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RESEARCH ARTICLE

Nerium oleander could be used for sustainable management of traffic-borne elemental-enriched roadside soils

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Abstract

Metal pollutants released from motor vehicles are deposited in roadside environments. Metals are non-biodegradable and biomagnify in the food chain causing significant health hazards at all levels of the ecosystem. Hence, management of contaminated roadside verges is critically important and should be kept in mind while planning specific management strategies of such areas. Native vegetation could help to decontaminate heavy metal polluted soils in the best sustainable way. Therefore, this study was designed to assess the potential of *Nerium oleander* to accumulate heavy metals commonly released by automobiles such as Pb, Cd, Ni, and Zn along with various C and N compounds from five different locations along a busy road in Punjab, Pakistan, during summer and winter seasons. *N. oleander* showed the ability to absorb C, N, and heavy metals Pb and Cd; the maximum concentration of Pb and Cd was 8.991 mg kg^{-1} and 0.599 mg kg^{-1}, respectively. These pollutants negatively affected photosynthetic pigments, gas exchange attributes, soluble proteins, and free amino acids. But antioxidant activity of *N. oleander* was found to be increased in both seasons. The metal accumulation in the plant was higher in the summer though. We highly recommend that by growing *N. oleander* at roadside verges for decontamination of vehicular pollutants could lead to sustainable management of these corridors.

Keywords Roadsides · Vehicle emissions · Phytomonitoring · *N. oleander*

Introduction

Vehicles have become a major source of heavy metal pollutants in areas with high anthropogenic pressure making roadside metal hotspots (Khalid et al. 2018b; Soleimani et al. 2018). This is particularly true in urban areas with high road traffic (Hong et al. 2020). Environmental and public health could be adversely affected if the roadways pass though farmlands, and metals could possibly enter the food chain via uptake by edible roadside plants (Khalid et al. 2019a; Willmert et al. 2018). The concentration of such vehicular origin metals in soils and plants tends to decay rapidly with distance from the road (Khalid et al. 2019b).

The most common metals released by vehicles and found in high quantities in roadside soils are lead (Pb), cadmium (Cd), zinc (Zn), nickel (Ni), and iron (Fe) (Khalid et al. 2018a; Malunguja et al. 2022). Gasolines, tires, lubricating and break oils, batteries, and automobile galvanized parts add significant metal impurities along roadside environments (Naeem et al. 2021). High concentrations of these metal elements in soil adversely

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impact the plants such as reduced growth and photosynthetic activity, chlorosis, necrosis, and poor yield (Aqeel et al. 2022; Chen et al. 2022; Khalid et al. 2021). As these metals tend to biomagnify, thus humans could have cytotoxicity, hypertension, anemia, cancers, and death of brain cells (Darwish et al. 2018). The bioavailability of metals to plants depends upon environmental conditions of the area, pH, and plant species (Wang et al. 2021). Seasonal and spatial variations may also influence on the concentration of metals in soil (Škrbić et al. 2018); higher concentrations of many metals have been reported in hotter times of the year (Noman et al. 2017; Škrbić et al. 2018).

Despite using various technologies to remove metals from contaminated soil and water, recently there has been a push to use "green" phytoremedial systems. Phytoremediation, where plants uptake, extract, and accumulate heavy metals in their aboveground parts, has become the cheapest and most reliable natural method for decontamination of the soil (Patra et al. 2020; Shah and Daverey 2020). Some wild and cultivated native plants can remove soil contamination efficiently by amassing high metal concentrations in their aboveground parts in polluted areas (Khalid et al. 2021). These plants are suitable for sustaining soil equilibrium balance because of their good metal extraction capacity (Zhou et al. 2020). Additionally, in developing countries like Pakistan, cost-effective techniques are necessary due to constraints in both funds and expertise. *Nerium oleander* L. is an exemplary ornamental hardy plant used by researchers in environmental studies because of its easy cultivation and cosmopolitan nature (Ibrahim and El Afandi 2020). We selected this plant in our roadside monitoring study because it tended to absorb metal elements and its esthetic beauty, which could be attractively employed in managing the roadside belts.

Sargodha and Faisalabad are the most populated cities in Punjab, a province of Pakistan. Both cities are connected by a single road known as the Grand Trunk Road (GT Road). The traffic load is very high and consists of cars, public transportation busses, oil tankers, trucks, loaders with multi-wheels, and carts driven by animals, which often use worn-out tires and may emit metals in high quantities. This wear and tear may be elevated due to high temperature during the summer season. Being very busy, crushed stone is transported through this road to almost all over the province to construct buildings and roads. No research study previously used *N. oleander* for the biomonitoring of this busiest road of the area. Therefore, this research aims to investigate the effect of transportation activities on the spatiotemporal variation of metal contents in soil and *N. oleander* across five different sites in order to determine the physiological responses of *N. oleander* and its phytoremediation potential.

Materials and methods

Experimental design

Phytomonitoring by *N. oleander*, five sites were selected at almost uniform distance from each other on GT Road (Fig. 1). The survey was replicated during summer and winter seasons of the year. The seasonal detail of the area is presented in Table 1. Traffic volume data (no. of vehicles/day) was also obtained for each site in summer and winter seasons.

Description of sites

To check spatial variation in metal contents at the selected five sites on GT Road, an average distance of 10 km was kept between the consecutive sites from each other. The sites were named Pull Dingro, Chiniot, Chenab Nagar, Pull-111, and Adda-46. The Pull Dingro site was near cultivated crops. The Chiniot site was selected on the road near the bridge of Chenab River. A large town was situated along the road at Chenab Nagar site. Adda-46 site, a local market, is situated on the road's edge. Pull-111 site was situated near to a stone crushing industry, where trucks and multi-wheeler loaders frequently run around the clock. The control soil and plant samples were also collected at a distance of 100 m away from the road (Jian-Hua et al. 2009; Khalid et al. 2019b).

Collection of plant and soil samples

Three leaves, selected at random from the middle of the *N. oleander* plant, were removed from the plant at each site during summers and winters. No young or senescence leaves were taken. Three soil samples were collected near the road edge at each site at a depth of 0–10 cm. Samples were stored in an iced cooler and brought to the laboratory in zip locked polythene bags. Leaf samples were washed with distilled water thoroughly before further processing.

