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EFFECTS OF LEAD ON ANTIOXIDANT ENZYME ACTIVITIES IN *NERIUM INDICUM* L. BYGROWING IN ROADSIDE SOIL OF LAHORE

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Abstract

Concentrations of different roadside soil amendment (0, 50, 75 and 100%) were prepared by mixing with garden (control) soil. All pollution parameters were determined in the different concentrations of soil *i.e.*, pH, TDS, EC, CO_3^{-2}, HCO_3. The highest values of pH (8.78), ECe (338 μS cm^{-1}), and TDS (696 mg L^{-1}), were observed in the 100% concentration, indicating a higher level of pollution compared to the control sample. The maximum value of bicarbonates (87 mg L^{-1}) recorded in 100% as compared to 0% (22 mg L^{-1}). Highest values of Pb were present in 100% (770 mg kg^{-1}) as compared tocontrol. With increasing soil concentrations, all of these indicators showed rising trends, with Pb>Cd being the most notable. *Nerium indicum* L. was grown in these concentrations for 35 days of the experiment. The growth parameters i.e., root length, shoot length; no. of roots and no .of leaves were recorded in all soil concentrations. The maximum growth was found to be in 100% and minimum in 50% and no growth was observed in 0% concentrations. The accumulation of heavy metals Pb and Cd was observed and increasing trend was Pb>Cd. The amount of protein was also detected, maximum found in the concentration of 100% which would be 20.54%, also determined in all plants that had grown in varying concentrations of soil. The amount of protein increased with increasing the concentrations. The SOD was determined in plants parts increased with increasing concentrations of soil; highest value was recorded in 100% (3.54 per mg^{-1} protein). The aim of the study was to check the effect of heavy metals in *Nerium indicum* L. by growing in different concentrations of roadside soil. The plant was further assessed to determine the role of protein and antioxidants activities.

Keywords: Antioxidant enzyme; Lead; *Nerium indicum* L; Toxicity

Introduction

Nerium indicum L. is the evergreen shrub that blooms with flowers. Urban gardens and public locations grow among the Dogbane family members as an ornamental plant. These plants are distributed globally, especially in mild temperate and subtropical regions. They started in Asia and the Mediterranean (Tinelli *et al*., 2023). Oleander is its common name because it resembles the unrelated olive *Olea*. It is widely cultivated and maybe native to Southwest Asia.

Oleander, a hot-growing subtropical plant, is commonly used as a landscape accent there and elsewhere (Potapenko *et al*., 2020). Global health is threatened by pollution. Climate change, biodiversity loss, ocean acidification, desert spread, and groundwater depletion undermine Earth's processes and threaten human life (Landrigan *et al*., 2018). Metallic contaminants are well-known to be harmful. Toxic environments increase headaches, nausea, exhaustion, sudden abortion, and dermatological disorders. Long-term pollution causes malignant tumors, leukemia, reproductive dysfunction, renal or hepatic impairment, and CNS dysfunction. Children are worse off than adults (Altaf *et al*., 2021).

Flora and soil samples are the cheapest and easiest technique to test roadside heavy metals. Alternative plant species, grasses, and fish can detect heavy metal contamination (Khan *et al*., 2023). This study examined how wild species along roadways absorb heavy metals due to the importance of wild plants and their habitats (Altaf *et al*., 2021).

Heavy metal pollution includes elements with atomic numbers and masses more than 20 and 5g cm-3, respectively. The metals are necessary or non-essential (Filipoiu *et al*., 2022). Copper, zinc, nickel, iron, chromium, and manganese are necessary for plant growth. These metals are essential for nature and human use. Lead, mercury, arsenic, and cadmium are non-essential HMs of great concern. Low concentrations of these toxic metals can damage plant physiology and biochemistry. Interesting, their biological actions are unknown (Shore, 2020). High lead concentrations inhibit flowering (Soliman *et al*., 2022). Higher soil metal concentrations affect seed germination, plant development, production, physiology, biochemistry, and genetics (Hussain *et al*., 2023). Soil chemistry, pH, and plant traits affect plant toxicity (Wang *et al*., 2020).

Lead (Pb), the most toxic heavy element, is most crucial (Singhal *et al*., 2023). The physico-chemical features date its use to ancient times. It's a vital but dangerous environmental chemical worldwide. Its unique features, including as pliability, workability, flexibility, limited conductivity, and corrosion resistance, make it difficult to abandon (Krivokapić, 2020). Because it is non-degradableand used repeatedly, its environmental levels rise, raising risks (Niu *et al*., 2023). Lead exposure comes through lead fuel, lead smelting and combustion, pottery production, ship construction, lead- based paint, lead-infused pipelines, battery recycling, grid production, arms manufacturing, pigments, and book printing (Menchini, 2023). High soil lead levels can damage fruits and vegetables (Akhtar *et al*., 2022).

