

Ecophysiological response of *Eucalyptus camaldulensis* to dust and lead pollution

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Abstract

Background: Air and soil pollution are among the main concerns in urban areas worldwide, and dust and heavy metals are major contributors to environmental pollution. Lead (Pb) is a highly toxic heavy metal that badly affects human health as well as plant's survival and growth. Vegetation can play an important role in ameliorating the effects of these pollutants. *Eucalyptus camaldulensis* is well adapted and cultivated throughout a wide range of urban environments from temperate to tropical climates.

Methods: A 90-day experiment was conducted to investigate the effects of lead (Pb) and dust pollution on the growth performance of young *E. camaldulensis* plants. Four months old seedlings were treated with a factorial combinations of Pb (0, 10 and 20 mg/L applied in irrigation) and dust levels (0, 5 and 10 g applied on foliage).

Results: All morphological traits (root length, shoot length, stem diameter) and biomass (root and shoot, fresh and dry mass) of *E. camaldulensis* were significantly reduced when exposed to higher Pb and dust levels. The highest Pb treatments exhibited greater Pb accumulation in plant roots (23.54 ± 1.61 mg/kg), shoots (15.53 ± 1.98 mg/kg), and leaves (13.89 ± 1.49 mg/kg). Dust load on leaves was greater (72.78 ± 8.1 mg/cm²) for those treatments with higher dust and Pb additions compared to the control (16.11 ± 2.0 mg/cm²). Chlorophyll content was greater at the start of the experiment (68.78 ± 0.74 mg/g FW) and progressively decreased over time consistently with the increase of Pb and dust levels applied.

Conclusions: The results of the experiment, suggest that *E. camaldulensis* could be successfully grown in minimum to moderate Pb and dust polluted urban environments.

Keywords: air pollution; urban forestry; environmental degradation; Pb; chlorophyll; tree growth

Introduction

Eucalyptus camaldulensis is a fast-growing tree species that belongs to the Myrtaceae family. It is native to Australia and introduced in Pakistan. It has high timber, firewood, paper production, and ornamental values. It can grow well up to 1027 m above sea level, air temperatures between 20 and 28°C, and rainfall

between 600 and 2900 mm per year (Nawaz et al. 2016). It has been cultivated in waterlogged areas to reclaim soils. It is considered a short rotation forest species able to tolerate drought, compacted soils or soils with high salt concentrations (Hirsch et al. 2020; Miltiadou et al. 2018). For current polluted urban areas, fast-growing and environmentally friendly trees that can mitigate the effects of pollution are highly valued. Consequently,

E. camaldulensis can play an essential role in controlling pollution and trapping dust through its leaves in rural and urban environments. However, growth and physiological responses of *E. camaldulensis* to polluted environments are largely unexplored.

Through dust storms, industrial processes, road traffic, and volcanic eruptions, various dust particles are released into the atmosphere (Rai 2016; Rai & Panda 2014a). Vehicular emissions and a massive quantity of dust accumulated on vegetation and soil, increase environmental pollution (Campos et al. 2020). Most studies have identified the genotoxic consequences of air pollution exposure on plants in urban environments (Campos et al. 2020; do Nascimento Rocha et al. 2018). The process of transpiration and photosynthesis is affected by the accumulation of alkaline dust-like cement on the leaves' surface, covering the stomata (Liao et al. 2020; Thompson et al. 1984). The dust has considerable physical and chemical impacts on plant growth and development (Liao et al. 2020). Industrialisation, population growth, and urbanisation are among the main causes of air pollution. Nowadays, the urban ecosystem is known to be affected by air pollution stress (Rai et al. 2014). Prajapati & Tripathi (2007) have shown that in urban atmospheres, the concentrations of PM10 and PM 2.5 exhibited good agreement with traffic-related pollutants and other combustion processes (Prajapati & Tripathi 2007).

