



Phytoremediation potential and Bioindicator Capabilities of *Nerium oleander* Under Traffic-Related Heavy Metal Stress

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DOI: <https://doi.org/10.54692/lgujls.2025.92418>

Vol 9 Issue 2, July- December 2025

Received: 08-01-2025

Revised: 26-09-2025

Accepted: 017-10-2025

Citation: Ali R, Butt S, Khan AS (2025). Phytoremediation potential and Bioindicator Capabilities of *Nerium oleander* Under Traffic-Related Heavy Metal Stress. LGU Journal of Life Sciences. 9(01):12-20.

ABSTRACT

Rapid urbanization and increasing vehicular traffic in Lahore have contributed to elevated levels of airborne heavy metals, posing risks to both ecosystems and public health. Identifying resilient plant species that can mitigate and signal such pollution is therefore critical for sustainable urban management. This study evaluated the phytoremediation potential and bioindicator capability of the ornamental shrub *Nerium oleander* growing in high-traffic zones of Lahore, Pakistan. Leaf samples were collected from two major polluted sites i.e., Canal Road and Ferozpur Road and compared with control samples from less polluted areas, including the Forman Christian College University (FCCU) botanical garden and adjacent residential zones. Heavy metal concentrations, particularly chromium (Cr) and lead (Pb), were quantified using Atomic Absorption Spectroscopy (AAS). Anatomical analysis through hand-sectioning revealed significant reductions in mesophyll and vascular tissue widths in plants from polluted sites. In addition, chlorophyll a and b contents showed a marked decline under metal stress. The accumulation of Cr and Pb in leaf tissues, along with associated anatomical

and physiological changes, suggests that *N. oleander* can serve as a potential phytoremediator and bioindicator of airborne pollutants in traffic-dense settings. These results indicate a strong association between traffic intensity and metal accumulation, though further controlled studies are needed to confirm causal relationships and long-term tolerance.

Keywords: Anatomical alterations, bioindicator, chlorophyll content, heavy metal stress, phytoremediation, urban environment.

INTRODUCTION

Environmental pollution is a growing concern in developing countries, including Pakistan. Among the various pollutants, heavy metals are particularly

persistent and harmful due to their non-biodegradable nature and toxicity. They are defined as metallic elements with high atomic weights and densities at least five times greater than water.

Common heavy metals of concern include arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb), and zinc (Zn). They are introduced into the environment through anthropogenic activities including mining, industrial discharges, application of fertilizers and pesticides, waste incineration, and vehicular emissions (Adamiec et al., 2016). In urban settings, vehicular emissions are recognized as a major contributor to airborne lead and other heavy metals (Angon et al., 2024). In plants, heavy metal stress induces oxidative damage, alters chloroplast ultrastructure, reduces photosynthetic pigment levels, and impairs enzymatic activity activities by decreasing the function of key enzymes like acid phosphatase, urease, catalase, and invertase, ultimately compromising growth and productivity (Abedi et al., 2022; Collin et al., 2022; Rehman et al., 2023). The persistence and toxicity of heavy metals highlight the urgent need for cost-effective and eco-friendly remediation strategies in urban ecosystems.

Cr and Pb are non-essential elements known for their toxicity to plants and animals. In plants, Cr toxicity is largely linked to its uptake and intracellular accumulation, which triggers many physiological and structural disturbances. These include root cell damage, disruption of chloroplast ultrastructure, reduction in photosynthetic pigments, inhibition of photosynthesis, and impairment of water and nutrient uptake. Cr exposure also hampers nitrogen assimilation and enzymatic activities, primarily by inducing excessive reactive oxygen species that disrupt the redox homeostasis of cells (Anjum et al., 2017; Sharma et al., 2020). Increased Cr(VI) levels are reported to elevate hydrogen peroxide production, resulting in lipid peroxidation and the upregulation of antioxidant enzymes such as superoxide dismutase and guaiacol peroxidase (Maiti et al., 2012). Pb contamination similarly poses severe ecological challenges. It contributes to soil acidification and phosphorus deficiency, ultimately reducing soil fertility and plant productivity (Collin et al., 2022). Pb²⁺ and its hydroxy complexes tend to accumulate predominantly in root tissues, adversely affecting

seed germination, chlorophyll synthesis, and overall plant growth (Ghani et al., 2021; Soliman et al., 2019; Yahaghi et al., 2019). Furthermore, Pb interferes with several key physiological processes by suppressing the activities of enzymes such as acid phosphatase, urease, catalase, and invertase. It also impairs nutrient uptake, disturbs water balance, and disrupts metabolic functions critical for plant development (Rehman et al., 2023).