Traditional diets have important antioxidants C and E. Many recent studies show these compounds' phytochemical composition and antioxidant capabilities (Rahaman *et al*., 2023). Antioxidants combat heavy metal-induced free radicals (Shi *et al*., 2022). Plants create ROS from heavy metal stress. However, excessive ROS production harms (Bardo *et al*., 2021). The balance between ROS production and antioxidant enzyme activity can cause heavy metal-exposed plants to produce too much ROS. SOD, CAT, APX, and POX are antioxidant enzymes. (A. Khan *et al*. 2020). The main antioxidants are synthetic and natural. They primarily protect cells from free radicals. Mechanism determines enzymatic or non-enzymatic antioxidants. SOD, catalase, and glutathione are enzyme antioxidants. Body enzymes boost serum antioxidant capability. 2022 (Martemucci *et al*.). Growing Pb concentrations in industrial roadside soil. Pb accumulates in topsoil and decreases with depth (Isinkaralar *et al*., 2022).

Plants easily absorb it from the soil and collect it in various organs during phytoremediation. Lead (Pb) contamination in soil reduces productivity, making agriculture difficult (Qin *et al*., 2021). The present study examined the effects of Pb on the antioxidant enzyme activities of *Nerium indicum* L. grown in varied concentrations of Lahore roadside soil.

Materials and Methods

Survey

The study was conducted on major roads of populated areas of Lahore. Roadside soil samples were collected in March 2021 during the spring summer season.

Collection of Soil

Soil samples were taken from the same stretch of road at two different depths (0-10 cm and 10-20 cm) using hand-driven stainless steel augers in order to determine whether or not the concentration of metals in the soil grew or decreased with depth. The samples were gathered from different distances adjacent to the chosen road sides, including 5, 10, and 15 feet. Control samples were collected from Garden of Punjab University, Lahore. Soil samples were then passed through a 2 mm sieve and stored in dry labeled plastic bags and taken to the laboratory for pretreatment and analyses.

Preparation of Soil Amendments

Drain soil was mixed with control soil for preparing different amendments. The following concentration of soil was prepared. Each pot filled with 5 kg of soil.

- 0% control soil (100 g roadside soil only).
- 50% (w/w) 50 g roadside soil mixed with 50 g of garden soil.
- 75% (w/w) 75 g of roadside soil mixed with in 25 g of garden soil.
- 100% (w/w) 100 g roadside soil only.

Experimental Design

There are four different soil concentrations (control = 100% soil), 50% soil (w/w), 75% soil (w/w), and 100% soil (w/w) were used in the experiment. After 35 days, plants were taken from one ofthree replicates of each soil concentration. Each experiment utilized 12 different pots. There were threereplicates of each concentration of soil and plants were harvested after 35 days. A total of 12 pots were used for each experiment as shown below.

Formation of soil extract for analysis

For the preparation of soil immersion extracts, 1000 g of soil samples were gathered and combined with a suitable quantity of water to create a saturated paste. The amalgamation was permitted to settle overnight until it displayed a glossy aspect when illuminated and did not adhere to the spatula. A soil immersion concentrate was subsequently acquired by employing suction pressure through a suction pump.

Soil sample treatment

Utilizing a 50 ml funnel, one gram of the soil sample was positioned and subjected to heat on a heated plate for a duration of 15-20 min. A blend of 20 ml nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a proportion of 1:4 was introduced into the funnel. The temperature was meticulously regulated within the range of 150-200˚C. After 20 minutes, 8 ml of a solution blend comprising nitric acid and perchloric acid in a 1:1 proportion was added. Subsequent heating was conducted until the volume diminished to 1-2 ml. The final volume was then adjusted to 100 ml by the addition of deionized water (Ghaedi *et al.*, 2008)

Table 01: Total replicates of *Nerium indicum* L. in different soil concentrations

Conc.	After 35 days	Total replicates	Total
0%	R1	6	24
	R2		
	R3		
50%	R1	6	
	R2		
	R3		
75%	R1	6	
	R2		
	R3		
100%	R1	6	
	R2		
	R3		

Soil Saturation Paste

In a 100 ml beaker, a mixture of soil and distilled water in a 1:1 ratio was prepared. The soil-water mixture was stirred continuously to create a soil saturation paste and allowed to equilibrate for 24 hours. Subsequently, the paste was filtered using a suction pump, and the resulting extract was collected in a funnel. Finally, the extract was transferred into a graduated cylinder (Amakor et al., 2013).