Heavy metals such as lead (Pb) are part of the urban environment, and air pollution has increased their concentration in soil, water, and the atmosphere (Sharma & Dubey 2005; Shu et al. 2012). The increased amounts of heavy metals have shown to cause serious human health problems. Plant morphology and biomass, mineral nutrition, cell division, and germination has been documented to be affected by Pb toxicity (McComb et al. 2012; Munzuroglu & Geckil 2002). The existence of Pb in the soil and water is mainly attributed to anthropogenic activities such as the usage of Pb paints, fuel, and pesticides since Pb usually occurs in small quantities inside the earth's crust (Gupta et al. 2013). It also has a major phytotoxic effect; the physiological features and the genotypes of plants depend on the metal sensitivity of a plant. It may cause retardation of root and leaf growth and changes in chloroplast (Gupta et al. 2011).

In order to use plants to remediate and clean heavy metal pollution, new research into plant resistance

mechanisms are needed (Zhang 1997). Plants show some morphological and physiological variations when grown in polluted areas. These variations may be in plant height, root and stem growth, leaf color, leaf shape, and leaf length (Leghari et al. 2014). Foliage is highly sensitive to air pollutants (Rai & Panda 2014b). Air pollutants affect the plant leaves, slowing the photosynthesis process (Uzu et al. 2010). Some species adapt to specific environmental conditions, particularly trees growing in urban zones play an essential role in removing poisonous material and improving ecological conditions. They generally reduce gaseous air contamination by uptake through leaf stomata and also accumulating particulate matter on leaf surfaces (Nowak et al. 2006). Besides, heavy metal accumulation in plant tissues by trees is an efficient approach for the restoration of polluted lands. (Panda & Rai 2015; Rai 2013; Rai & Panda 2014a; Uaboi-Egbenni et al. 2009).

Vegetation plays a vital role in mitigating soil and environmental pollution problems. *Eucalyptus camaldulensis* is a fast-growing perennial tree which is known to extract heavy metals from the soil storing them in the plant tissues. However, so far, and to the best of our knowledge, no research has been carried out to check the combined effect of lead and dust on *Eucalyptus camaldulensis* growth and morphological attributes. Therefore, the current study was designed to evaluate the combined effect of lead (Pb) and dust application on some morphological and physiological variables of *Eucalyptus camaldulensis*.

Methods

Plant material and site description

The experiment was carried out using four-month-old healthy seedlings cultivated in the open at the Punjab Forest Research Institute (PFRI), Faisalabad, Pakistan. The seedlings were of similar age, with an average height of 15.45 ± 1.5 cm and diameter 0.6 ± 0.15 mm at the start of the experiment.

The research station lies between 30.35 to 31.47°N Latitude and 72.08 to 73°E longitude at an elevation of 150 m above the sea level. The site's climate is classified as semi-arid with long hot summers, mild to cold-short winters, and a rainy Monsoon season. Climatic conditions during the experiment are shown in (Table 1).

TABLE 1: Climatic conditions during the Experiment

Months	Average max temp (°C)	Average min temp (°C)	Average Rainfall (mm)	Relative Humidity (%)	PAN Evaporation (mm)	Sunshine (Hours)
November (2018)	24.4	11.8	1.5	84.6	1.1	3.7
December (2018)	22.0	7.6	4.2	69.3	1.4	6.0
January (2018)	21.1	5.5	0.0	75.9	1.4	6.4
February (2019)	24.0	9.5	9.5	73.3	.19	6.5
March (2019)	31.2	16.4	12.5	61.4	3.2	8.6
April (2019)	36.4	20.8	7.9	47.3	9.1	4.2

Experimental Design

The experiment was carried out from December 2018 to March 2019 in a research area at the Department of Forestry and Range Management, University of Agriculture Faisalabad, Pakistan. The plants were planted in pots of 0.045 m³ volume and 7 kg of substrate were added in the pots. After 15 days of the plant's shifting, two pollutants (Pb and dust) were applied at three levels each, Pb at 0, 10 and 20 mg/L (identified henceforth as Pb₀, Pb₁, and Pb₂) and dust at 0, 5 and 10 g dust (identified henceforth as D₀, D₁, and D₂). Treatments were therefore Pb₀D₀ (No Lead + No Dust), Pb₁D₀ (10 mg/L Lead + No Dust), Pb₂D₀ (20 mg/L Lead + no Dust), Pb₀D₁ (No Lead + 5g Dust), Pb₁D₁ (10mg/L Lead + 5g Dust), Pb₂D₁ (20mg/L Lead + 5g Dust), Pb₀D₂ (No lead + 10g Dust), Pb₁D₂ (10mg/L Lead + 10g Dust), Pb₂D₂ (20mg/L Lead + 10g Dust) treatments.

