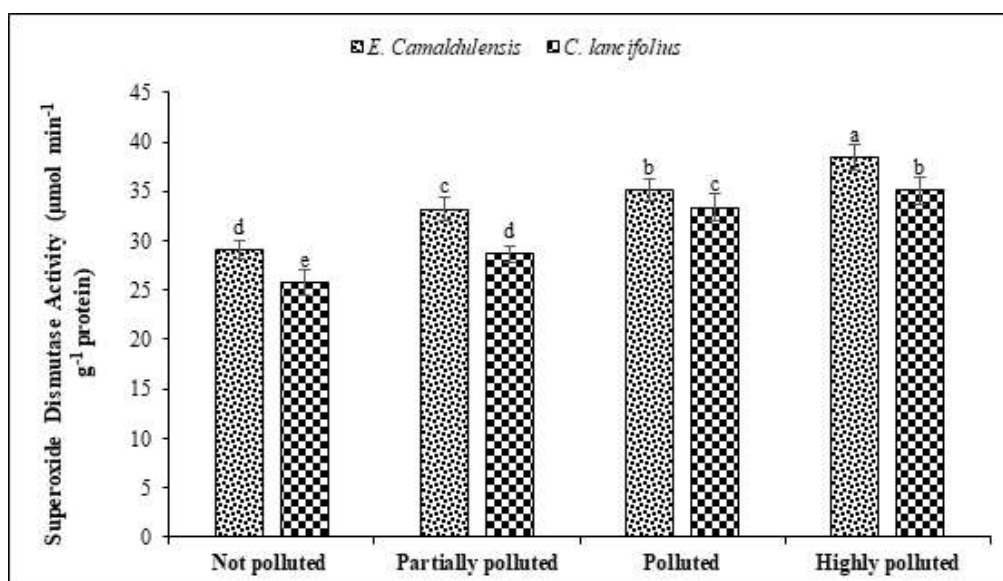


Figure 5. Superoxide dismutase activity in *E. camaldulensis* and *C. lancifolius* under different environments. Different lowercase letters show significant differences among various environments and species.

different types of the environment was in the range of 25.80–38.42 U mg⁻¹ protein. The maximum superoxide dismutase activity (38.42 U mg⁻¹ protein) was measured in *E. camaldulensis* in a highly polluted environment, followed by *C. lancifolius* (35.05 U mg⁻¹ protein) in the same environment. While minimum superoxide dismutase activity for *E. camaldulensis* and *C. lancifolius* were 29.14 U mg⁻¹ protein and 25.80 U mg⁻¹ protein, respectively, in a

“not polluted” environment. The order of species response under different types of environments for the superoxide dismutase activity was *E. camaldulensis* > *C. lancifolius*, while for the different environments was highly polluted > polluted > partially polluted > not polluted.

3.4. Protein contents

The results showed that both tree species in the study (*E. camaldulensis* and *C. lancifolius*) depicted significant