In recent years, the concentration of airborne heavy metals has risen sharply in Lahore due to rapid urbanization and increased vehicular activity (Bibi et al., 2016; von Schneidmesser et al., 2010). Conventional remediation methods such as chemical treatment, incineration, and excavation are often expensive, invasive, and ecologically damaging. In contrast, phytoremediation has emerged as a sustainable, low-cost, and eco-friendly alternative (Azizi et al., 2023; Yan et al., 2020). Phytoremediation refers to the use of plants and their associated rhizospheric microorganisms to remove, degrade, or stabilize environmental contaminants. Different mechanisms include phytoextraction, phytostabilization, phytovolatilization, phytodegradation, and rhizodegradation (Tangahu et al., 2011; Yan et al., 2020). In phytoextraction, plant roots absorb heavy metals and translocate them to aerial parts, making the process effective for remediation of contaminated soils and air. Despite the toxicity, many plants have evolved detoxification strategies that enable them to tolerate and accumulate heavy metals (Viehweger, 2014).

N. oleander, an ornamental and medicinally valuable shrub, is widely grown in urban landscapes for its aesthetic and aromatic appeal. Besides its decorative value, it has been traditionally used to treat anxiety, digestive disorders, and infections due to its antioxidant-rich components. Given its hardy nature, adaptability, and widespread cultivation, it presents potential as a phytoremediator for urban air pollutants. The aim of this research is to evaluate the Cr and Pb uptake capacity of *N. oleander* growing along high-traffic roads in Lahore, and to analyze associated anatomical and physiological changes in the plant. This study also seeks to advocate the

strategic plantation of heavy metal-accumulating species such as *N. oleander* in polluted urban areas to mitigate airborne pollution and improve urban environmental health.

MATERIALS AND METHODS

Field sampling

At each site, a total of 20 foliar samples of *N. oleander* were collected (4 leaves from each of 5 plants) from three locations in Lahore, Pakistan. Canal Road sample (31.5226° N, 74.3505° E) is a six-lane dual carriageway approximately 24 m wide, with an estimated daily traffic volume exceeding 90,000 vehicles. Ferozpur Road sample (31.4758° N, 74.3259° E) is an eight-lane urban arterial road approximately 30 m wide, carrying an estimated 80,000 vehicles per day. Other potential sources of heavy metals were controlled by selecting control plants from the FCCU botanical garden and adjacent low-traffic residential areas due to negligible vehicular flow. Control plants were located in the FCCU botanical garden (31.5154° N, 74.3403° E), adjacent to low-traffic residential streets with negligible heavy vehicular flow (<10 vehicles/day) (Fig. 1). At each site, leaves of similar age and position were collected from five trees situated 2–3 m from the road edge (for experimental sites) and internal garden paths (for control). Sampling height was maintained at 1.5–2.0 m above ground level, and collections were made during early morning hours in late spring to minimize diurnal variation in leaf water status. Each treatment consisted of five biological replicates, and all measurements were performed in technical triplicate to ensure precision.

Slide preparation: Transverse sections of control and experimental leaf samples were prepared using hand-sectioning, stained with toluidine blue, and observed under a light microscope (MT5300H – Meiji Techno Co., Ltd, Japan) at 10× magnification. Measurements of vascular and mesophyll tissues were recorded using an ocular micrometer and converted into micrometers (μm) using a stage micrometer.