Pollution parameters

Determination of pH

To determine the pH of samples, solutions containing various concentrations of soil mixed with tap water were utilized. The pH measurements were conducted using an Electrical pH meter (Model HI 98107). The pH of samples was determined at four different concentrations of solutions (0, 50, 75, and 100%). There were four distinct concentrations of solutions 0, 50, 75, 100% pH determined of the considerable number of samples similarly.

Determination of EC

The solution was also employed to define the electrical conductivity (ECe) of the solutions by an EC meter (Model: HI 9811-5). Beforehand, the probe handle was cleansed with purified water, and subsequently, the electrode was immersed directly into the different solutions. After each sample, the electrode was rinsed with purified water.

Determination of TDS

In order to ascertain the Total Dissolved Solids (TDS) of the solutions, a TDS meter was utilized. The process involved immersing the electrode into the solution sample and initially calibrating the meter. After obtaining the reading of each sample, the electrode was rinsed with purified (distilled) water. All the values were expressed in mg L^{-1}.

Determination of Chlorides

Samples were subjected to titration using a standardized solution of 0.01 N $AgNO_3$ to determine the chloride content. An indicator, potassium chromate, was used during the titrationprocess by using standard procedure. The endpoint of the titration was indicated by the formation of red precipitates of silver chromate (Poupard *et al.*, 2004). Here N is the normality of $AgNO_3$.

$$Cl\ (mg\ L^{-1}) = \frac{N\ of\ AgNO_3 \times Vol\ of\ AgNO_3 \times 35.5 \times 1000}{Volume\ of\ sample\ used}$$

Determination of CO3^{-2} and HCO3^{-1}

To regulate the presence of CO_3^{-2} and HCO_3^{-1}, a method provided by (Moss *et al* 2014) was employed. In carbonate and bicarbonate ion estimation, a 10 ml sample was taken with phenolphthalein added. Pink color indicates presence of carbonates. Titration with 0.01 N H_2SO_4 measured X. Absence of pink color suggests no carbonates. Methyl orange served as bicarbonate indicator. Another titration with 0.01 N H_2SO_4 determined carbonate and bicarbonate levels using the following formulae in which X stands for the normality, Y indicates the volume of H_2SO_4.

For Carbonates

$$CO_3^{-2}\ (mg\ L^{-1}) = \frac{2X \times N\ of\ H_2SO_4 \times 1000}{Vol.\ of\ sample\ used}$$

For Bicarbonates

$$HCO_3\ (mg\ L^{-1}) = \frac{Y - 2X \times N\ of\ H_2SO_4 \times 1000}{Vol.\ of\ sample\ used}$$

Plant digestion for metal analysis:

After collection, the plants were washed, dried, and separated into leaves, roots, and shoots. They were air-dried on absorbent paper and further dried in an electric oven at 80°C for 24 hours. Once dried, the samples were ground with a mortar and pestle. A 1 g portion of the dried plant sample was mixed with 5 ml of nitric acid and 15 ml of perchloric acid (in a 1:3 ratio) in a cup on a hot plate until the volume reduced by half. The mixture was then allowed to cool and filtered through Whatman filter paper no.4 into a measuring chamber. The filtrate was diluted with distilled water to a final volume of 50 ml (Greenberg *et al.,* 1998). The prepared sample was then used to determine the concentration of heavy metals.

Dtermination of Antioxidant ActivityPreparation of supernatant

For protein and enzyme assays, 1 g of fresh plant material (leaves, shoots) was finely powderedin liquid nitrogen using a pestle and mortar that had been chilled with ice. The powdered tissue was then mixed with 2.0 ml of 0.1 M phosphate buffer, pH 7.2, and 0.1 g of Polyvinyl-pyrrolidone (PVP). The mixture was subjected to centrifugation at 14,000 rpm and 4°C for 30 minutes using a Sorval RB-5 refrigerated super speed centrifuge. The resulting supernatant was collected and stored at 0°C for subsequent analysis of soluble protein content and superoxide dismutase levels.

Assessment of soluble Protein content

The estimation of soluble protein content was carried out using the Biuret method developed by Racusen and Johnstone (1961). In a total volume of 1,000 mL, the reaction mixture consisted of 2.0 mL of Biuret reagent, containing 3.8 g $CuSO_4{\cdot}5H_2O$, 1.0 g KI, 6.7 g Na-EDTA, and 200 ml of 5 N NaOH. Then, 0.1 mL of the supernatant was added to this mixture, while 0.1 mL of distilled water was used as acontrol. The protein content was determined by comparing the optical density readings with a standard curve of known protein concentrations prepared using bovine serum albumin.