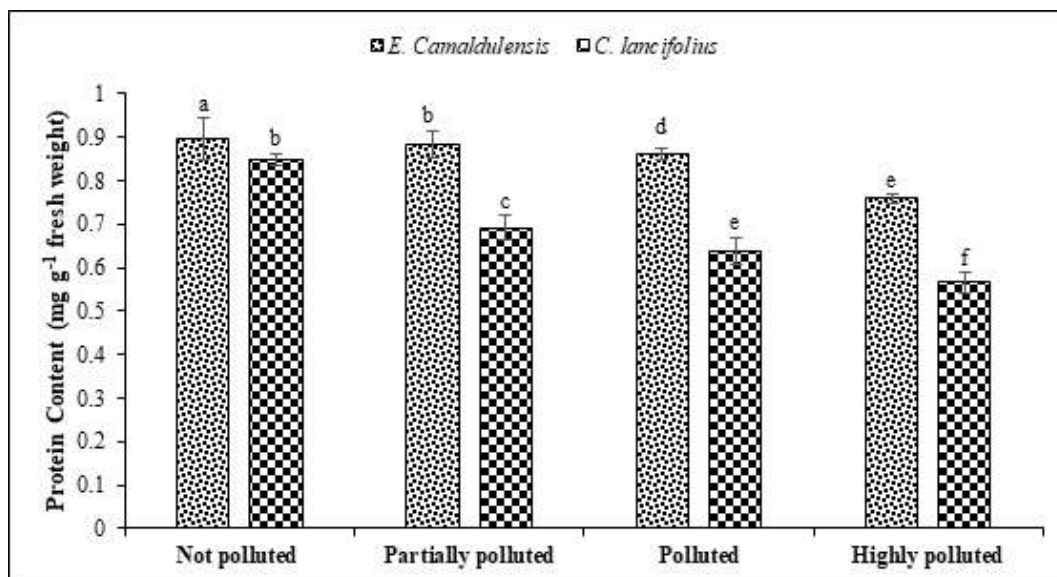


Figure 6. Protein content in *E. camaldulensis* and *C. lancifolius* under different environments. Different lowercase letters show significant differences among various environments and species.

variations regarding the protein contents under different types of environments, as indicated in Figure 6. The protein contents of two tree species under different types of environments were in the range of 0.56 – 0.89 mg g⁻¹ fresh weight. Significant variations were recorded in the protein contents under different types of environments as well. Likewise, the interaction of tree species and different types of the environment exerted a significant influence on protein contents as well. Results revealed that significantly more protein contents were found in *E. camaldulensis* in comparison to *C. lancifolius* under all types of environments, whereas these showed a declining trend with the increase in pollution status of the environment. The provision of a pollution-free environment (control) resulted in significantly higher protein contents than all other environments with different concentrations of pollution (Figure 6). The interactive influence of tree species and environments revealed that protein contents were significantly more in *E. camaldulensis* (0.89 mg g⁻¹ fresh weight) under a “not-polluted” environment. The least protein contents were measured in *C. lancifolius* (0.56 mg g⁻¹ fresh weight) in a highly polluted environment. The order of species response under different types of environments for the protein contents was *E. camaldulensis* > *C. lancifolius*, while for the different environments was not polluted > partially polluted > polluted > highly polluted.

3.5. Proline contents

The main effects and the interactive influence of tree species and different environment types were significant regarding the proline contents (Figure 7). *C. lancifolius* had

more proline contents in comparison to *E. camaldulensis* under all types of environments. Both tree species' overall range of proline contents was 2.46–8.19 $\mu\text{mol g}^{-1}$ fresh weight. Proline contents kept on increasing with the environment becoming more and more concentrated in pollutants, thus, the maximum proline contents among different types of environments were recorded in a highly polluted environment. The interactive influence revealed that significantly more proline contents (8.19 $\mu\text{mol g}^{-1}$ fresh weight) were measured in *C. lancifolius* under a highly polluted environment, whereas the least (2.46 $\mu\text{mol g}^{-1}$ fresh weight) were measured in *E. camaldulensis* under a “not polluted” environment. The order of species response under different types of environments for the proline contents was *C. lancifolius* > *E. camaldulensis*, while for the different environments was highly polluted > polluted > partially polluted > not polluted.