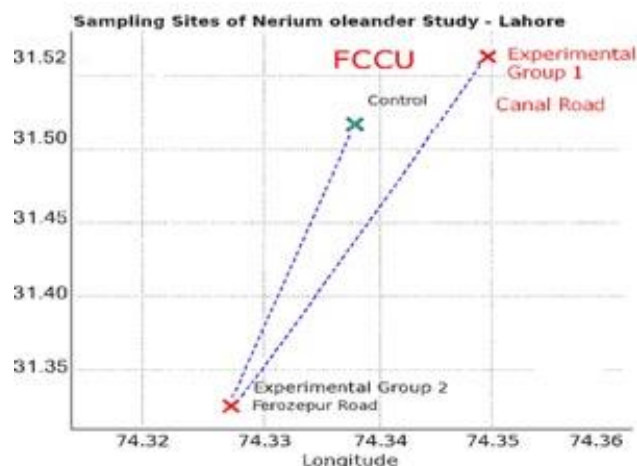


Figure 1: Sampling Sites Map - Map showing the locations of the three sampling sites in Lahore, Pakistan. Canal Road and Ferozpur Road represent high-traffic sites where *N. oleander* leaves were collected. The FCCU Botanical Garden site serves as the low-traffic control. High-traffic sites are marked in red, and the control site is marked in green.

Chlorophyll estimation: Acetone was used as the solvent. A 100 mg sample was used and crushed using a pastel and mortar for 3-5 minutes until it was devoid of green color. For mixing of chlorophyll, 4ml of 80% acetone was used. It was then further rinsed with 2ml acetone and the final concentration was raised to 10 ml using acetone (80%). It was then transferred to a centrifuge tube, and then centrifuged at 10,000 rpm for 5 minutes. After centrifugation, 3ml of the supernatant was extracted and transferred to a cuvette which was then analyzed for the amount of chlorophyll 'a' and 'b' present at different wavelengths by a UV/VIS spectrophotometer. In spectrophotometry, acetone was used as a blank. For estimating chlorophyll 'a' and 'b' the absorbance of samples at wavelengths of 663nm and 645nm respectively were recorded (Arnon, 1949).

Chlorophyll concentrations were calculated using the following equations:

$$\text{Chlorophyll a (mg/g)} = [(12.7 \times A_{663}) - (2.69 \times A_{645})] \times \text{Volume of acetone (mL)} / \text{Weight of leaf tissue (mg)}$$

AAS analysis: Prior to analysis, plant samples were thoroughly washed with tap water followed by distilled water to remove surface dust and atmospheric deposits. Cleaned samples were air-dried under shade, then oven-dried at 70 °C until constant weight. The dried tissues were ground using

a pre-cleaned stainless-steel grinder and stored in acid-washed airtight polyethylene bags at room temperature to avoid contamination or analyte loss. Cr and Pb concentrations in plant tissues (roots and shoots) were quantified using atomic absorption spectroscopy (AAS) after 20, 40 and 60 days. Dried and ground samples (0.10 g) from each plant were digested in a mixture of concentrated nitric acid (15 mL) and sulfuric acid (10 mL) using a hot-plate digestion method. The digest was heated until a clear solution was obtained, which was then diluted to 25 mL with distilled water (Anwar et al., 2023). Absorbance was measured at 357.9 nm for Cr and 217.0 nm for Pb using a Varian AA240FS, Fast Sequential AAS.

Statistical analysis: All experimental measurements, including anatomical parameters (vascular and mesophyll tissue widths), chlorophyll content (chlorophyll a and b), and heavy metal concentrations (Cr and Pb), were statistically analyzed to assess the significance of differences between control and experimental groups. Each treatment was conducted in triplicate, and all measurements were performed on five biological replicates to ensure reliability. Data were expressed as mean \pm standard error of the mean (SEM). A one-way analysis of variance (ANOVA) was applied to determine whether there were statistically significant differences among the groups. Following ANOVA, Tukey's HSD test was used to compare each experimental group directly with the control group to identify specific significant differences at ($p \leq 0.05$).