Determination of metal elements, nutrient ions, carbon, and nitrogen

Plant leaves and soil samples were oven dried at 65 °C for 72 h, and the method of Arsenic et al. (1996) was used for their digestion. An inductively coupled plasma-atomic emission spectrometer (ICP-AES) was used for the determination of Pb, Cd, Ni, Zn, and Fe using a Thermo iCAP Model 6300. Standard solutions of high purity standards were used for the calibration. A standard reference material was run after every 10 samples. The C and N in both dried soil and plant samples were analyzed through NC

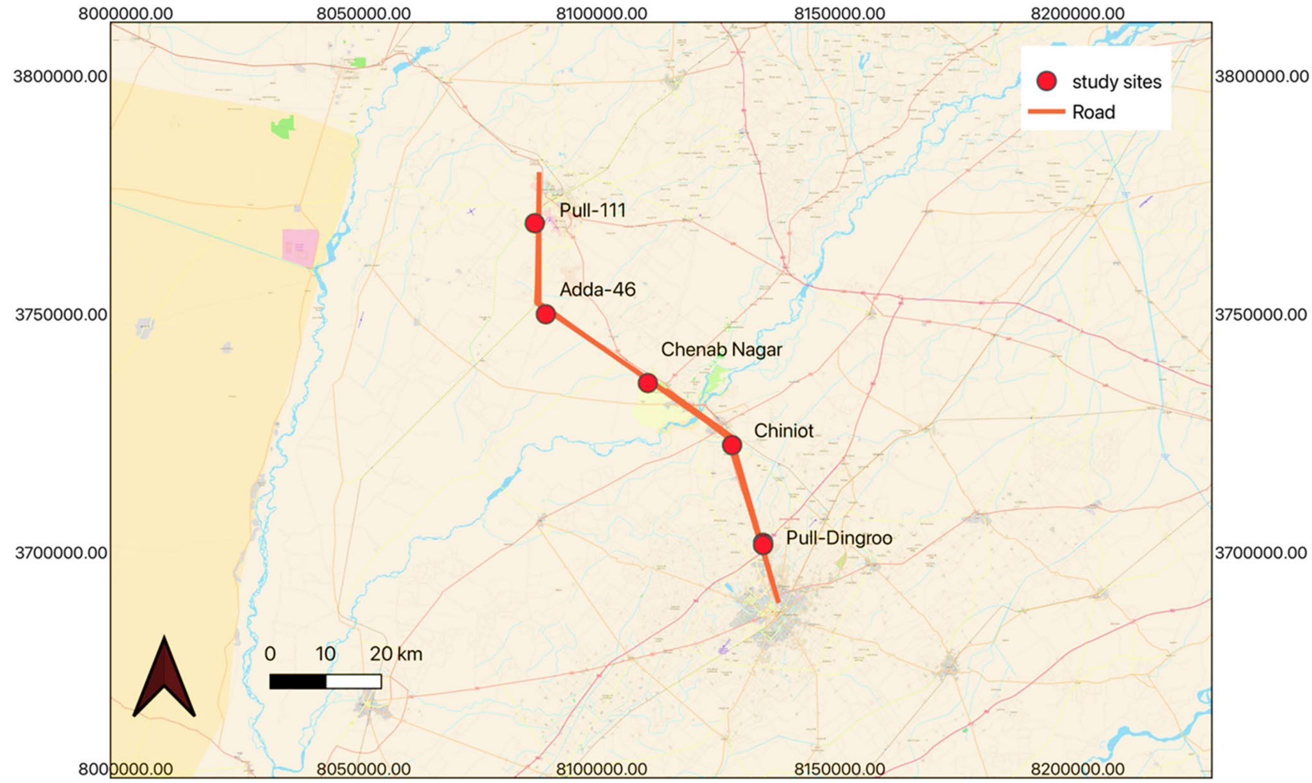

Fig. 1 Map of the study area showing locations of different sites

Table 1 Concentration of heavy metals Pb, Cd, Zn, Ni, and Fe (mg kg^{-1}); nutrient ions Na^+, K^+, and Ca^{+2} (mg g^{-1}); and C and N contents (%) in plant and soil samples in summer and winter seasons