3.6. Dust load

Dust load showed significant variable responses among tree species, different types of environments, and their interaction (Figure 8). Among tree species, significantly more dust load was settled on *C. lancifolius* in comparison to *E. camaldulensis*. The dust load of two tree species under different types of environments was observed to be in the range of 0.42–1.045 g/ft². Likewise, the highly polluted environment had significantly more dust load in comparison to all other types of environments. The interactive influence revealed that *C. lancifolius* grown under a highly polluted environment recorded the maximum dust load of 1.045 g/ft², whereas the least amount of this load (0.42 g/ft²) was found

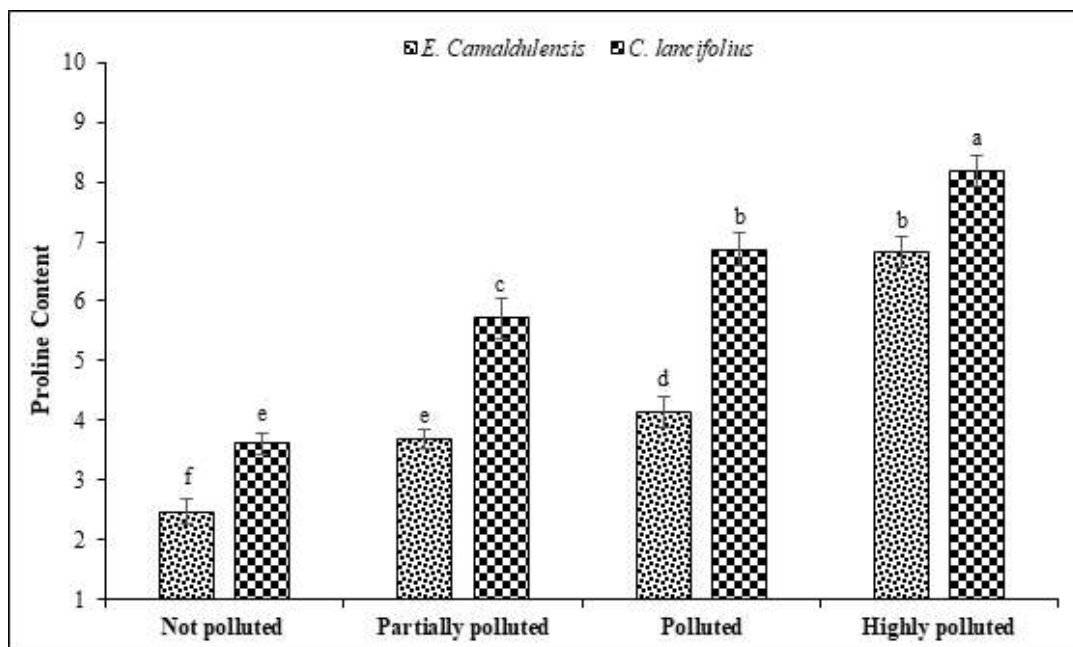


Figure 7. Proline content in *E. camaldulensis* and *C. lancifolius* under different environments. Different lowercase letters show significant differences among various environments and species.