Determination of Superoxide dismutase (SOD) activity

The spectrophotometric assay was employed to determine the activity of superoxide dismutase by measuring its capability to hinder the photochemical reduction of nitro blue tetrazolium (NBT), as described by Maral *et al.* (1977).

Morphological parameters

The shoot lengths (cm), number of leaves, nodes were determined after 8 days of germination. The root length (g), fresh and dry weight (g) of cultivated *N. indicum* were evaluated after 35 days when plant is harvested.

Statistical analysis

All the data from laboratory bioassays were subjected to analysis of variance followed by LSD test to differentiate the treatment means at probability 5% through Statistics 8.1.

Results

Zero analysis

The analysis of these factors yields a gradient of pollutant concentrations, enabling scientists to calculate plant tolerance to soil contaminants. Using a 0% concentration as a control (garden soil, presumably with no or minimal pollutants) and increasing concentrations of polluted soil, we may determine the maximum level of contamination that plants can endure before they begin to show signs of stress. When selecting or breeding plants for phytoremediation (the practice of utilizing plants to clean up polluted soils), knowing this threshold is essential. The gradient also helps illustrate how contaminants may have a gradual impact on plant growth, shedding light on whether the reduction in plant health is linear or whether there are discrete thresholds beyond which the plant's health rapidly declines. Soil amendments provide a diverse view of plant resilience. Even while plants in 100% roadside soil are growing, they may not be healthy. Researchers can assess plant vitality and identify phytoremediation candidates by studying growth across pollution concentrations. This approach also illuminates roadside plant tolerance thresholds to contaminants, improving plant selection for different pollution circumstances. By mimicking real-world pollution levels, researchers can learn how plants react to varied pollution intensities and forecast plant behavior as pollution changes.

The measurements of different pollution indicators in soil samples from roadsides were documented in Table No. 1. The values of these pollution indicators, including pH, electrical conductivity (EC), total dissolved solids (TDS), NaCl, chlorides, carbonates (CO_3^{-2}), and bicarbonates (HCO_3), exhibited an upward trend with increasing concentrations of roadside soil, namely 0%, 50%, 75%, and 100%. The highest values of pH, ECe, and TDS were observed in the 100% concentration, indicating a higher level of pollution compared to the control sample.

The highest value of pH was calculated in 100% concentration because it had high amount of heavy metals which make it more contaminated as compared to other concentrations (0, 50, 75%). The value of pH in 0% (Control) was 7.76 less than the

100% (8.78) which was roadside soil. The value of pH was high in 100% concentration because it was more polluted than control. In 50% pH was 7.83 and in 75% (7.87). EC was high in 100% (338 μS cm^{-1}).

The highest value of Electrical conductivity was found in 100% concentration of contaminated soil because roadside soil has toxic metals indicating more toxicity. Conductivity in 75% concentrationwas 296 μS cm^{-1} which is higher than the 50% (274μS cm^{-1}). The TDS levels were lowest in the 0% concentration (518 mg L^{-1}), while the highest TDS value was observed in the 100% concentration (696 mg L^{-1}). Contaminated soil generally contains higher amounts of total dissolved solids, thus resulting in increased TDS levels compared to the control (518 mg L^{-1}). The 50% concentration had a lower TDS amount (568 mg L^{-1}) compared to the 75% concentration (612 mg L^{-1}). The table illustrates that the pH, EC, and TDS levels increased with the increasing concentration of polluted soil. The 0% concentration had the lowest values for pH, ECe, and TDS. The presence of a higher quantity of heavy metals in the 100% concentration contributed to its higher TDS value, indicating higher pollution levels.

The maximum value of bicarbonates recorded in 100% as compared to 0%. In 50% the amount of bicarbonates (30 mg L^{-1}) was less than the 75% (45 mg L^{-1}). The lowest concentration was presentin control group of soil (22 mg L^{-1}). Due to vehicles the maximum Pb were absorbed by roadside soil. Highest values of Pb were present in 100% (770 mg kg^{-1}) as compared to control. In 75% the amount of Pb is (319 mg kg^{-}1) that is maximum than 50% (256 mg kg^{-1}). The lowest concentration of Cd was present in control group thatis 55 mg kg^{-1}. By the increasing the concentration of soil value of Cd increased. In 75% the amount of Cd (175 mg kg^{-1}) was greater than the 50% (80 mg kg^{-1}). The highest concentration was present in100% (402 mg kg^{-1}).