RESULTS

The anatomical parameters such as width of vascular region and width of palisade as well as spongy mesophyll region were checked for control as well as experimental groups of the *N. oleander*. Width of vascular region, width of palisade as well as spongy mesophyll region showed decrease in experimental groups as compared with control of this species. The

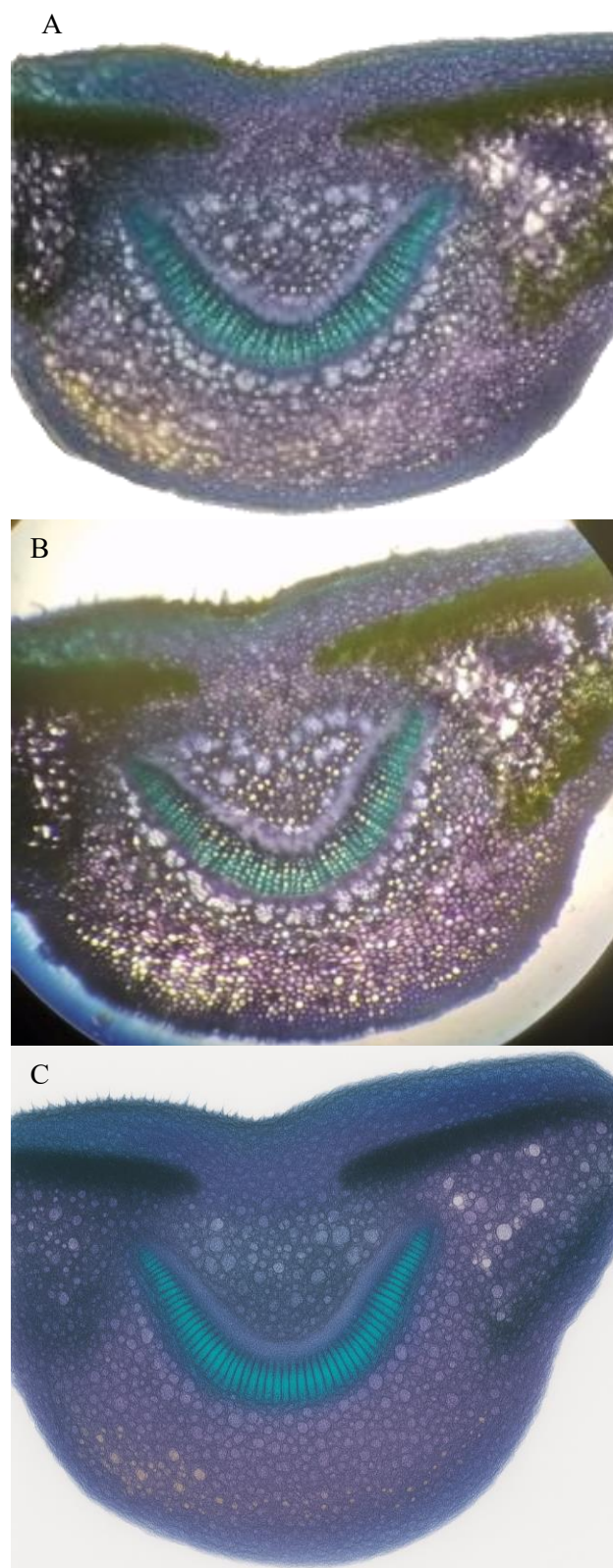


Figure 2 (A-C): (A) Transverse sections (T.S.) of *N. oleander* leaf under 10 \times magnification: (a) Control group (unpolluted site); (b) Canal Road Group (c) Ferozepur Road Group

Canal Road group showed decrease of 24.01% in width of vascular region while Ferozpur Road group showed 20.83% decrease in width of vascular region (Table 1, Fig. 2). The Canal Road showed 27.07% reduction in width of palisade mesophyll region while it also showed reduction of 13.64% in width of spongy mesophyll region (Table 1, Fig. 2). The Ferozpur Road group showed decrease of 18.17% in width of palisade region while it showed decrease of 13.64% in width of spongy mesophyll region.

Table 1: Comparative effects of Cr and Pb stress on vascular and mesophyll tissue widths (palisade and spongy) in control and experimental groups of *N. oleander* (values represent mean \pm SEM; n = 5)

Treatments	Vascular region (μm)	Palisade mesophyll (μm)	Spongy mesophyll (μm)	P value
Control	318.01 \pm 0.541	35.060 \pm 0.037	72.005 \pm 0.754	—
Canal Road	242.168* \pm 0.520	25.061* \pm 0.539	62.985 \pm 0.127	0.018
Ferozpur Road	252.32* \pm 1.829	29.890* \pm 0.778	62.183* \pm 0.231	0.027

* experimental groups are substantially different from the control group at $p \leq 0.05$ according to the Tukey's HSD test.