Nine replications per treatment were used. Pb was applied in the form of Pb (NO₃)₂ through mixing in the irrigation water after preparing a stock solution, while the dust was applied through a foliar application on seedlings. An equal and adequate amount of water was applied to control and treated plants throughout the experiment. About 25 liters of water was applied to each pot during the whole experiment. Consequently, a small perforated and sterile PVC bottle covered with porous cloth was used to apply the measured dust on each plant. For Pb₁ and Pb₂, 225 mg/pot and 450 mg/pot of Pb were supplied to each pot during the whole experiment, respectively. For D₁ and D₂, 120 g/pot and 240 g/pot of dust were applied during the whole experiment, respectively. The soil physico-chemical properties of the substrate are given in Table 2. Data were collected three months after the trial started.

Measurement of morphological traits and biomass partitioning

Eucalyptus seedlings were harvested and divided into different sections: leaves, stems, and roots. Root length and shoot length were measured with a measuring tape. Stem diameter was measured with a digital Vernier caliper. Root, shoot and foliage fresh masses were measured, and then placed into a drying oven at 70°C for 24 hours and then dry masses taken. The root-shoot ratio was also calculated to observe biomass partitioning across treatments.

Determination of leaf chlorophyll content

To estimate leaf chlorophyll concentration, a SPAD meter (SPAD-502, Minolta Camera Co. Japan) was used.

Determination of dust load and lead (Pb) concentration

Foliage samples were collected from the seedlings of *E. camaldulensis*, and the leaf area measured using graduated millimetric paper. After measuring the leaf area, samples were washed, and dust filtered using Whatman 42 filter paper. The filter paper was weighed before and after filtering the dust, dried and then dust mass determined as their difference. Based on the leaf area, we express dust load in mg/cm².

Measurement of physiological traits

The concentration of Pb was determined by using an atomic absorption spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan) following the conditions described in AOAC (1990). Calibrated standards were prepared from the commercially available stock solution (Applichem®) in the form of an aqueous solution (1000 ppm). Highly purified de-ionised water was used for the preparation of working standards. All the glassware used throughout the analytical work process were immersed in 8N HNO₃ overnight and washed with several changes of de-ionised water before use. The instrumental operating conditions for the any reported element are summarised in Table 3.

Statistical analysis

The experiment was set as a Completely Randomised Design (CRD) in order to assess the effect of lead and dust on growth performance, biomass, morphological and physiological variables of *Eucalyptus camaldulensis*. Two-way ANOVA and Duncan's test ($P < 0.05$) were used to compare treatment means and significance levels using SPSS 19.0.

Results

Plant growth

Pb and dust treatments showed a significant impact on root length of *E. camaldulensis* ($p = 0.003$). Root length was maximum (41.3 ± 3.39 cm) at the Pb₁D₀ treatment,

TABLE 2: Physico-chemical properties of the substrate used

Parameters	Sand (%)	Silt (%)	Clay (%)	pH	EC (dSm ⁻¹)	TSS (ppm)	Nitrogen (%)	Phosphorous (ppm)	Potassium (ppm)	SOC (%)
0-15 cm	40 ± 0.8	45 ± 0.7	15 ± 0.4	8.0 ± 0.06	1.7 ± 0.02	1176 ± 48	0.1 ± 0.005	3.9 ± 0.2	280 ± 12	1.54 ± 0.2

TABLE 3: Operational conditions employed in the determination of Pb by atomic absorption spectrophotometer. (OGC= Oxidant gas pressure)

Parameters	Wavelength (nm)	Slit Width (nm)	Lamp Current (mA)	Burner Head	Flame	Burner Height (mm)	OGC (kpa)
Set Values	283.3	1.3	7.5	Standard type	Air-C ₂ H ₂	7.5	0.077 ± 0.005

while it gradually decreased (28.52 ± 2.43 cm) at higher Pb and dust levels. All treatments under Pb_1 showed greater root length compared to the other treatments (Figure 1a).