Determination of Morphological parameters

After 35 days the growth parameters were observed in all soil concentrations i.e., 0, 50, 75 and 100%. There were three replicates of each concentration to evaluate the content of metals and its effect on the plant (*N. indicum*). All the results of morphological parameters were compared with the control group (0%). The lengths of cuttings were taken 9 inch in all concentrations i.e., 0, 50, 75 and 100%. In control there was no growth of shoots, leaves and roots. In 50% concentration the length of shoot, sprouting and number of leaves and roots observed. The lowest number of shoot was present in 50%. The maximum growth of shoots, leaves, roots and sprouting was shown in 100% concentration. The maximum shoots calculated in 100% as compared to 75%. The minimum sprouting appeared in 50% and maximum was seen in 75 %.

Fig: 1 *Nerium indicum* L. grown in different concentration of soil at the start of the experiment

Fig: 2. Cuttings of *Nerium indicum* L. grown in different concentration of roadside soil after 35 days of experiment

Table 02: Various pollution parameters of different concentrations of roadside soil

Parameters	**Concentrations**				**Effect of concentration on various parameters**
	0%	**50%**	**75%**	**100%**	
pH	7.76	7.83	7.87	8.78	NS
ECe (µS cm^{-1})	249	274	296	338	NS
TDS (mg L^{-1})	518	568	612	696	S
Carbonates (mg L^{-1})	0	0	0	0	-
Bicarbonates (mg L^{-1})	22	30	45	87	NS
Chlorides (mg L^{-1})	32	45	77	154	S
Pb (mg kg^{-1})	116	256	319	770	S
Cd (mg kg^{-1})	55	80	175	402	S

RS=Roadside soil , 0%= soil only, 50%= soil contaminated with 50% RS, 75%= soil contaminated with % RS 100%=RS only, Values are mean ± Standard deviation from 4 replicates, Groups with the same letters do not show significant differences at a significance level of $P \leq 0.05$, The significance (S) or non-significance of the values at $P \leq 0.05$ is determined using the F test.

The leaf count reached its peak in the 100% concentration. The 100% group displayed a higher number of developed cuttings compared to the control group, exhibiting a significant increase in the abundance of leaves and shoots. In the 50% concentration, three shoots were observed in each replicate, measuring 13.5cm, 11cm, and 15cm in length respectively. Additionally, the leaf count in each replicate of the 50% concentration was recorded as 20cm, 21cm, and 24cm respectively. The root count in the 50% concentration was observed to be 4cm, 8cm, and 11cm respectively.

In 75% concentration the length of shoots was medium as compared to 50% but shown better results than 8cm, 11cm and 9cm. All replicates were shown number of sprouting and the number of leaves was 20cm, 26cm and 22cm respectively. The number of roots calculated in all replicates of 75% was 10cm, 8cm and 6cm respectively. The maximum growth of shoot was observed in 100% concentration, the length of shoot in each replicate of 100% was 5.5cm, 3.2cm and 2.7cm respectively. It had the number of leaves 10cm, 11cm and 14 with the root length which was calculated 2cm, 5cm and 11 cm respectively. The length of leaves observed was 5cm, 8.5cm and 7cm respectively.

Assessment of Metal in Plants

The concentrations of heavy metals in the soil samples were determined using an Atomic Absorption Spectrophotometer (AAS). Higher concentrations of soil were associated with higher levels of heavy metals. The order of metals observed was Cd < Pb. The amounts of heavy metals in the roots were highest in the 100% concentration and lowest in the 0% concentration, as shown in Table 4.2. The levels of Cd and Pb increased with the increasing concentration of contaminated soil, following the sequence of 0% < 50% < 75% < 100%. This indicates that *Nerium indicum* L. has the ability to absorb heavy metal ions and can be utilized to mitigate environmental pollutants resulting from toxic metallic ions released by vehicles and traffic.

The roots of the 100% concentration exhibited a significant increase in Cd content, measuring at 1,567 mg kg^{-1}, compared to the 0% concentration.

Table 03: Growth parameters in 35 days old plants of *Nerium indicum* L. grown in different concentrations of roadside soil