Both experimental groups of *N. oleander* showed decrease in content of both chlorophyll 'a' and 'b' as compared with control of this species. The Canal Road group showed decrease of 30.76% and 3.78% in content of chlorophyll 'a' and 'b' respectively (Fig. 3).

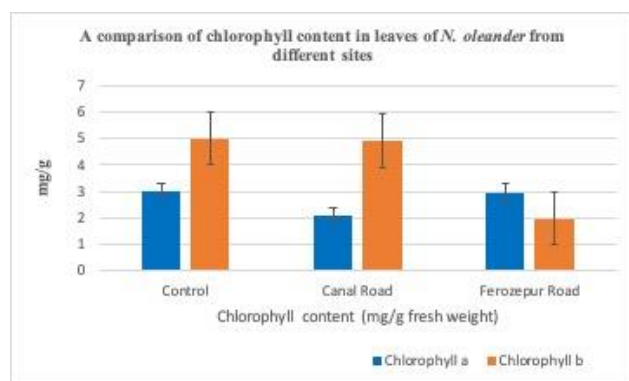


Figure 3: Comparative analysis of chlorophyll *a* and *b* content in control and experimental groups of *N. oleander*. An asterisk denotes a statistically significant difference from the uncontaminated control under different soil conditions, as determined by Tukey's HSD test ($p \leq 0.05$)

In *N. oleander*, Canal Road group exhibited the highest accumulation of heavy metals, with a Cr concentration of 41.56 mg/kg and Pb concentration of 33.56 mg/kg in leaf tissues. These elevated values reflect the heavy traffic load and higher pollutant exposure at Canal Road, which likely results in greater deposition and foliar absorption of airborne contaminants. In comparison, Ferozpur Road group also showed accumulation, though to a slightly lesser extent, recording 33.56 mg/kg of Cr and 33.78 mg/kg of Pb (Fig. 4a and b).

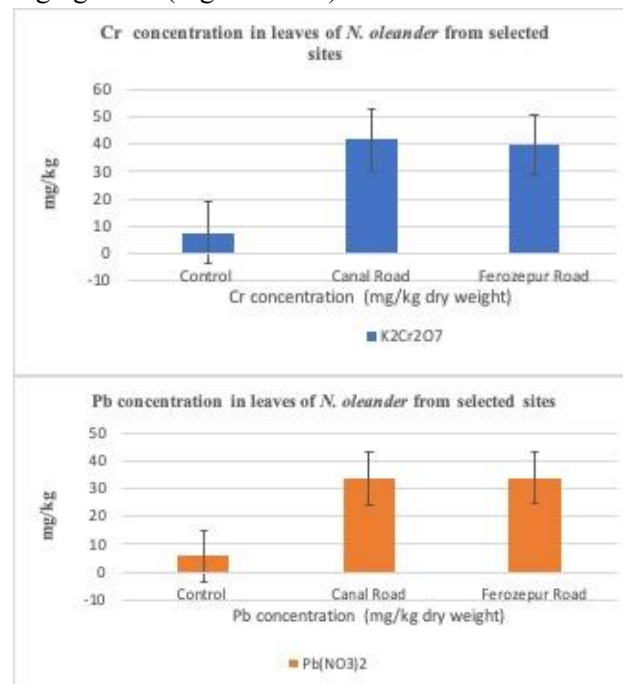


Figure 4 (a and b) Cr and Pb (mg/kg) accumulated in leaf tissues of *N. oleander* from experimental groups. An asterisk denotes a statistically significant difference from the uncontaminated control under different soil conditions, as determined by Tukey's HSD test ($p \leq 0.05$)

While still indicative of substantial pollution, the slightly lower traffic density and potentially different urban layout of Ferozpur Road may contribute to the reduced concentrations observed as compared with control residential site (Fig. 5).