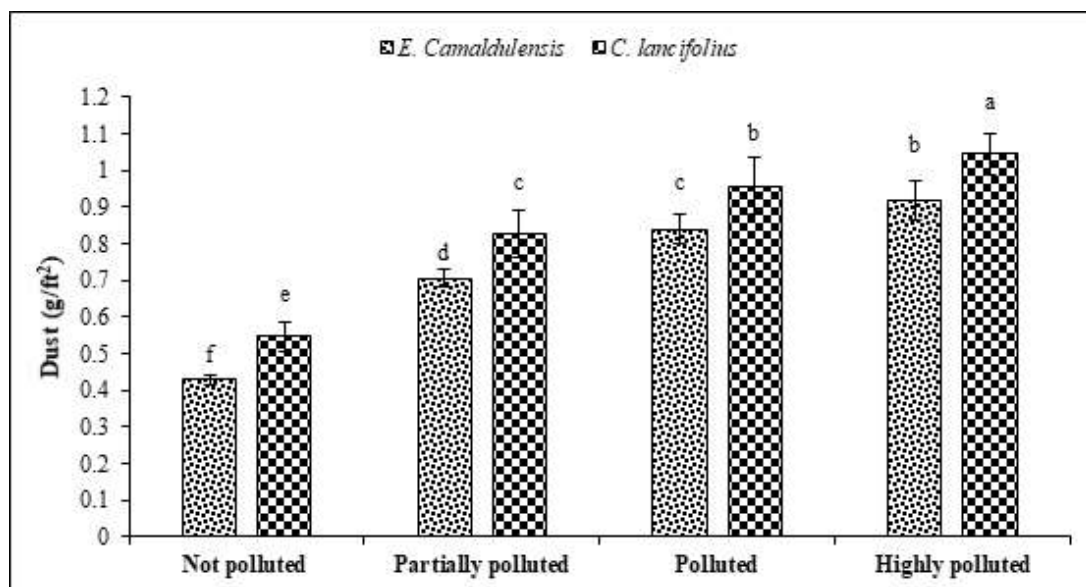


Figure 8. Dust load in *E. camaldulensis* and *C. lancifolius* under different environments. Different lowercase letters show significant differences among various environments and species.

in *E. camaldulensis* under a “not polluted” environment. The maximum dust load of 1.045 g/ft² was observed in the *C. lancifolius* in a highly polluted environment, followed by *C. lancifolius* at 0.95 g/ft² for polluted. The order of species responsible for the dust load under different types of environments was *C. lancifolius* > *E. camaldulensis*, while for the different environments was highly polluted > polluted >

partially polluted > not polluted environment.

3.7. Lead content

Significant variations in the lead (Pb) contents were recorded for different tree species and environments. The interactive influence of both factors regarding Pb contents was significant as well (Figure 9). Among tree species, significantly more Pb contents were accumulated

by *C. lancifolius* than *E. camaldulensis*. The lead contents of two tree species under different types of environments ranged from 4.13–15.13 ppm, whereas among different environments, a gradual increase in the Pb contents were recorded with the increase in the concentration of pollution. Thus, the highly polluted environment recorded significantly more Pb contents than other types of environments. Interaction revealed that significantly more Pb content (15.13 ppm) was recorded for *C. lancifolius* under a highly polluted environment. The provision of a pollution-free period to *E. camaldulensis* resulted in the least Pb contents (4.13 ppm). The order of species response under different types of environments for the lead contents was *C. lancifolius* > *E. camaldulensis* while for the different environments was highly polluted > polluted > partially polluted > not polluted.

4. Discussion

Hazardous impacts of atmospheric pollutants on the growth of the plant and overall yield have been reported by Ulrich, (1984). However, the growth pattern and final plant development vary depending on the variant visible physical, physiological, and biochemical nature of the pollutants (Pandey et al., 1999). Farmer, (1993) studied the effect of different pollutants on vegetation and described that higher level of PM could damage vegetation due to

high surface loads, while later reports by Grantz et al. (2003) indicated that higher concentrations of cement dust damages the plants even at lower surface loads due to the presence of reactive constituents like heavy metals and other chemicals. The damages caused by air pollutants to plants include chlorosis, necrosis, and epinasty (Khare and Baruah, 2010). The impact of air pollution on plants is reflected in reducing photosynthetic pigments, inhibiting certain physiological processes, and changing metabolic functions and enzyme activities (Heath, 2008).

Air pollutants interfere with the biochemical and physiological processes of plants to the extent that ultimately leads to lower growth rates (Calatayud and Barreno, 2004). They are responsible for producing reactive oxygen species (ROS) that affect the cellular and molecular structures of plants (Apel and Hirt, 2004; Zulfiqar et al. 2019). Under abiotic stress, the enzymes responsible for ROS accumulation are usually decoupled from metabolic pathways, such as singlet oxygen (O_2), superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^\cdot), and others cause oxidative stress (Asada et al., 2006). The reaction of hydroxyl radicals with pigments may affect proteins, lipids, and DNA (Møller et al., 2007). Under oxidative stress, plants protect themselves from the damaging effects of ROS by synthesizing various enzymatic and nonenzymatic ROS scavenging and detoxification