Growth parameters	Conc.	R1	R2	R3	Effect of concentration on various parameters
Length of cutting (inch)	0%	9.0±1.00	8.5±0.5	8.0±1.00	NS
	50%	9.0±0.50	8.0±0.76	8.5±0.50	NS
	75%	9.0±0.76	6.0±0.76	7.5±0.50	S
	100%	9.0±0.76	7.0±0.76	8.0±1.32	NS
Length of shoots (cm)	0%	1.5±0.50	1.0±1.5	2.0±1.53	
	50%	13.5±1.3	11.0±2.0	15.0±1.0	NS
	75%	8.0±2.1	12.0±1.04	9.0±1.30	S
	100%	5.5±2.02	3.2±0.57	2.7±0.68	NS
Length of leaves (cm)	0%	1.4±0.85	3.1±0.67	2.0±1.01	
	50%	8.7±1.65	6.0±1.00	7.0±0.76	
	75%	5.0±1.0	8.5±0.5	7.0±0.76	S
	100%	4.0±2.5	7.0±0.76	8.7±1.08	S
No. of leaves (cm)	0%	2.0±1.5	1.9±1.76	2.5±1.64	
	50%	21.0±1.8	20.0±2.2	24.0±3.06	S
	75%	20.0±2.2	26.0±3.0	22.0±1.5	S
	100%	10.0±2.08	11.0±1.53	14.0±1.0	NS
	50%	2.4±1.31	3.0±2.65	4.1±1.52	
	75%	2.5±0.36	1.9±0.67	3.2±1.21	
	100%	2.0±2.65	3.0±3.62	4.0±2.84	NS
No. of roots (cm)	0%	5.0±1.00	7.0±0.76	4.0±0.5	
	50%	4.0±0.00	8.0±1.00	11.0±1.5	S
	75%	10.0±1.53	8.0±0.76	6.0±1.00	S
	100%	2.0±2.65	5.0±2.08	8.0±1.72	S
No. of sprouting	0%	5.0±1.00	3.0±1.82	1.0±3.51	NS
	50%	3.0±3.62	4.0±2.84	5.0±1.00	NS
	75%	1.0±0.76	2.0±1.53	3.00±2.65	S
	100%	3.0±1.00	4.0±0.50	7.00±1.26	S

Conversely, the Pb content in the 0% concentration was 44 mg kg-1, whereas it significantly increased to 2,236 mg kg-1 in the 100% concentration. In the 50% concentration, the roots contained 322 mg kg-1 of Cd and 550 mg kg-1 of Pb, indicating a higher uptake of Pb by the plants compared to Cd. The roots of the 75% concentration showed a notable Cd content of 708 mg kg-1. Additionally, the leaves of the 100% concentration contained 688 mg kg-1 of Cd and 11 mg kg-1 of Cd in the 0% concentration.

The foliage of the 75% concentration exhibited a higher concentration of Cd, measuring at 345 mg kg-1, compared to the 50% concentration, which recorded 150 mg kg-1. On the other hand, the 100% concentration showed the highest Pb content at 1,035 mg kg-1, while the lowest amount was observed in the 0% concentration at 44 mg kg-1. The leaves of the 50% concentration contained 234 mg kg-1 of Pb, whereas the 75% concentration recorded 532 mg kg-1. It is evident that the concentration of metals increased with the higher levels of soil concentrations.

The leaf count reached its peak in the 100% concentration. The 100% group displayed a higher number of developed cuttings compared to the control group, exhibiting a significant increase in the abundance of leaves and shoots. In the 50% concentration, three shoots were observed in each replicate, measuring 13.5cm, 11cm, and 15cm in length respectively. Additionally, the leaf count in each replicate of the 50% concentration was recorded as 20cm, 21cm, and 24cm respectively. The rootcount in the 50% concentration was observed to be 4cm, 8cm, and 11cm respectively.

In 75% concentration the length of shoots was medium as compared to 50% but shown betterresults than 8cm, 11cm and 9cm. All replicates were shown number of sprouting and the number of leaves was 20cm, 26cm and 22cm respectively. The number of roots calculated in all replicates of 75 was 10cm, 8cm and 6cm respectively. The maximum growth of shoot was observed in 100% concentration, the length of shoot in each replicate of 100% was 5.5cm, 3.2cm and 2.7cm respectively.It had the number of leaves 10cm, 11cm and 14 with the root length which was calculated 2cm, 5cm and 11 cm respectively. The length of leaves observed was 5cm, 8.5cm and 7cm respectively.

Determination of Antioxidant parameters

Table 4.3 displays the protein content measured in 35-day-old Kaner plants (*Nerium sp*.). Plants cultivated in soils with varying amounts of contaminants have progressively higher protein levels i.e., 0<50<75<100%. High amount of protein was recorded in 100% after 35 days (20.54) as compared to other concentration. After harvesting the plants, the high amount of protein was recorded in 100% is 20.54 as compared to 0%. The amount of protein in 50% is 13.50 mg g^{-1} of tissue and 75% is 15.87 mg g^{-1} of tissue. The amount of protein is increased with the increased the concentrations of soil.