Shoot length also significantly changed with Pb and dust ($p < 0.001$). The maximum shoot length (46.39 ± 3.5 cm) was observed at the Pb_0D_0 treatment, while the minimum (33.21 ± 1.1 cm) at the Pb_2D_2 . In all treatments, shoot length gradually decreased as the Pb concentration increased from D_0 to D_1 and D_2 (Figure 1b).

In terms of stem diameter, the interaction between Pb and dust were significant ($p = 0.004$). Stem diameter

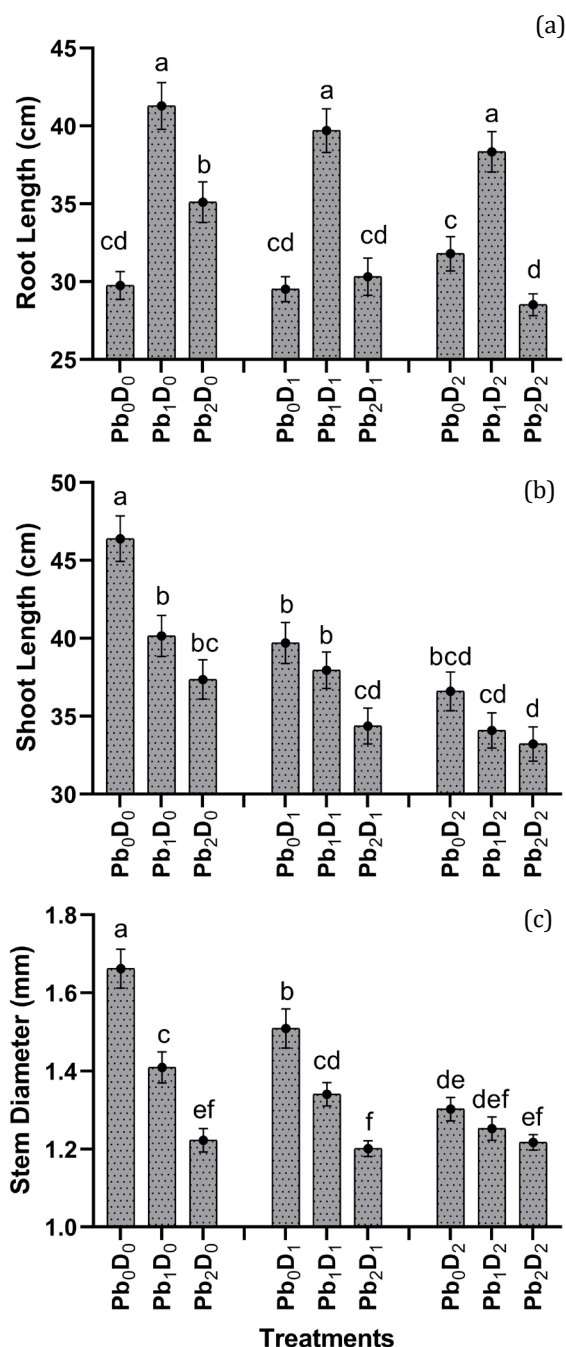


FIGURE 1: Effect of different levels of Pb and dust on *E. camaldulensis* plants. (a) root length; (b) shoot length; and (c) stem diameter.

was maximum (1.66 ± 0.18 mm) at the Pb_0D_0 treatment, being much higher than the response observed at Pb_2D_1 (1.20 ± 0.02 mm). Results showed that the stem diameter generally decreased with increasing dust and Pb concentrations, in the order: $D_0 > D_1 > D_2$ and $Pb_0 > Pb_1 > Pb_2$ (Figure 1c).

Biomass partitioning

Root (fresh and dry) weight significantly change with Pb ($p = 0.001$) and dust ($p < 0.001$) concentrations. Results showed that, Pb_1D_1 treatment revealed greater root biomass while lower root biomass observed in response to the Pb_2D_1 treatment (Figure 2a, b). Shoot biomass production varied significantly with Pb and dust ($p = 0.01$). Shoot represented the highest (fresh and dry) biomass in Pb_1D_0 treatment while the reduction in shoot biomass was recorded in response to higher Pb and dust concentrations (Figure 2c, d). The root-shoot ratio increased with increasing Pb and dust (Figure 2e), although a significant interaction was found ($p = 0.003$).

