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Original Research Article

EFFECT OF AUTOMOBILE RELATED LEAD POLLUTION ON SPATIO-TEMPORAL DISTRIBUTION OF BIOCHEMICAL ATTRIBUTES ALONG ROADSIDE VEGETATION

AHMAD I[1], HUSSAIN T[2*], LIAQUAT M[2], ANJUM S[3*], SARWAR M[4], AHMAD S[5], ALI Q[6], NASEEM W[7], RIZVI SA[7]

[1]*Department of Botany, Govt. Post-Graduate College Satellite Town Gujranwala, Pakistan*
[2]*Department of Horticulture, PMAS Arid Agriculture University, Rawalpindi, Pakistan*
[3]*Institute of Botany, Faculty of Life Sciences, University of the Punjab, Lahore, 54590, Pakistan*
[4]*Department of Horticulture, Faculty of Agricultural Sciences, University of the Punjab, Lahore, 54590, Pakistan*
[5]*Department of Entomology, Faculty of Agricultural Sciences, University of the Punjab, Lahore, 54590, Pakistan*
[6]*Department of Plant Breeding and Genetics, Faculty of Agricultural Sciences, University of the Punjab, Lahore, 54590, Pakistan*
[7]*Soil and water conservation research Institute Chakwal, Pakistan*
**Corresponding author's email address: sumreen38@gmail.com, dr.tanveer05@uaar.edu.pk*

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Abstract *This research was carried out for the phyto-monitoring of pollution to check the effect of Pb2+ pollution on the spatio-temporal distribution of biochemical attributes resulting along Motorway M-2 and G.T. road Kharian to Taxila. The result showed that maximum decrease of total soluble sugars in Nerium oleander (0.023 mg/g F.wt) along M-2 and Calotropis procera (0.042 mg/g F.wt) along N-5, total free amino acid in Datura alba (0.501 mg/g F.wt) and Cenchrus ciliaris (0.62 mg/g F.wt) and total soluble proteins in Cenchrus ciliaris (3.91 mg/g F.wt) and Ricinus communis (0.73 mg/g F.wt) due to high concentration of Pb2+ (2.05-1.33 mg/kg dry wt.) along roadside. Findings may have their application as bio-indicators to perceive the occurrence of atmospheric pollutants on plants for identification and prediction of environmental hazards.*

Keywords: *Vehicular Pb^{2+} pollution; Seasonal variation; Biochemical attributes; environmental hazards*

Introduction

Various factors influence the release of different pollutants due to road traffic.. These include various vehicles, engine types, vehicle use, quality of maintenance, vehicle age, fuel type and quality tyre type. Environmental pollution, mostly air pollution, alters plants' physiological and biochemical pathways by limiting productivity (Woo *et al.*, 2007). Metal released from the exhaust of motor vehicles gets highly deposited in plants compared to other metals (Celik, 2005). The vehicle pollution showed marked changes in photosynthetic pigments, cysteine and protein contents. Photosynthesis was inhibited by metal exposure (Singh *et al.*, 1999). Metals, other metals, and arsenic reduced the photosynthetic activity in plants (Miteva and Merakchiyska, 2002). The *Eucalyptus calmadulensis* exposed to air pollution showed higher chlorophyll contents, soluble sugars and high proline contents in polluted plants compared to the control one (Seyyednejad and Koochak, 2011). Mir *et al.* (2008) studied the total chlorophyll and protein analyzed in leaf samples of *Alstoniascholaris*, *cusreligiosa* and *Neriumodorum* subjected to vehicular pollution. All the parameters mentioned above are reduced with an increase in traffic density. This study selected M-2 and G.T. roads of Punjab, Pakistan, for the above analysis. Both roads vary greatly not only in their traffic density but also in the categories of vehicles.

Aim and Objectives

The objectives of this study are as follows.

- To examine the effect of metal pollution caused by automobiles on vegetation.
- To examine the adverse effects of automobile-related Pb2+ pollution on spatio-temporal variations of biochemical attributes of natural vegetation along two roads.
- To provide a baseline study for future investigation.

Material and Methods

Experimental sites

The following five sites were selected on both roads, given below.

Sr.No.	G.T. Road	Motorway (M-2)
1.	Kharian	Lilla
2.	Deena	Kalarkahar

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3.	Gujjar khan	Balkaser
4.	Bahriya town	Chakry
5.	Texila (G)	Texila (M)

Motorway (M-2) has relatively less traffic density and residential particles on its sides than G. T. Road (N-5), which has heavy traffic and factories along the roadside. M-2 road is well maintained than G.T road.