DISCUSSION

In the present study, several anatomical and physiological parameters including leaf color, area, vascular width, palisade and spongy mesophyll thickness, chlorophyll content, and heavy metal

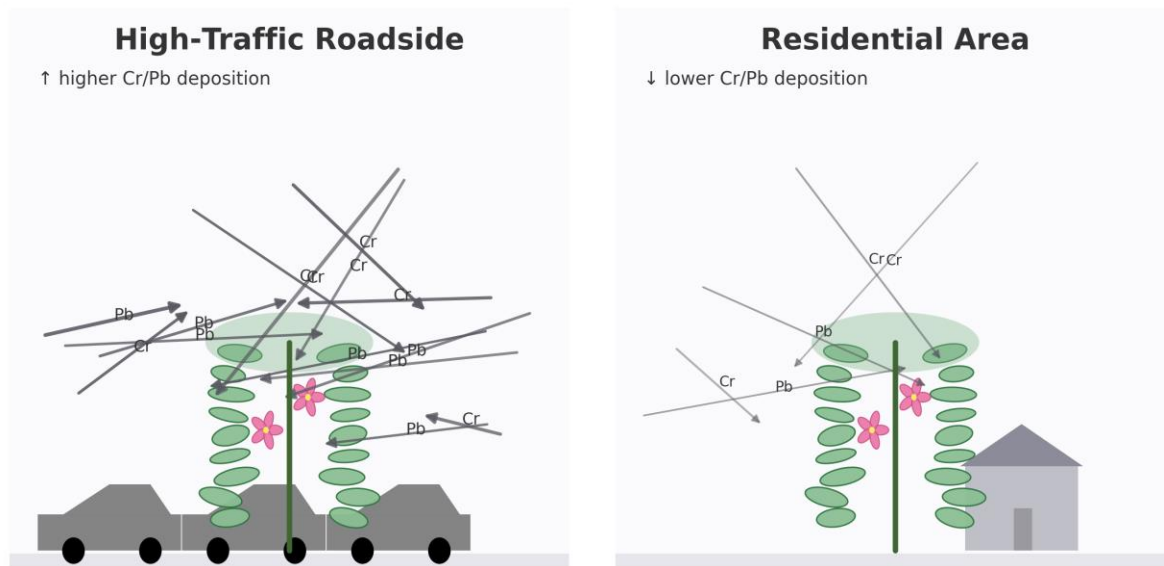


Figure 5: Conceptual illustration: foliar uptake of Cr and Pb in *N. oleander* across contrasting urban microenvironments. Arrows indicate directional entry of Cr and Pb into leaves; arrow density reflects relative magnitude. This figure is an original conceptual illustration prepared by the authors for this study. All rights are retained by the authors.

accumulation were evaluated in *N. oleander* collected from high-traffic locations in Lahore. The experimental groups consisted of leaf samples from Canal Road and Ferozpur Road, while the control group comprised samples from the FCCU botanical garden and nearby low-traffic residential areas.

The width of the vascular region showed a significant reduction in both experimental groups when compared with the control, with Canal Road group showing a greater reduction than Ferozpur Road group (Fig 2 A-C). This decrease aligns with previous findings that Cr and Pb exposure can lead to cellular disruption and collapse of vascular tissues, ultimately impairing translocation of water and nutrients (Khan et al., 2025; Rehman et al., 2023). The more pronounced reduction observed in Canal Road group may be due to higher pollutant exposure at Canal Road, which experiences denser and more continuous vehicular traffic compared to Ferozpur Road, resulting in greater accumulation of airborne particulates and metal stress. Similarly, the palisade mesophyll showed a 27.07% decrease in thickness in Canal Road group compared to 18.17% in Ferozpur Road group, while the spongy mesophyll width was reduced by 13.64% in both

groups. These reductions suggest compromised leaf internal architecture and impaired gaseous exchange due to metal-induced oxidative stress and inhibition of cellular expansion. Literature supports that heavy metals such as Cr and Pb can damage mesophyll structure and reduce intercellular spaces, thereby limiting CO₂ diffusion and photosynthetic activity (Liza et al., 2020; Suri et al., 2023).