According to the analysis of variance, foliage biomass of *E. camaldulensis* significantly varied ($p < 0.01$) after the addition of different concentrations of Pb and dust into the growing media. Results revealed that, Pb_1D_1 treatment exhibited greater foliage biomass (fresh and dry), compared to the Pb_2D_1 treatment (Figure 2f, g).

Dust load on leaves

There were significant differences ($p = 0.04$) in dust load across treatments. Dust concentration was greater at the Pb_1D_2 treatment (0.72 ± 0.08 mg/cm²) compared with the control Pb_0D_0 (0.16 ± 0.02 mg/cm²) (Figure 3).

Chlorophyll Content (SPAD-value)

The chlorophyll content showed a significant difference ($p = 0.001$) under different concentrations of Pb and dust. Higher chlorophyll content was found in Pb_0D_0 (68.78 ± 0.74 mg. g⁻¹FW) compared to the other treatments (Figure 4).

Pb concentration in plant organs

There were significant interactions between Pb and dust levels on root ($p = 0.0004$), shoot ($p = 0.0002$) and foliage ($p = 0.0001$) biomasses. Greater root Pb concentration was observed at the Pb_2D_0 treatment (23.54 ± 1.61 mg/kg), compared to the Pb_0D_2 (4.19 ± 0.28 mg/kg) treatment (Figure 5a). Greater shoot Pb concentration (15.53 ± 1.98 mg/kg) was observed at the Pb_2D_2 treatment compared to Pb_0D_2 (3.03 ± 0.34 mg/kg) treatment (Figure 5b). As depicted in Figure 5c, foliage Pb concentration was higher (13.89 ± 1.49 mg/kg) at the Pb_2D_2 compared to the Pb_0D_0 treatment (2.17 ± 0.23 mg/kg). Overall, Pb concentration was consistently higher in roots compared to shoots and foliage.

Discussion

Seedlings of *E. camaldulensis* were grown outdoor in pots under contrasting Pb and dust levels. Several investigations have revealed that the Pb toxicity adversely affects plant growth, metabolism and soil microbial diversity. It may impact plant growth and