Metals	Season	Control		Pull-Dingro		Chiniot		Chenab Nagar		Adda-46		Pull-111	
		Leaves	Soil	Leaves	Soil	Leaves	Soil	Leaves	Soil	Leaves	Soil	Leaves	Soil
Pb	Summer	0.001±0.001	0.011±0.000	5.440±0.334	4.05±0.042	5.277±0.279	4.144±0.032	5.435±0.259	4.391±0.073	7.411±0.366	6.760±0.077	8.481±0.444	7.610±0.289
	Winter	0.000±0.000	0.007±0.005	4.477±0.123	4.034±0.041	4.392±0.310	4.059±0.026	5.269±0.246	4.5±0.204	6.375±0.633	5.256±0.029	7.699±0.358	6.594±0.232
Cd	Summer	0.000±0.000	0.004±0.005	0.430±0.013	0.415±0.003	0.444±0.030	0.42±0.002	0.492±0.033	0.443±0.004	0.577±0.018	0.506±0.005	0.576±0.032	0.56±0.008
	Winter	0.000±0.000	0.001±0.000	0.370±0.058	0.370±0.058	0.369±0.066	0.369±0.066	0.256±0.028	0.256±0.028	0.511±0.065	0.501±0.065	0.527±0.025	0.528±0.025
Zn	Summer	9.325±0.054	20.61±0.106	81.08±0.265	124.3±0.907	81.58±0.289	127.7±1.001	81.92±0.361	131.8±0.9	90.77±0.827	135.4±1.242	94.98±0.687	144.1±1.101
	Winter	8.674±0.290	20.25±0.429	80.25±0.532	120.5±0.1	79.78±1.156	122.9±0.781	77.66±2.005	127.4±0.472	87.9±1.155	130.1±0.608	90.68±0.494	131.2±0.907
Ni	Summer	0.814±0.018	1.7±0.157	7.630±0.388	10.84±0.245	8.064±0.282	11.62±0.058	9.672±0.263	11.97±0.043	10.46±0.445	13.48±0.5	11.90±0.494	17.83±0.16
	Winter	0.772±0.060	1.443±0.100	6.444±0.483	9.696±0.165	6.933±0.711	9.86±0.269	6.788±0.295	10.37±0.234	8.820±0.076	11.62±0.258	10.42±0.680	15.70±0.138
Fe	Summer	2.225±0.117	4.036±0.037	4.376±0.180	6.506±0.160	4.608±0.147	6.88±0.1	5.156±0.165	7.713±0.235	10.11±0.621	10.83±0.076	10.13±0.809	12.75±0.172
	Winter	2.070±0.068	4.013±0.015	4.411±0.163	5.758±0.081	6.145±0.702	6.69±0.321	6.973±0.074	7.729±0.246	9.449±0.448	10.50±0.418	10.28±0.777	10.88±0.1
Na^+	Summer	242.5±33.84	301.3±1.159	580.3±9.279	725.6±40.42	594.2±8.750	756.1±32.70	772.6±23.15	856.7±21.06	883.5±10.03	909.5±4.416	987.3±7.724	1000.4±0.1
	Winter	201.2±0.901	199.0±0.608	470.9±29.21	701.6±1.664	561.3±7.077	786.2±15.87	641.7±18.68	903.9±2.324	697.9±4.650	928.2±20.64	819.4±9.254	987.5±8.563
K^+	Summer	29.59±0.256	30.82±0.134	20.62±0.260	23.63±1.106	19.95±0.653	22.64±0.265	13.84±5.096	22.23±0.083	16.07±0.185	20.6±0.144	14.78±0.520	17.40±0.156
	Winter	27.24±0.228	32.78±0.790	20.72±0.615	21.15±0.410	19.7±0.403	21.48±0.165	17.35±0.952	20.5±0.147	16.51±0.194	19.59±0.162	14.73±0.321	18.34±0.105
Ca^{+2}	Summer	56.30±0.563	60.40±0.141	41.55±0.375	45.39±0.721	42.51±0.273	42.58±1.200	41.96±1.143	43.82±1.009	44.24±0.809	42.58±0.550	45.71±0.230	47.15±1.041
	Winter	55.66±0.603	60.42±0.229	40.46±0.539	43.68±0.175	41.88±0.785	43.80±1.276	40.72±1.237	42.60±1.536	40.09±0.542	41.54±1.229	39.52±1.047	40.42±0.303
C	Summer	41.25±0.575	1.629±0.005	42.94±0.748	3.023±0.051	44.96±1.058	3.42±0.407	45.96±0.711	3.826±0.041	47.43±0.395	4.153±0.151	48.22±0.618	4.743±0.127
	Winter	40.56±0.312	1.64±0.036	41.36±0.118	3.483±0.281	41.67±0.399	3.706±0.155	43.48±0.346	3.946±0.145	45.12±0.677	4.094±0.103	46.28±0.425	4.286±0.083
N	Summer	2.226±0.113	0.117±0.006	4.233±0.032	0.945±0.020	4.447±0.030	0.974±0.021	4.704±0.048	1.218±0.305	4.892±0.008	1.551±0.010	4.967±0.031	1.730±0.008
	Winter	2.296±0.031	0.116±0.005	3.229±0.115	0.563±0.015	3.359±0.026	0.741±0.018	3.513±0.059	0.846±0.032	3.651±0.012	0.856±0.017	3.727±0.058	0.891±0.006

Leaves Soil

soil analyzer, Thermo Scientific model Flash 2000. Nutrient ions, i.e., Na^+, K^+, and Ca^{+2}, were also determined in plant and soil samples using the solution obtained after digestion from the previous step in a flame photometer.

Bioconcentration factor (BCF)

The bioconcentration factor (BCF) has been applied to evaluate the plants' ability to absorb metals from the soil and to study the relationship between the metal contents of soils and plants. BCF is defined as the ratio of metal concentration in plants to that in soil. Many researchers have calculated the BCF for various elements (Khalid et al. 2019a, 2021; Khan et al. 2016). BCF was determined by employing the following formula:

$$\mathbf{BCF} = \frac{\text{metal concentration in plant leaves}}{\text{metal concentration in the soil}}$$

Road traffic data

Traffic volume data (number of vehicles per day) was also obtained for each site in summer and winter seasons. The number of vehicles was manually counted at each study site for a specific time, and then, data was calculated for 24 h (Supplementary Table 1).

Determination of soil physicochemical characteristics

Soil samples were passed through a sieve mesh and stored in refrigerator in ziplock plastic bags before the analysis. pH, EC, and TDS of the soil samples were determined using a pH and EC meter in the lab. Organic carbon in the soil was determined following the method of Bakr and El-Ashry (2018) and Heiri et al. (2001).

Determination of chlorophyll content

Chlorophyll and carotenoid contents in the leaves of *N. oleander* were analyzed following the formulae given by Akhter et al. (2022b) and Arnon (1949). For that, plant extract was prepared by crushing the leaves with 80% acetone at − 4 °C and left overnight. It was then centrifuged using a centrifuge machine and supernatant was collected. Optical density of the supernatant was measured for carotenoids and chlorophyll a and b at 480, 645, and 663 nm wavelength, respectively, on a spectrophotometer (IRMECO U2020).

Determination of gas exchange characteristics

A portable IRGA (Infrared Gas Analyzer) Model C1-340 was taken to the field in a bright sunny day for at the spot measurement of photosynthetic rate, transpiration rate, stomatal conductance, and internal CO_2 concentration from healthy leaves of *N. oleander*. Ambient concentration of CO_2 in the air was 348 µmol mol^{-1}. Leaf chamber temperature was in a range of 27–37 °C. Water vapor pressure inside the chamber was 7–10mbars.

Determination of total free amino acids and total soluble proteins

Fresh leaves of *N. oleander* were extracted with sodium phosphate buffer of pH 7. The supernatant was collected after centrifugation and utilized for the determination of total free amino acids and total soluble proteins following the methods established by Bradford (1976) and Kielkopf et al. (2020), respectively.