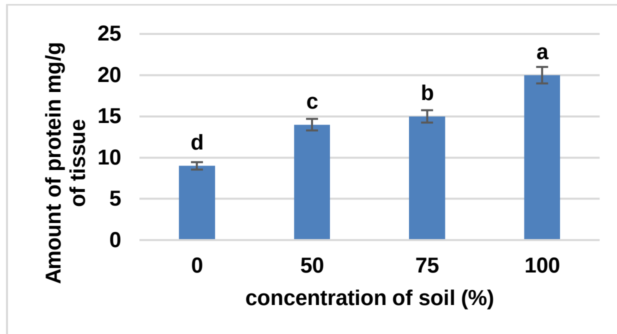

Fig: 3 Amount of protein observed in the leaves of *Nerium indicum* L. plant

Amount of SOD (superoxide dismutase) was recorded in plants of *Nerium indicum* L. (Kaner) are shown in table: 4.3. Amount of SOD in control group is minimum (1.02µg) which is increased with the increased in concentration of contaminated soil in order 0<50<75<100%. High amount of SOD was recorded in 100% after 35 days (3.54 per mg^{-1} protein) as compared to other concentration. In 50% the amount of SOD is 2.65 and 75% is 2.98 per mg^{-1} protein. The amount of SOD is increased with the increased the concentrations of soil.

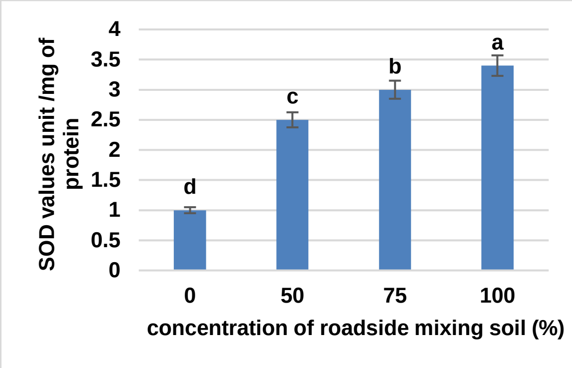

Fig 4: Amount of SOD (superoxide dismutase) was recorded in plants of *Nerium indicum* L.

Discussion

Air pollution can arise from various origins, encompassing both natural and anthropogenic sources. Natural sources comprise events like volcanic eruptions, forest fires, the decay of organic matter, the dispersion of pollen grains, wetlands, and the presence of radioactive materials.

Conversely, human activities contribute significantly to air pollution through the operation of thermal power plants, industrial processes, emissions from vehicles, the use of combustion devices in households, the burning of fossil fuels, and agricultural practices. Pollution denotes the introduction of harmful substances into the environment, resulting in detrimental changes to the ecosystem (Aithal, 2021) and affect soil, plant, ground water and consequently human health. Contaminated soils and waters pose a major environmental and human health problem (Hadjipanagiotou *et al.*, 2020). Heavy metals are found soluble in water, so that, it can enter in ecosystems by water pathways (Ahmad *et al.*, 2021).

In the present study, pollution parameters increase with increasing lead in soil concentration.

Hence pH, ECe, TDS, CO_3 and HCO_3 was also increased within the concentrations of the soil.

The pH values varied among different concentrations of soil, with the highest value observed in 100% and the lowest in 0%. Previous studies (Boguta *et al.*, 2020; Hosseini *et al.*, 2022) have supported these findings, indicating that roadside soil tends to become more neutral or even alkaline. An increase in pH can enhance the adsorption of metals and the solubility of organic matter, as stated by Boguta and Sokołowska (2020).

The maximum electrical conductivity (ECe) values were found in the 100% concentration (338 µS cm^{-1}), which indicated a high alkaline nature. The ECe values increased with increasing soil concentration, a trend supported by Huang *et al.* (2022), who noted that higher ECe values indicate greater concentrations of soluble salts and heavy metals in the soil. The minimum ECe value was observed in the 0% soil concentration (249 µS cm^{-1}).

Regarding total dissolved solids (TDS), all concentrations of soil exhibited varying amounts. The highest TDS value was recorded in the 100% concentration, while the lowest was observed in the 0% concentration. These findings align with the results reported by Adimalla *et al.*, (2020), who noted higher TDS values in polluted areas, ranging from 120 mg L^{-1} to 329 mg L^{-1}.

The heavy metals Pb was observed in all concentrations of soil. The amount of metals were increased with the increasing the value of soil concentration. The maximum value was recorded in 100% soil concentration and the minimum was found in 0% concentration of soil. The increasing trendof metals were Pb > Cd. The value of Cd was maximum in 100% and lowest was recorded in 50 %. According to (Guo *et al.*, 2023), the accumulation capacity for wild plant changed along the different high ways. The results show that transportation and various kinds of vehicles on road caused maximum cadmium accumulation in the plants.

The growth parameters i.e., root length, shoots length; numbers of leaves, number of sprouting were observed in all soil concentrations. The highest growth was calculated in 100% and the lowest growth was observed in 50% concentration. There was no growth observed in control group of soil. Similar findings were confirmed by Arif *et al.,* (2017) reported that maximum growth showed in highly alkaline soil.