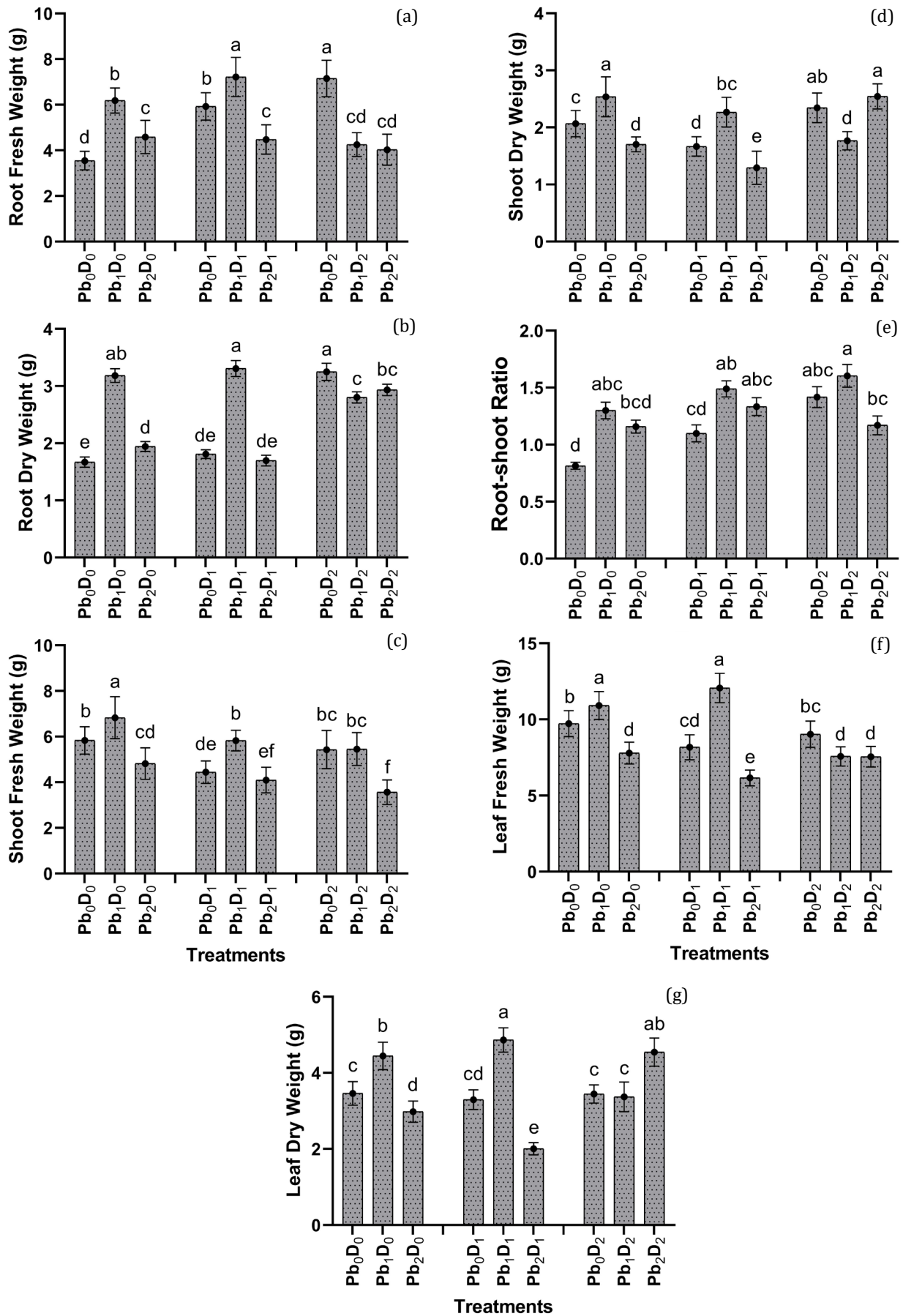


FIGURE 2: Effect of different Pb and dust levels on (a, b) root fresh and dry masses; (c, d) shoot fresh and dry masses and (e) root-shoot ratio; (f, g) leaf fresh and dry masses of *E. camaldulensis* respectively.

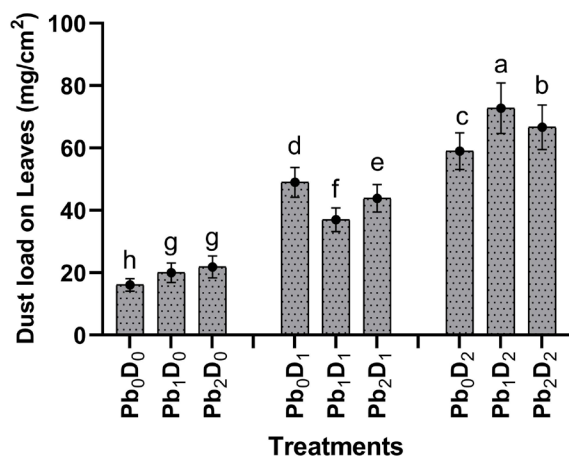


FIGURE 3: Dust load on leaves of *E. camaldulensis* under different concentrations of Pb and dust.

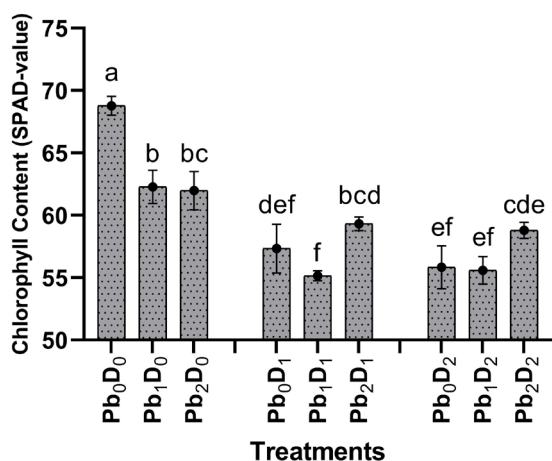


FIGURE 4: Chlorophyll content in leaves of *E. camaldulensis* under different concentrations of Pb and dust.

development, root elongation, and transpiration (Fatemi et al. 2021; Kushwaha et al. 2018; A. Singh et al. 2016; Xie et al. 2017). Besides, dust particles with high hazardous metal concentrations become one of the major concerns responsible for stunted plant growth (Jia et al. 2021; Karami et al. 2017).