Determination of total antioxidant activity

For the estimation of total antioxidant activity, oven dried grounded leaves of *N. oleander* were heated at 40 °C in a water bath for 20 min. Then, supernatant was collected after centrifugation to determine total antioxidant activity following the Rahmat et al. (2003) study. For this purpose, a 1: 1 mixture of plant extract and absolute alcohol was taken and 2.52% linoleic acid was added and incubated the mixture in an incubator for 1 day. At small intervals, 0.1 ml of this solution was mixed with ammonium thiocyanate and ferrous chloride and absorbance of the solution was measured at 500 nm wavelength on a spectrophotometer (Hitachi 220, Japan).

Statistical analysis

We tested two factorial analysis of variance (ANOVA) and comparison between different sites and seasons were done by least significant difference (LSD) in "SPSS (Version: 23)" (SPSS I 2015). Pearson's correlation (2-tailed test) and principal component analysis were executed to test the relationship between different attributes and factors using the "ggbiplot," "factoextra," "ggplot2," "corrplot," and "tidyverse" packages in "R (v 4.0.5)" (R Development Core Team 2021).

Results

Metal contents in soil

The statistical analysis of the data for soil contents of the studied sites exhibited highly significant differences ($P < 0.001$) in metal contents in both seasons (Tables 1 and 2). The sites with high road traffic were more contaminated by metals. The Pull-111 contained a maximum concentration of the different elements such as Pb, Cd, Zn, Ni, Fe, Na^+, C, and N. The soil sample of Adda-46 also contained a high amount of metals than the other mentioned sites but lesser than the Pull-111 (Table 1).

Table 2 Analysis of variance for soils collected from various sites for Pb, Cd, Zn, Ni, Fe, Na^+, K^+, Ca^{+2}, C, and N contents

Source	Seasons	Sites	Interaction seasons and sites	Error
Degree of freedom	1	5	5	24
Soil Pb content	1.545	35.17	0.683	0.016
Soil Cd content	0.026	0.221	0.006	0.001
Soil Zn content	251.7	12085.3	25.72	0.635
Soil Ni content	19.15	148.5	0.679	0.051
Soil Fe content	2.478	50.94	0.768	0.040
Soil Na^+ content	468.0	427804.4	4316.2	327.4
Soil K^+ content	3.021	138.1	4.253	0.187
Soil Ca^{+2} content	22.40	294.8	11.19	0.839
Soil C content	0.032	6.136	0.149	0.029
Soil N content	1.590	1.094	0.139	0.008

Significant at $p<0.001$ Significant at $p<0.01$ Significant at $p<0.05$ Non-significant

Heavy metals and nutrient contents in the plant

N. oleander leaves showed statistically significant differences for various parameters among sites and for seasons (Table 3). The highest Pb, Cd, and N concentration was present in plants collected from Pull-111 compared to the other sites (Table 1). The concentration of the aforementioned elements was higher in the plant even compared to the soil at this site. The samples from other sites exhibited an increase in these metals in the following trend as Adda-46 > Chenab Nagar > Chiniot > Pull-Dingro. The concentration of Ni, Fe, and C was enhanced in plant shoots by the increase of these elements in the soil. The Pull-Dingro site with a smaller number of vehicles has shown less amount of Ni, Fe, and C in the plant after the control plants compared to other sites. The maximum values of these metals were noted at Pull-111 site followed by Adda-46, Chenab Nagar, and Chiniot in winter as well as in summer. The Ni and Fe concentrations were lesser in leaves than in the soil samples collected from the same sites, while C contents were greater in the plant compared to its concentration in the soils at all the sites. The higher concentration of heavy metals contents in the soil hindered the absorption of essential elements from the soil. The Na^+, K^+, and Ca^{2+} contents in plant samples in

Table 3 Analysis of variance for *N. oleander* collected from various sites for Pb, Cd, Zn, Ni, Fe, Na^+, K^+, Ca^{+2}, C, N, chlorophyll a, chlorophyll b, and carotenoid contents

Source	Seasons	Sites	Interaction between seasons and sites	Error
Degree of freedom	1	5	5	24
Shoot Pb content	3.672	45.93	0.292	0.107
Shoot Cd content	0.061	0.240	0.009	0.001
Shoot Zn content	54.22	5897.9	3.947	0.729
Shoot Ni content	17.50	76.44	1.269	0.177
Shoot Fe content	1.855	58.99	1.473	0.206
Shoot Na^+ content	111444.6	343482.6	6063.2	299.1
Shoot K^+ content	0.480	154.5	5.322	2.373
Shoot Ca^{+2} content	48.60	201.5	8.016	0.561
Shoot C content	37.88	35.56	1.160	0.340
Shoot N content	8.104	3.594	0.386	0.003
Chlorophyll a	0.035	0.231	0.008	0.003
Chlorophyll b	0.003	0.024	1.803	0.000
Carotenoids	0.000	0.000	0.000	0.000
Photosynthetic rate	22.07	35.73	1.748	0.135
Transpiration rate	0.305	0.668	0.005	0.001
Stomatal conductance	768.2	9584.5	26.25	4.768
Leaf internal CO_2 conc.	2765.0	23228.9	124.3	27.75
Total soluble proteins	0.624	115.5	1.850	0.115
Total free amino acids	12.88	79.11	0.662	0.121
Total antioxidant activity	6.596	100.0	0.720	0.163

Significant at $p<0.001$ Significant at $p<0.01$ Significant at $p<0.05$ Non-significant summer were in the order as Pull-111 > Adda-46 > Chenab Nagar > Chiniot > Pull-Dingro. However, during winter, generally, we got the trend for K^+ and Ca^{2+} in plants as Pull-Dingroo > Chiniot > Chenab Nagar > Adda-46 > Pull-111. The comparison of seasons exhibited less accumulation of all the studied metals in soil and plant samples in the winter season compared to the summer (Tables 1 and 2).