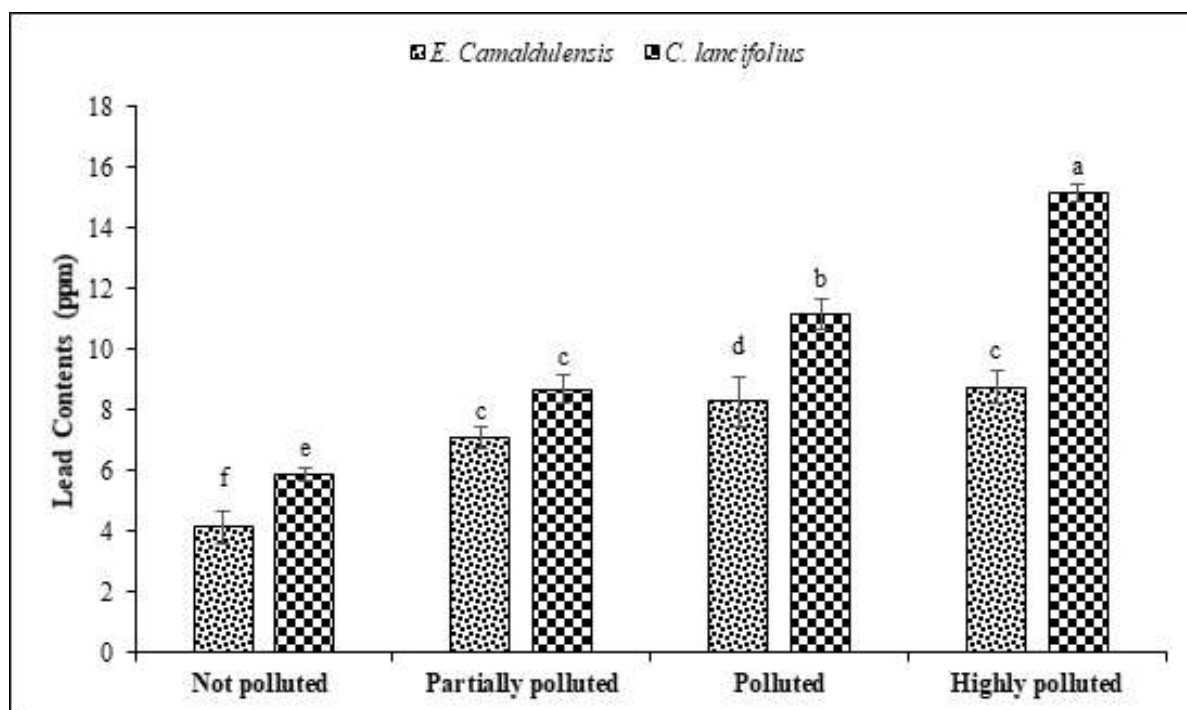


Figure 9. Lead contents in *E. camaldulensis* and *C. lancifolius* under different environments. Different lowercase letters show significant differences among various environments and species.

mediators (Apel and Hirt, 2004). Chen and Gallie, (2005) proposed that the damage induced by oxidative stress can be minimized by various enzymatic and nonenzymatic antioxidant defense mechanisms. The antioxidant defense system includes enzymes such as SOD, CAT, POD, protein, and proline, and various other compounds such as ascorbic acid, glutathione Peptides, and alpha-tocopherol (Gill and Tuteja, 2010). In the present study, the order of species response under different types of environments for the catalase and peroxide contents was *E. camaldulensis* > *C. lancifolius* while for SOD and proline contents, the response was in the order of *C. lancifolius* > *E. camaldulensis* in a highly polluted environment. The elevated concentration of SOD, POD, CAT, proline and dust exhibited by vegetation in highly polluted areas were reported by several researchers (Alscher et al., 2002; Ruley et al., 2004; Qureshi et al., 2007; Gill and Tuteja, 2010;). Similarly, Ram et al. (2015) have also reported a substantial increase in antioxidants and dust levels while the decline in total chlorophyll contents in different tree species such as *Shorea robusta*, *Alstonia scholaris*, *Peltophorum pterocarpum*, *Albizia lebbek*, *Tectona grandis*, *Lagerstroemia speciosa* and *Drosera regia* exposed to industrial pollution. To one side from the amplified concentration of these antioxidants under air pollutants and other abiotic stress, a number of different responses to oxidative stress have been documented e.g., regulation of heat shock protein (Zhen et al., 2007).