Our results indicated that the high concentration of Pb and dust had adverse effects on plant morphological growth and biomass. Root and shoot length and stem diameter decreased at high Pb and dust concentration, as discussed by (Karami et al. 2017). Shu et al. (2012) found that root growth and the amount of root hairs decreased with increasing Pb, observing also changes in root color. Pb directly affects the plant root system, and roots quickly react by removing and synthesizing callose and discontinuing the intake of Pb by establishing a barrier (Fahr et al. 2013). Different mechanisms involved in reducing Pb uptake and allocating towards above-ground parts of plant, tolerance, and uptake depend on roots condition (Fahr et al. 2013). In other studies, Pb accumulation and cell showed a different response in primary and adventitious root systems in sunflower.

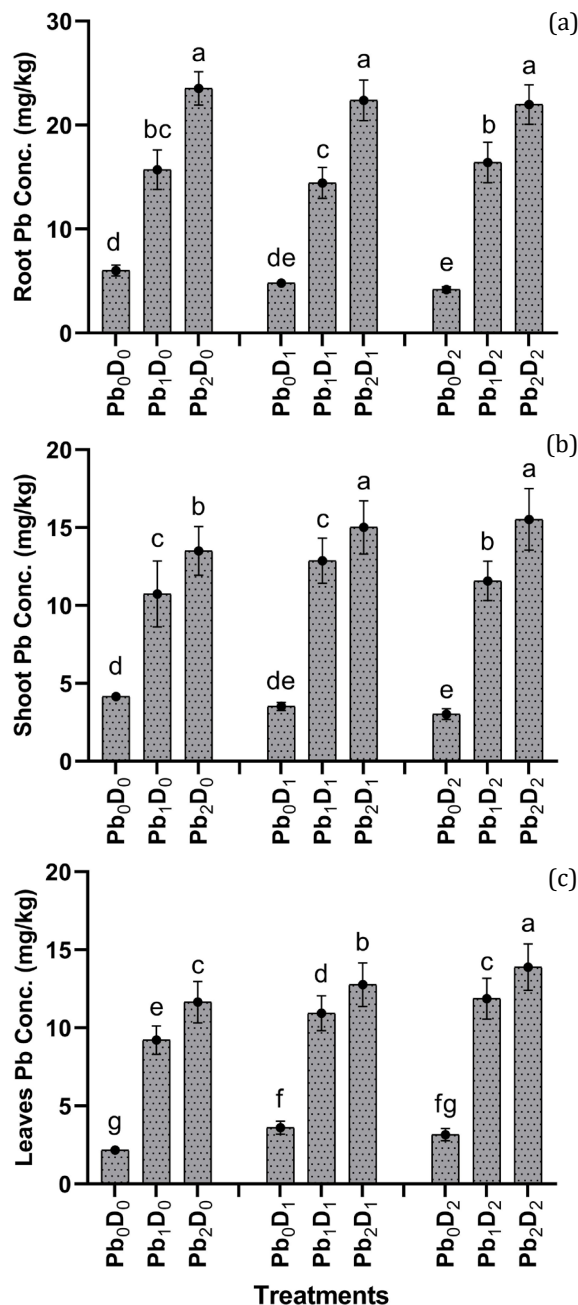


FIGURE 5: Pb concentration in the (a) root, (b) shoot, and (c) leaves of *E. camaldulensis* under different concentrations of Pb and dust.