Plants under study

The following plant samples were collected from M-2 and G.T. Road.

1-*Calotropis procera* , 2-*Datura alba*, 3-*Nerium oleander*, 4-*Ricinus communis*, 5-*Cenchrus ciliaris.*

Collection of leaf samples

Five plant species were selected for this study. Three plants of each species were selected at each site along both the left and right sides of the road and from the road's central green belt at roads M-2 and N-5 for analysis. These leaf samples were collected in different seasons of the year i.e spring (last week of April, 2013), summer (last week of July, 2013), autumn (last week of October, 2013), and winter (First week of January 2014).

Digestion procedure

The samples of leaves were processed with the method of sulphuric peroxide. Fresh sample measuring 0.1 g of dry powder of plant leaves of each plant species was digested in 0.42g of selenium and 14g of $LiSO_4.2H_2O$ by slowly adding hydrogen peroxide (H_2O_2), mixed well, and finally added 420 mL of sulphuric acid and made the volume up to 50 mL.

Pb^{2+} determination

Pb^{2+} concentration was determined by Flame-Atomic Absorption Spectrophotometer (Analyst 300 Perkin-Elmer Germany). A calibration curve was prepared using various concentrations of Pb^{2+} standard.

Biochemical attributes

A 0.1g fresh leaf sample of all under study species was kept in sodium buffer for one day and analyzed to determine:

a- Total free amino acids
b. Total soluble proteins
c. Total soluble sugars

a) Determination of total free amino acids

The calculation of total free amino acids was performed by following the formula outlined by Hamilton and Van Slyke (1973),

$$\text{Total free amino acids} = \frac{\text{Sample Readings x Sample Volume x dilution factor}}{\text{Fresh Leaf Weight x 1000}}$$

b) Determination of total soluble proteins

Total soluble proteins were calculated by the method of Lowry *et al*. (1951).

c) Determination of total soluble sugars

Total soluble sugars were calculated according to Yemm and Willis's (1954) method.

Experimental design and statistical analysis of data

Two ways blocking (Two Way Randomized Block Design) was used for randomized stratified sampling. The data for various ecological attributes was analyzed using a COSTAT (Cohort Software, Berkeley, California USA). The mean values were compared with the least significant difference (LSD) test following Steel and Torrie (1980).

Results and Discussion

Pb^{2+} concentration in plant leaves

It is evident from Figure 1 that the concentration of Pb^{2+} in selected plant species showed significant variation during different seasons at M-2 and G.T. road ($p < 0.001$) technique. The interactions between sites and seasons, sites and plants, seasons and plants, and among sites x seasons x plants were also highly significant as $p<0.001$. A comparison among sites indicated that Pb^{2+} concentration in plants varied significantly along the M-2 and G.T. road sites compared to the control. Maximum Pb^{2+} concentration (2.9571 mg kg^{-1}dry wt.) was observed at M2 Kalar kahar and Texila (2.753 mg kg^{-1}dry wt.) followed by that recorded at Ckakry, Balkasar, whereas the minimum Pb^{2+} concentration (1.1806 $mgkg^{-1}$dry wt.) was observed at Lila. The maximum increase was observed at Bahyria town (3.4735 $mgkg^{-1}$dry wt.) and Texila (G) (3.2471 mg kg^{-1}dry wt.) followed by G.T. road Gujjar khan, Kharian whereas the minimum Pb^{2+} concentration was found at Deena. The higher concentration was found during the summer, followed by that recorded during spring, while the minimum Pb^{2+} concentration was recorded during winter.

The Pb^{2+} concentration was significantly higher in roadside leaves than in control plants growing on the roadside of M-2 and G.T. road. All plant species are significantly different from each other at different sites in different seasons. Plants accumulate heavy metals depending on their leaf morphology and anatomy (Srivastava *et al*., 2015). In this study concentration of Pb^{2+} in different plants was found from 2.04501-1.3233 mg kg^{-1} dry wt. This level of Pb^{2+} concentration is higher than the permissible limit according to WHO (Alexander, 2015). The maximum Pb^{2+} concentration in soil dust was 0.37 mg kg^{-1} along M-2 and G.T road respectively. The level of Pb^{2+} in soil dust also crossed the maximum permissible limit devised by WHO (0.015 mg kg^{-1}). *Nerium oleander* and *Calotropis procera* accumulated the highest Pb^{2+} concentrations around both roads. D'Souza *et al*., (2010) and Barthwal et al. (2008) recorded high Pb^{2+} concentrations in *Calotropis*

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procera. It was found that plants growing along the roads deposit many toxic metals, particularly Pb2+, in leaves (Tong, 1991; Jaradat *et al*., 2004).