Leaf photosynthetic pigments

The addition of harmful metals in soil severely influenced the photosynthetic rate of the plant by declining the chlorophyll contents. The data of photosynthetic pigments exhibited significant differences between the factors such as sites ($P < 0.001$) and seasons ($P < 0.05$). The interaction differences of these two factors were non-significant ($P > 0.05$) except for carotenoids which showed highly significant differences for sites ($P < 0.001$) while non-significant differences in data of seasons as well as in their interaction (Table 3). The deterioration of Chl-a contents was calculated maximum in *N. oleander* leaves collected from Pull-111 compared to the control plants. The collected plant samples from areas situated away from the industry and having fewer vehicle numbers exhibited less decline in this pigment such as the plants in Pull Dingro seemed to have more Chl-a contents compared to the other studied areas excluding control plants. The decline in Chl-b contents were recorded in the order as Pull-111 > Adda-46 > Chenab Nagar > Chiniot > Pull Dingro > control. The carotenoid contents were enhanced in plants present near the more polluted areas such as Pull-111 < Adda-46 < Chenab Nagar < Chiniot < Pull Dingro < control because these chemicals act as antioxidants and increased the resilience level of plants under stress conditions. The comparison of seasons indicated that Chl-a contents of plant collected from studied areas were decreased by 48.5% in winter and 51.5% in summer. The Chl-b contents were more deteriorated in summer (47.5%) than in winter (52.5%) while this difference for carotenoids was recorded at 51.2% in winter and 48.8% in summer (Fig. 2a–c).

Gas exchange characteristics

The gas exchange attributes of plants collected from various locations near the stone grinding factory in the winter and summer seasons showed a significant ($P < 0.01$) waning (Table 3 and Fig. 3). The comparison of plants (collected from various areas) with control demonstrated notable perturbation in these parameters. In the winter season, the extremely reduced photosynthetic rate was recorded in plants located near the Pull-111, while in summer, Pull-111 and Adda-46 exhibited a maximum decline in the rate of this attribute. The *N. oleander* samples taken from the sites with low traffic volume showed a slight decrease in this parameter due to less pollution. The transpiration rate of plants was highly reduced by the pollutants than the control in winter and summer. The rate of transpiration was calculated in collected samples from different sites in the order as Pull Dingro > Chiniot > Chenab Nagar > Adda-46 > Pull-111. The

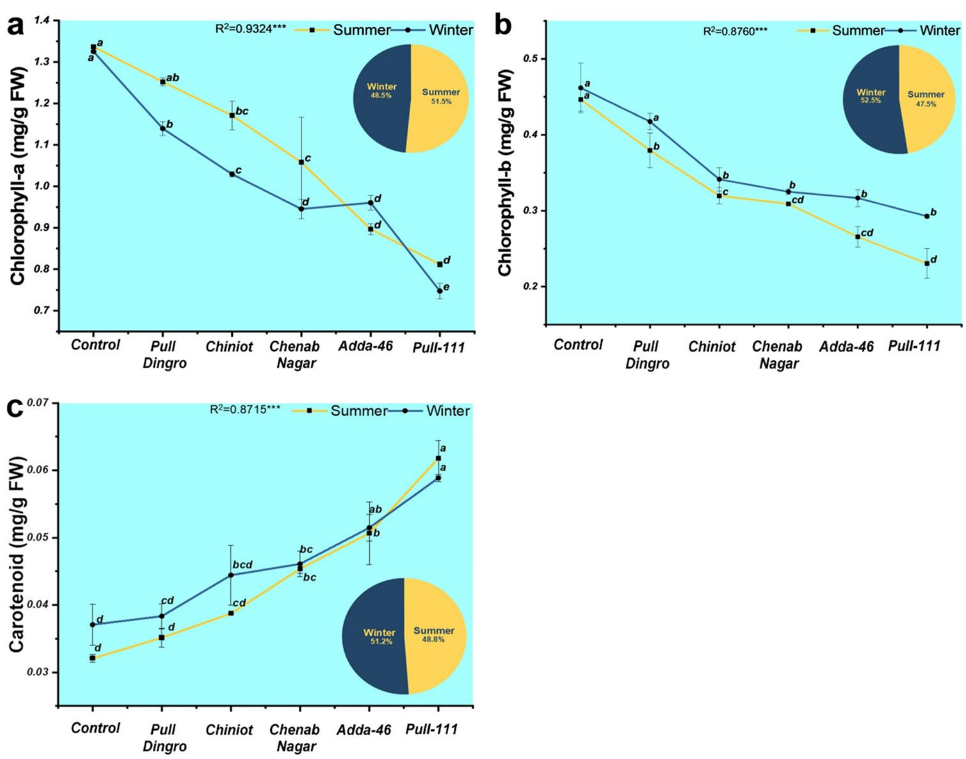

Fig. 2 Photosynthetic pigments, i.e., chlorophyll a (**a**), chlorophyll b (**b**), and carotenoid contents (**c**) in *N. oleander* at various sites in summer and winter seasons. Letters a–e represent LSD among the sites