It was observed that the adventitious root system was more tolerant to Pb in *Helianthus annus* L. and *Allium cepa* (Michalak et al. 1998; Strubińska & Hanaka 2011). However, the response of plants towards Pb causes a decrease in root growth and biomass production (Fargašová 2001), as observed in our study (Figure 2a, b). Pb accumulates in all plants, but its highest levels were measured in roots while lowest in shoots (Di Lonardo et al. 2011). Pb and Cd caused a reduction in shoot length in *Albizzia lebbek* (Farooqi et al. 2009). In our study, stem diameter of *E. camaldulensis* was significantly influenced by Pb and dust concentration i.e., stem diameter decreased gradually with the increase of

Pb and dust concentrations (Figure 1c). By increasing Pb concentration, height and ground diameter also decline in *Ligustrum vulgare* seedlings (Zhou et al. 2018), which are similar to our findings.

In our study, the biomasses of root, shoot, and leaves (fresh and dry) decline at higher Pb and dust concentration (Figure 2). These findings are in agreement with (Fatemi et al. 2021; Karami et al. 2017; Shu et al. 2012), who concluded that high Pb concentration inhibits plant biomass production of cuttings and seedlings of *J. curcus*. Similar results were reported by (Abdul 2010) and (Shafiq et al. 2008). However, a decreasing trend was observed with the increase of Pb concentration by (Karami et al. 2017; Lakshmi 2014). Similarly, under high dust levels, commonly plant biomass declines (Leghari et al. 2014).

Applying dust on leaves may impair its photosynthetic capacity by setting a thick layer on them, which limits sunlight reducing photosynthesis, causing the deterioration of leaves in the form of premature senescence, and reducing the growth of sensitive plants (Brandt & Rhoades 1973; Karami et al. 2017; Raina et al. 2008). According to our study, foliage dry and fresh weight was higher at the Pb₁D₁ treatment while it decreased at the highest doses of both factors (Figure 2e, f). Shu et al. (2012) showed that Pb inhibits foliage growth and stimulated membrane's structural damage. Pb accumulation mainly happens in leaves (Stefanov et al. 1995), with a concomitant decrease in leaf biomass as Pb concentration increase (Shafiq et al. 2008). Under high Pb exogenous additions, a decline in transpiration and stomatal conductance occurred in plants (Shu et al. 2012).

As expected, the dust load on leaves increased with the increase in dust levels (Figure 3), which results in a decline in foliage growth (Rai & Panda 2014b). (Leghari et al. 2014) estimated the effect of dust pollution on the leaf expansion and total chlorophyll content in *Vitis vinifera*, finding a decreasing trend in chlorophyll content with increasing Pb concentration similar to our study. These results are similar to those found by Shu et al. (2012) who showed that Pb concentration had a positive effect on chlorophyll content under low concentration and showed inhibition at higher Pb levels. Dust also has a drastic impact on chlorophyll and plant growth (Leghari et al. 2014; Swain et al. 2016). The response of seedlings to dust accumulation causes degradation of chlorophyll and inhibit the photosynthesis process (Gajewska et al. 2006), due to lipid peroxidation degradation (S. Singh et al. 2006).

The Pb concentration and its accumulation in different parts of the plant (root, shoot, and leaves) have been studied before. As expected, we found that Pb tissue concentration increased with increasing exogenous Pb levels. Roots can act as barriers to prevent Pb being transported towards the shoot (Shu et al. 2012). In our case, the root exhibited a consistently higher Pb concentration compared to the shoots and leaves although the response to the treatments was similar, being possible that roots acted as a barrier to the movement of Pb. Higher Pb concentration was found at

a high exogenous dose of Pb (Pb₂D₀, Pb₂D₁, and Pb₂D₂), consistently with the findings of (Peng et al. 2012; Shafiq et al. 2008).

Conclusions

Different Pb and dust concentrations significantly affected the morphological characteristics, biomass, chlorophyll content, dust load, and Pb concentrations in plant tissues. The consequences of Pb and dust on the plant cause slow and stunted growth. By decreasing Pb and dust pollution, we may enhance plant growth and development of *E. camaldulensis*.

Competing interests

The authors declare that they have no conflicts of interest.

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Authors' contributions

Conceptualisation: (MFN); Methodology: (MZA and MAS); Validation: (SG); Formal analysis: (MHUR and THF); Investigation: (MFN); Data curation: (MFN); Writing—original draft preparation: (MHUR); Writing—review and editing: (MFN, THE, and MHUR); Visualisation: (NPG); Supervision: (MFN and MHUR); Funding acquisition: (MFN).

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