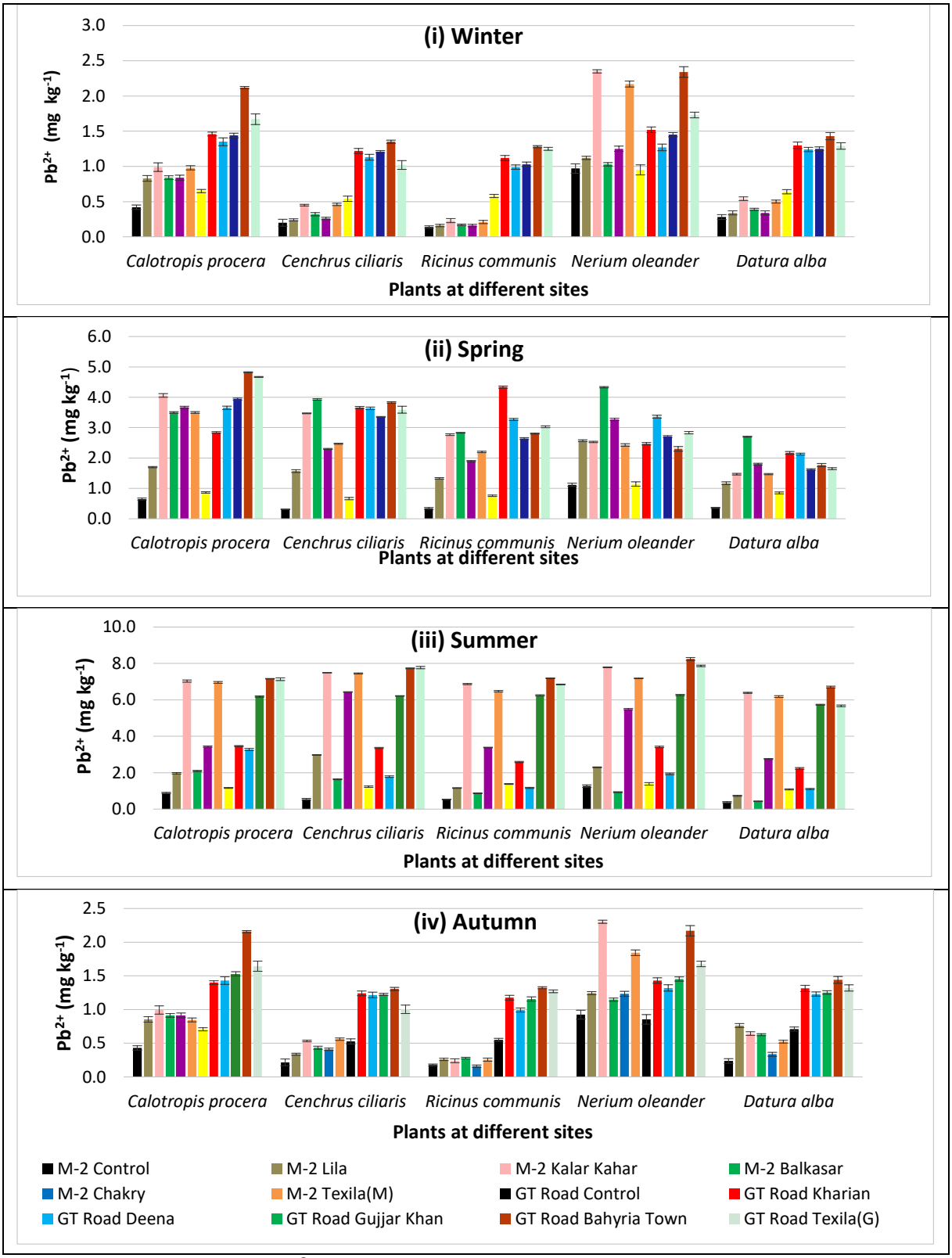

Figure 1. Concentrations of Pb^{2+} during different seasons in plant species along Motorway (M-2) and G.T. road (N-5) in the province of Punjab, Pakistan