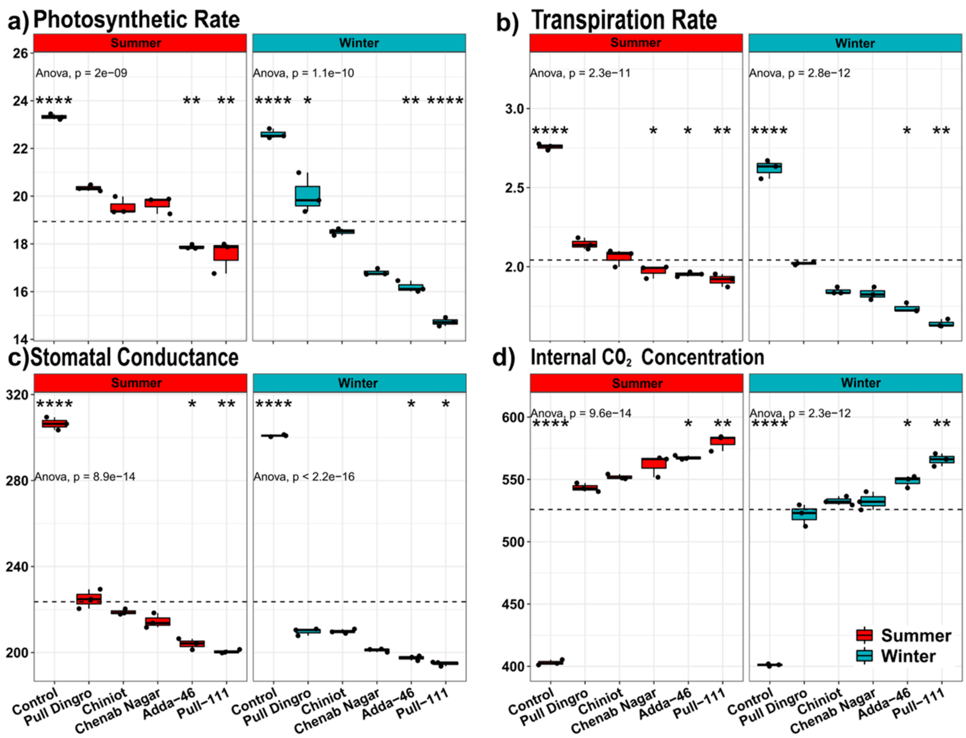

Fig. 3 Gas exchange characteristics: **a** photosynthetic rate (μmol CO_2 m^{-2} s^{-1}), **b** transpiration rate (H_2O m^{-2} s^{-1}), **c** stomatal conductance (H_2O m^{-2} s^{-1}), and **d** internal CO_2 concentration (μmol CO_2 mol^{-1}) of *N. oleander* at various sites in summer and winter seasons

reduction in stomatal conductance was recorded in both seasons. The maximum deterioration in this characteristic was in plants taken from Pull-111 and then from Adda-46. In the winter season, higher reduction in this parameter was observed. In case of internal CO_2, the samples picked from Pull-111 exhibited the highest values for CO_2 than the control. In summer, greater amount of CO_2 was measured than during the winter in plants collected from various sites.

Total soluble proteins, free amino acids, and antioxidant activity

The pollutants in soil significantly ($P < 0.05$) reduced the total soluble proteins in plants at all the sites compared to the control (Table 3). Pull Dingro, Chiniot, and Chenab Nagar sites could be considered less polluted sites because the protein contents of plants were less affected at these locations compared to the other sites (Fig. 4). The maximum deterioration in this biomolecule was determined in samples taken from Pull-111 followed by Adda-46. The more decline in this attribute was calculated in summer (50.4%) than in winter (49.6%). The total amino acids and antioxidants were significantly ($P < 0.01$) enhanced with the increase in the concentration of heavy metals in samples collected from all the sites in winter and summer seasons. Both phytochemicals are involved in the defensive mechanism of plants that is why the prominent increase in these parameters was recorded in samples collected from areas with high pollutant concentrations (Batista-Silva et al. 2019). In summer, 52% elevation in amino acids was recorded and 48% in winter. The maximum values of this attribute were determined in samples collected from Pull-111 followed by Adda-46, Chenab Nagar, Chiniot, and Pull Dingro in both seasons compared to the control. In both seasons, the minimum value for antioxidant activity was noted in control plants. The sites with heavy traffic load exhibited higher values of antioxidant activity in *N. oleander* such as Pull-111 > Adda-46 > Chenab Nagar > Chiniot > Pull Dingro.

Bioconcentration factor (BCF)

An examination of the BCF revealed that *N. oleander* has quite an ability to absorb heavy metal ions from the soil and accumulate in its leaves (Table 4), especially for Pb and Cd at all sites in both seasons. The highest value (1.343) of BCF was noted for Pb at Pull-Dingro site and for Cd; the highest value, i.e., 1.140, was recorded at Adda-46 site in summer. However, the BCF values for Zn, Ni, and Fe remained below 1.00 in both seasons.

Correlation

The significantly negative correlations were noted in the samples of various polluted sites collected in both seasons concerning the stomatal conductance, photosynthetic and transpiration rate, soluble proteins, Chl-a, Chl-b, K^+, and Ca^{2+} demonstrated in Pearson's correlation (Fig. 5a, b). This test also exposed a positive correlation of plants taken from

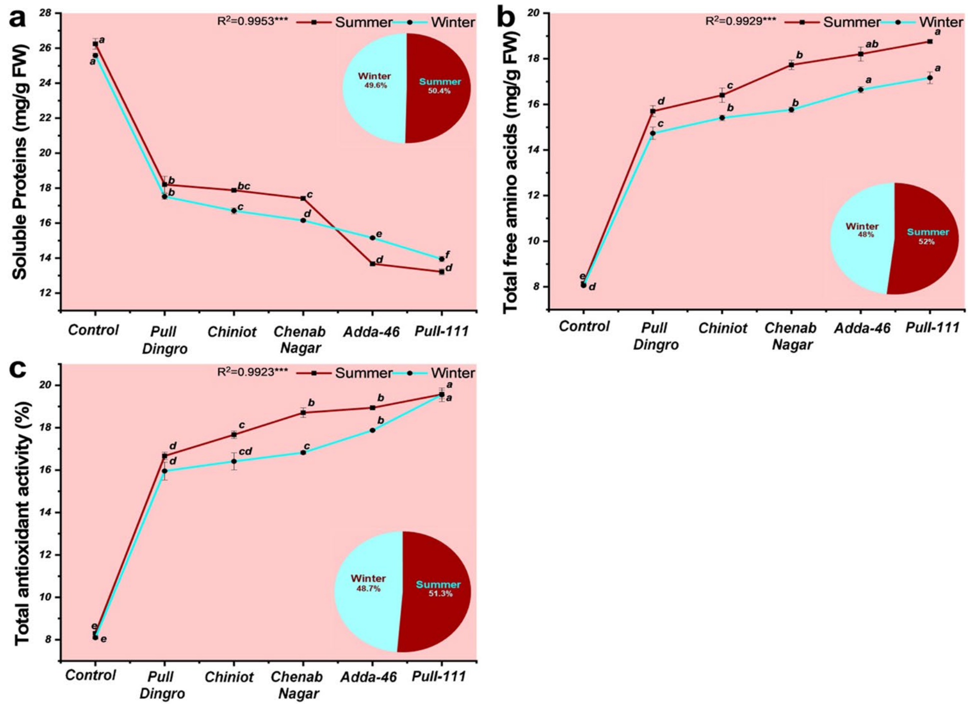

Fig. 4 Soluble proteins (**a**), total free amino acids (**b**), and total antioxidant activity (**c**) of *N. oleander* at various sites in summer and winter seasons. Letters a–e represent LSD among the sites