In plants, environmental stress often causes the abrupt generation of ROS, producing SOD, POD, and CAT to scavenge the ROS generation (Alscher et al., 2002). The biological harmfulness of Pb ions alone results in lower growth in plants by blocking the cellular mitosis (Sharma and Dubey, 2005). Much of the research work has been done on this aspect to understand the response of different types of plants under polluted environments and reported an increase in the production of antioxidant enzymes (Gill and Tuteja, 2010). The increase in these antioxidant enzymes' activity in response to different levels of pollutants has been reported in several plant species viz. *Sesbania drummondii* (Ruley et al., 2004), *Curcuma angustifolia* (Ram et al., 2015) and *Jatropha curcas* (Shun and Du, 2009). Overexpression of catalase in the cytoplasm or mitochondrial compartment has also been shown to protect cells from oxidative damage (Anderson et al., 1999). Schutzendbel and Polle (2002) believe that the increase in CAT activity may be considered a general response to the production of ROS by tree species cells in severely polluted areas. Under various pollutants and abiotic stresses, this increase in CAT activity has been reported in various plants, such as *Macrotyloma uniflorum* and *Cicer arietinum* (Reddy et al., 2005), and *Vicia faba* (Wang et al., 2008). In the present study, the maximum concentration of CAT was recorded in the highly polluted location of Faisalabad city as compared to polluted and nonpolluted areas. It was reported

that an increased concentration of CAT in plant cells has been correlated with the development of increased tolerance to a variety of chemical compounds and physical stresses (Miller et al., 2008). Apart from the increased concentration of these antioxidants under pollutants and abiotic stress, several different responses to oxidative stress have been documented e.g., regulation of heat shock protein (Zhen et al., 2007). The results regarding antioxidants and protein contents of the present study are in line with the findings of Sharmin et al. (2012), Gill and Tuteja (2010), and Ram et al. (2015).

5. Conclusions

Urban vegetation is an indicator of atmospheric conditions, which acts as a pollutant scouting. Urban vegetation directly reduces the concentration of several environmental pollutants, such as lead concentration and dust load. These pollutants disturb plant functioning by reducing the enzymatic and phenolic activity in leaves, which indirectly lowers the growth and productivity of plants. In *E. camaldulensis* and *C. lancifolius* the antioxidant activity was maximum in a highly polluted environment while minimum in a nonpolluted environment. Regarding lead uptake and dust removal, *C. lancifolius* performed better as compared to *E. camaldulensis*. The above study is helpful in the quantification of services and assessing the diversity of urban trees in Faisalabad city. Furthermore, it will be helpful for the formulation of future planting policies in urban area.

Declarations

Ethical approval

Not applicable

Competing interests

Not applicable

Authors' contributions

Muhammad Azeem Sabir: Original draft preparation. Muhammad Farrakh Nawaz: Conceptualization. Tanveer Hussain Khan: Data analysis. Usman Zulfikar: Investigation, writing-reviewing. Junaid Naseer: Investigation. Sadam Hussain: Writing-reviewing. Sadaf Gul: Writing-reviewing. Muhammad Faisal Maqsood: Methodology. Muhammad Farrakh Nawaz: Supervision. Rashid Iqbal: Visualization. Baber Ali: Editing. Rana Roy: Editing.

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Availability of data and materials

Data used in this study is available online.

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