The chlorophyll content also declined in both experimental groups, further confirming the stress response. Chlorophyll *a* and *b* levels in Canal Road group decreased by 30.76% and 3.78%, respectively, whereas Ferozpur Road group showed a smaller reduction of 6.59% in chlorophyll *a* but a much greater decline in chlorophyll *b* (64.96%) (Figure 3). This asymmetrical decline in pigment concentrations suggests that different traffic zones may influence pigment synthesis and degradation pathways in distinct ways. Canal Road group overall higher reduction in total chlorophyll reflects its exposure to more prolonged and severe pollution stress. Meanwhile, the significant drop in chlorophyll *b* in Ferozpur Road group may point to greater sensitivity of chlorophyll *b* synthesis pathways or preferential degradation under Pb exposure. Previous

studies confirm that heavy metals can interfere with magnesium chelation, enzyme activity, and the stability of chlorophyll molecules (Amin et al., 2018; Anwar et al., 2023; Supriatno and Rahmatan, 2019).

AAS further confirmed metal accumulation in *N. oleander* leaf tissues. Canal Road group accumulated 41.56 mg/kg of Cr and 33.56 mg/kg of Pb, while Ferozepur Road group accumulated slightly less i.e., 39.87 mg/kg Cr and 33.78 mg/kg Pb in comparison with control where Cr and Pb concentration was significantly low (Fig 4a and b). The relatively higher concentrations in Canal Road group corroborate its more intense traffic-related pollution load and explain the stronger anatomical and physiological impairments observed in these samples. These findings are consistent with earlier research suggesting that plants located near high-traffic zones experience higher rates of heavy metal deposition, uptake, and physiological stress (Ramana et al., 2013).

Reductions in mesophyll and vascular region thickness, chlorophyll pigment degradation, and confirmed metal accumulation validate the hypothesis that *N. oleander* is both sensitive to and capable of accumulating airborne Cr and Pb. The plant physiological responses are consistent with known mechanisms of metal toxicity, such as reactive oxygen species generation, membrane lipid peroxidation, chloroplast damage, and inhibition of metabolic enzymes. Importantly, the plant did not show complete failure in growth or tissue formation, indicating a level of tolerance and detoxification capacity. *N. oleander* demonstrates a dual role in polluted urban environments: it not only absorbs and tolerates heavy metals confirming its potential as a phytoremediator but also exhibits measurable morphological and physiological changes, highlighting its function as a bioindicator. Its wide availability, ornamental value, and visible responses to pollution make it an ideal candidate for green belt development and ecological monitoring in urban centers like Lahore.

Conclusion

This study indicates that *N. oleander* has potential as both a phytoremediator and a bioindicator of traffic-related heavy metal pollution in urban environments. Plants from high-traffic sites in Lahore, particularly Canal Road and Ferozepur Road, showed measurable anatomical alterations and declines in chlorophyll pigments, coinciding with elevated Cr and Pb concentrations. While these patterns are consistent with heavy metal stress responses, they represent an association rather than definitive proof of causal effects or inherent tolerance. Broader, multi-season studies incorporating environmental co-variable measurements are recommended to validate and expand these findings. Future research could explore the use of *N. oleander* in controlled remediation trials and assess its capacity to accumulate other traffic-related metals (like cadmium or zinc), as well as the long-term effects of such accumulation on the plant health.

Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

The research concept and experimental design were developed by Aisha Khan, who also supervised this project. Ali Raza conducted field experiments and collected laboratory data. Saba Butt contributed to the preparation and application of statistical design of this research work. The manuscript was primarily written by Ali Raza with contributions and editing support from Aisha Khan. All authors reviewed and approved the final version of the manuscript.

Acknowledgements

The authors gratefully acknowledge the Prof. Dr. Kauser Abdulla Malik School of Life Sciences (KAM-SLS), Dean of Postgraduate Research and Dr. Bilal Sadiq, Head of School of Life Sciences at Forman Christian College (A Chartered University), Lahore, for providing access to laboratory, greenhouse, and microscopy facilities essential to this study. Financial support in the form of equipment uses and field resources was provided in part by departmental research funds.

Ethical Statement: Not Applicable

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