Table 4 Bioconcentration factor (BCF) of *N. oleander* for Pb, Cd, Zn, Ni, and Fe at various sites

	Summer				
	Pb	Cd	Zn	Ni	Fe
Control	0.09	0.00	0.452	0.478	0.551
Pull-Dingro	1.343	1.036	0.652	0.703	0.672
Chiniot	1.273	1.057	0.638	0.693	0.669
Chenab Nagar	1.237	1.110	0.621	0.808	0.668
Adda-46	1.096	1.140	0.670	0.775	0.933
Pull-111	1.114	1.028	0.659	0.757	0.794
	Winter				
Control	0.00	0.00	0.428	0.534	0.515
Pull-Dingro	1.109	1.00	0.665	0.664	0.909
Chiniot	1.082	1.00	0.649	0.703	0.918
Chenab Nagar	1.170	1.00	0.609	0.654	0.902
Adda-46	1.212	1.019	0.675	0.759	0.899
Pull-111	1.167	0.998	0.691	0.663	0.944

Values of BCFs above 1 or equals to 1.

various polluted sites in winter and summer with cellular CO_2, total free amino acids and antioxidant activity, C, and other ionic and heavy metal contents (like C, N, Na^+, Fe, Ni, Zn, Cd, and Pb). To confirm the results of this test, further statistical tests were also run such as PCA-biplot (Fig. 6 and Supplementary Table 2). The results obtained from the biplot revealed a trend of calculated attributes in winter and summer seasons due to the differences in the sites, shown by dim1 and dim2. The variance by dim1 was 88.4% compared to dim2 (5.5%). The PC analysis displayed a very clear dissociation of the determined parameter according to the seasons (winter and summer).

Discussion

In this study, *N. oleander* leaves showed higher concentration of Pb, Cd, N, and C compared to the soil samples showing the great ability of this plant to absorb and accumulate these elements to its aboveground part and thus rendering itself a plant suitable for phytoremediation. Like many other plants, it can store a larger amount of toxic metals in its body without causing any noticeable harm to itself. By continued cultivation of this plant in contaminated roadsides, eradication of vehicular heavy metal pollutants could be possible (Ibrahim and El Afandi 2020; Mahar et al. 2016).

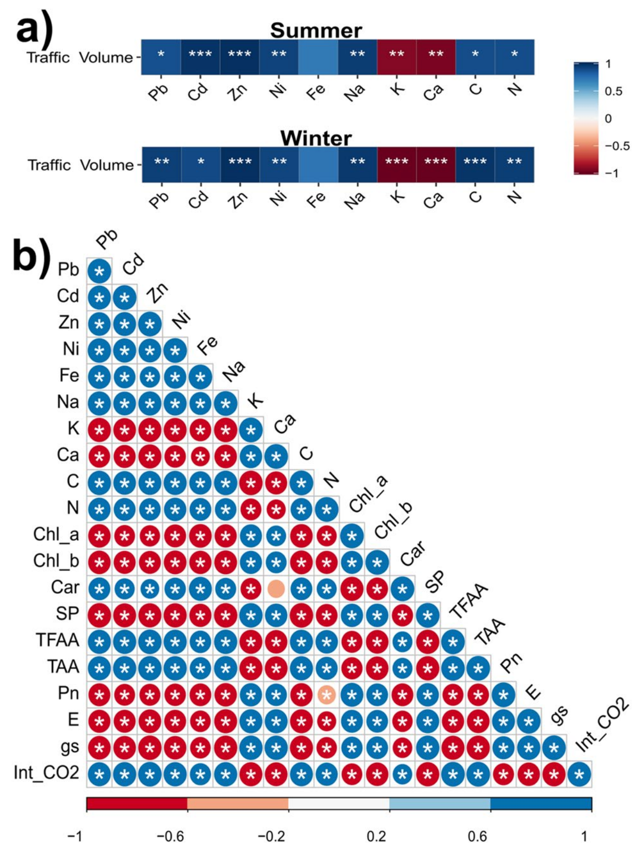

Fig. 5 Pearson's correlation between traffic volume and seasons (**a**) and autocorrelation among various measured variables (**b**) of the data collected from various sites in two seasons. Pb, lead; Cd, cadmium; Zn, zinc; Ni, nickel; Fe, iron; Na, sodium; K, potassium; Ca, calcium; C, carbon; N, nitrogen; Chl_a, chlorophyll a; Chl_b, chlorophyll b; Car, carotenoid; SP, soluble proteins; TFAA, total free amino acids; TAA, total antioxidant activity; Pn, photosynthetic rate; E, transpiration rate; gs, stomatal conductance; Int_CO2, internal carbon dioxide

The heavy metals and other pollutants released by the vehicles also influence the plant growth by affecting the physiological machinery of plants (Nasir et al. 2022; Neelab et al. 2022; Noman and Aqeel 2017), such as a reduction in photosynthesis due to a decline in chlorophyll contents. The absorption of heavy metals through the soil generates oxidative stress which disrupts the structure of chlorophyll contents (Chl-a and Chl-b), while the pollutants in the air especially dust particles are deposited on the surface of the leaf and block the light required to run the photosynthesis cycles (Aqeel et al. 2021; Bamagoos et al. 2022). That is why the plants collected from Pull-111 and Adda-46 exhibited a waning in chlorophyll contents and photosynthetic rate than the control due to high vehicle exhaust pollution in the air and soil because of high traffic load there. While in these sites, the increase in the concentration of carotenoids is due to the activity of this pigment under stress conditions. This chemical acts as an antioxidant and enhances the resilience of plants to environmental stresses (Khilji et al. 2021; Naheed et al. 2022; Noman et al. 2017; Tahaei et al. 2022). Among the sites, Pull-Dingro is the least polluted; the minimum carotenoid contents have been estimated in plants collected from this site because the generation of reactive oxygen species (ROS) is lesser in these samples that is why large numbers of antioxidants are not needed.