The amounts of heavy metals were calculated in different parts of plant i.e., roots, leaves and shoots. The highest value of metals were recorded in leaves and lowest was observed in plant shoot. The highest amounts of metals were determined in leaves which grow in the 100% concentration of soil. Similar results were reported by (Shareif *et al.*, 2022). They observed that minimum concentration was found in the stem of *Oleander* plant the control treatment and the maximum concentration was observed in the leaf of the *Oleander* plant at 100%. The minimum value of metals observed in roots of 50%.

According to (Sharma *et al.*, 2022), the identification of plant species with high biomass production and the ability to tolerate or accumulate pollutants is crucial for the restoration or purification of heavy metal-contaminated sites using phytoremediation. The concentration analysis results of both roots and leaves/stems demonstrated that as the concentration of Pb increased in the plant, the cadmium concentration in both roots and leaves also increased. These findings are consistent with the results obtained in the current study.

The amount of protein observed in all concentrations of soil which was increased with the increasing of soil concentration. The maximum amount of protein observed in 100%. Similar results confirmed by Díaz-Domínguez *et al.,* (2022).

The result of current findings revealed that the maximum amount of SOD was observed in 100% concentration. And the lowest was calculated in 50% which is increased with the increased of soil concentration. These results were confirmed by (Weerasooriyagedara *et al.*, 2020) .The antioxidant activities of SOD increased with the increased of concentration of soil contaminated with lead.

Same results were confirmed by Guedes *et al.,* (2021) that the concentration of Pb led to a notable rise in SOD activity in both leaf and root tissues. Their observations indicated that the leaf exhibited the highest SOD activity, while the root displayed the lowest, as compared to the control. Overall, the findings indicate an elevated level of SOD activity in plants subjected to lead treatment.

Conclusion

The findings of the current study revealed the presence of all pollution parameters, which exhibited an increase with higher soil concentrations. The results of the study highlight the adaptabilityof *N. indicum* L., and more specifically *N. Oleander*, to contaminated settings. This plant grew exceptionally well in increasingly polluted environments, and its leaves showed a remarkable ability to absorb metals, especially lead. Its potential for pollution mitigation is strengthened by the fact that protein levels and antioxidant indices rise sharply in response to elevated levels of pollution. Additionally, the 100% concentration of SOD signifies the plant's response to oxidative stress. N. oleander emerges as a promising choice for monitoring and treating airborne metal pollution in urban settings in light of these findings and the increasing emphasis on phytoremediation using invasive species. Roadside soil (100% concentration) demonstrated the highest growth in both shoot and root, while the minimum growth was observed in the 50% concentration. No growth of roots, shoots, and leaves was observed in the (0% concentration) control group. The uptake of metals was highest in the 100% concentration compared to other concentrations, with Pb showing a greater accumulation than Cd in *N. indicum* L. Lead content was found to be highest in the 100% concentration, particularly in the leaves compared to the roots. Additionally, the presence of free radicals and heavy metal uptake led to an increase in antioxidant parameters. After plant harvest, the highest protein content was recorded in the 100% concentration, indicating an increase in protein levels in highly polluted soil. Thepresence of SOD was also observed in Nerium indicum L., with the lowest amount detected in the 0% concentration and the highest amount in the 100% concentration.

Table 04: Heavy metals accumulation in roots, shoots, and leaves of *Nerium indicum* L. grown in different levels of roadside soil

Conc.	**Plant parts**	**Metals (mg kg^{-1})**		**Effect of conservation on various parameters**
		Cd	**Pb**	
0%	Root	20.0±0.71	44±4.51	S
	Leaves	11.0±1.53	30±7.09	S
	Shoot	5.0±1.53	18±2.52	NS
50%	Root	322±3.61	550±7.64	S
	Leaves	150±5.57	234±7.55	S
	Shoot	80.0±3.61	100±5	NS
75%	Root	708±9.01	976±7.55	S
	Leaves	345±5.00	532±6.11	S
	Shoot	165±4.51	208±7.02	NS
100%	Root	1567±7.5	2,236±5.03	S
	Leaves	688±12.9	1035±5	S
	Shoot	322±5.13	866±5.03	S

Values are mean ± Standard deviation from 4 replicates

Values are significant (S) at P≤ 0.05 according to F test

Considering the future prospects of phytoremediation, employing invasive plants for pollution mitigation has gained significant attention. These plants have emerged as a prominent solution to combat pollution in contemporary times. The findings collectively suggest that N. Oleander, possessing metal resistance through metal exclusion mechanisms, serves as a viable choice for utilizing as a bio monitoring tool to assess airborne metal pollution in urban regions.

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