The reduction in stomatal conductance, photosynthetic, and transpiration rate in the sample was also high. The metal stress stimulates the overproduction of ROS which disintegrates the membranous structures of the cell, especially the electron transport chain. The decrease in chlorophyll contents and disturbance in PSII are the main causes of the fall in the photosynthetic rate (Bamagoos et al. 2022; Hussain et al. 2022; Ma et al. 2022). The ROS also disrupts cell homeostasis and generates water scarcity leading to a reduction in transpiration rate and stomatal conductance (gs). The reduction in photosynthesis also enhances the internal CO_2, which causes a reduction in stomatal conductivity (Akhter et al. 2021; Awan et al. 2022; Naheed et al. 2021).

In metal stress, the concentration of antioxidant activity is enhanced to quench the activity of ROS. Amino acids are directly involved in the synthesis of proteins. Under metal toxicity, their number gets increased to protect the plant from damage. Increased free amino acids could also be attributed to degradation in total protein content which could directly be caused by metal toxicity (Akhter et al. 2022a; Khalid et al. 2019a). The amino acids and other antioxidants control the stomatal regulation and maintain the cell's water level by preventing ROS formation. In plants at various growth stages, the increase in the activity of antioxidants is noted under metal toxicity. Same observations have been made by studying the various polluted sites. The plants from highly polluted sites (Pull-111 and Adda-46) exhibited an increase in amino acid contents and antioxidants activity, while there is a decline in protein contents compared to less polluted sites such as Pull-Dingro (AbdElgawad et al. 2020; Mahmud et al. 2019; Shekari and Javanmardi 2017; Tripathi et al. 2018).

Although the photosynthetic rate and gas exchange attributes have been affected by the adverse effects of heavy metals and other pollutants, *N. oleander*'s defense system seemed to be boosted to cope with the adverse effects of these pollutants. Also, an increased concentration of metals in the soil restricted the absorption of essential mineral nutrients. An examination of the BCF values of *N. oleander* for Pb and Cd metals also indicated that it could accumulate these toxic metals in its body. Previous investigations have also used BCF values to appraise the phytoremediation potential of different plant species (Sajad et al. 2020). BCF varies between different plants and metal types (Khan et al. 2016; Li et al. 2021). A difference in BCF of metals among various plant species could be due to different soil properties or due to physiological phenomenon of plants, i.e., growth

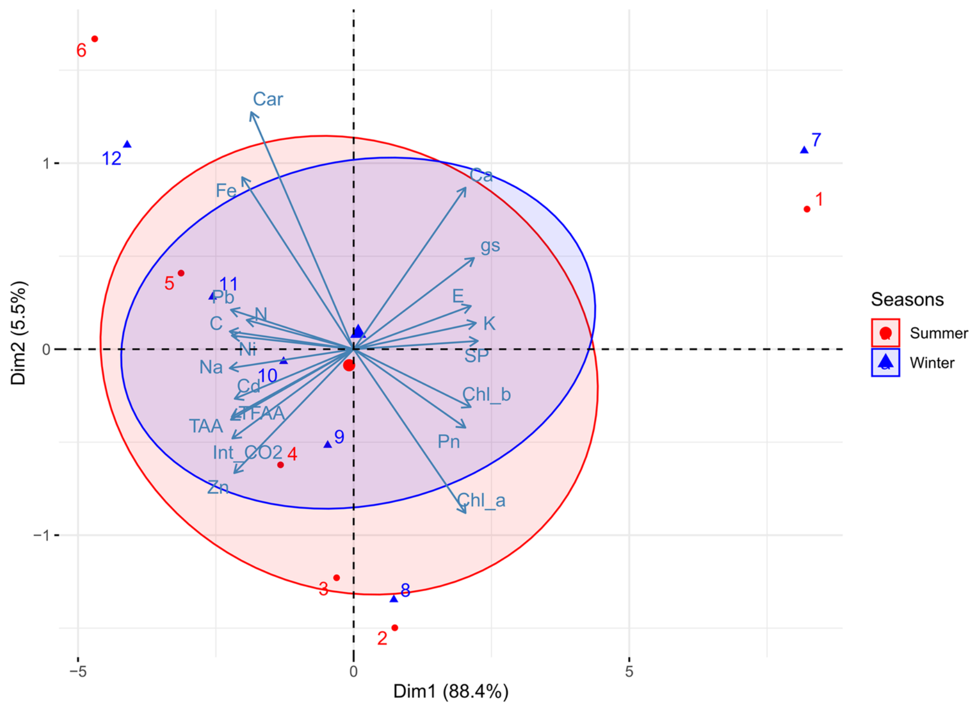

Fig. 6 PCA-biplot of the data of *N. oleander* for various variables in summer and winter. Pb, lead; Cd, cadmium; Zn, zinc; Ni, nickel; Fe, iron; Na, sodium; K, potassium; Ca, calcium; C, carbon; N, nitrogen; Chl_a, chlorophyll a; Chl_b, chlorophyll b; Car, carotenoid; SP, soluble proteins; TFAA, total free amino acids; TAA, total antioxidant activity; Pn, photosynthetic rate; E, transpiration rate; gs, stomatal conductance; Int_CO2, internal carbon dioxide

factor dynamics and metal uptake efficiency of the plant species (Hasan et al. 2021).

Conclusion

N. oleander leaves collected from various sites along the road depicted high concentrations of Pb, Cd, Ni, Zn, and Fe metals and C and N compounds. The concentration of these elements was also high in the soil samples taken from these sites. Interestingly, the concentration of Pb and Cd metals was higher in the plant compared to the soil in both seasons. These two heavy metals are the ones which are most commonly found along the roads because they are mainly released by the automobiles especially in developing countries like Pakistan. And *N. oleander* has shown a great potential to extract them from the contaminated roadside soils. In that case, it could be the best solution for decontamination and sustainably managing these areas. We also recommend studying this plant for a broad spectrum of pollutants at diverse environmental locations.

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Author contribution NK and MA: conceived the idea and designed the experiment; NK, MA, AN, and AN: writing, review, and editing; NK and MA: methodology and visualization; NK, AT, NH, SI, and NA: gathered literature, resources, and experimentation; FMA and MH: help in the interpretation of the results; NK and MA: critically revised the MS. All authors approved the final version of the manuscript.

Data availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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