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Anatomical studies of leaves might be useful for evaluating the effect of pollution due to vehicles (Pal *et al*., 2002). The same finding was reported by Munendra *et al*. (2004) that Pb^{2+} concentrations decreased in spring and increased in the monsoon to winter seasons (Pb^{2+} ranged from 0.0021 to 0.00282 mg g^{-1}dry weight). The study indicated that the predominant source for the accumulation of Pb^{2+} in leaves of (*Dalbergia sisso*) was directly related to exposure time of automobile emission. Higher concentration of Pb^{2+} was measured i.e. 0.00132 mg g^{-1} in 1994 to 0.00019 mg g^{-1} in 2002 at the road side plants compared to the urban areas. Among seasons, the highest Pb^{2+} content was found during the summer season in plants along both roads. The level of Pb^{2+} recorded during the present study was lower than those reported for some other sites of word (Teju *et al*., 2012; Silva *et al*., 2016). Pb^{2+} content in soil along the roadside in Nigeria was 26.7 mg kg^{-1} (Adedeji *et al*., 2013). Martin *et al*., 2015 found a 126 % reduction in Pb2+ content in soil collected from sites in Valencia city, Spain over the past 70 years due to controlled Pb^{2+} fuel consumption. Automobiles are the main source of Pb^{2+} in plants and soil of Quetta city, Pakistan (Khattak *et al*., 2013). Leaded gasoline is the main source of leaded pollution (Bhowmick *et al*., 2015).

Biochemical Parameters

a) Total soluble sugars

The data (Figure 2) about total soluble sugars revealed that this attribute was decreased in all plants on the roadside of M-2 and G.T. road while comparing the plant species on M-2, the maximum decrease in total soluble sugars was observed in *Nerium oleander* (0.023 mg/g F.wt) growing on roadside M-2 while the minimum was in *Datura alba* on roadside comparative to their controls, however, the other plant species showed an intermediate trend for a decrease in total soluble sugars respective to their controls. The trend among the plants of G.T. road, the maximum decrease was found in *Calotropis procera* (0.042 mg/g F.wt) and the minimum decrease was recorded for total soluble sugars in *Cenchrus ciliaris* while the other plants like *Riccinus communis* and *Nerium oleander* showed inconsistent decrease in total soluble sugars (Figure 3) roadside plants as compared to controls.

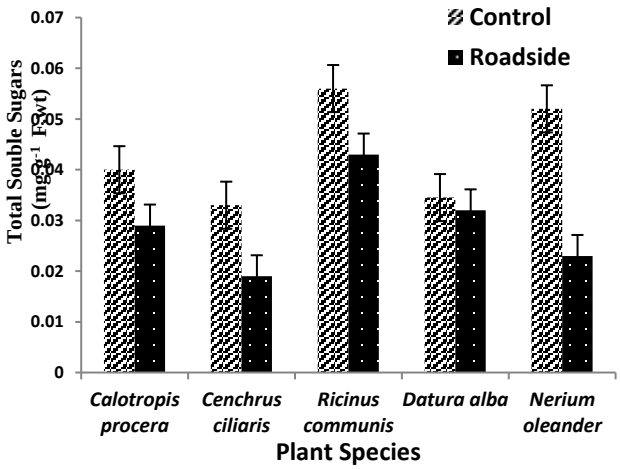

Figure 2. Concentration of total soluble sugars in all plant species growing on Motorway (M-2)

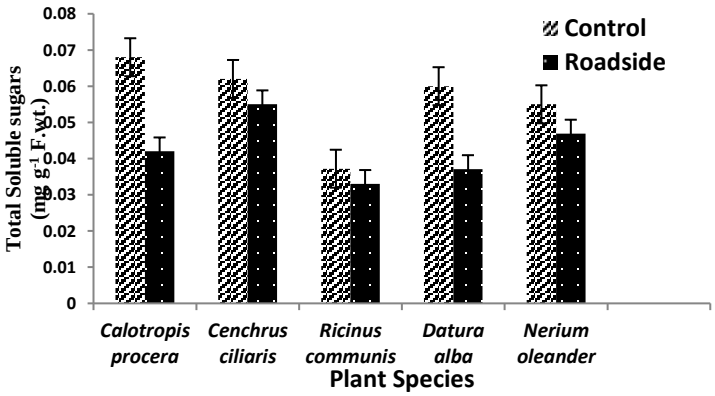

Figure 3. Concentration of total soluble sugars in all plant species growing on G. T. road (N-5).

b) Total free amino acids

Overall, a decreasing trend in total free amino acids was seen in roadside vegetation compared to control plants on M-2 and G.T. road (Figure 4). The *Datura alba* (control) had maximum free amino acids, while the plants growing on the roadside had quite low amino acid concentration. In *Nerium oleander* (0.501 mg/g F.wt) growing on the roadside, the total free amino acids decreased less as compared to other plant species i.e *Ricinus communis*, *Cenchrus ciliaris* and *Calotropis procera* as compared to their controls. Compared to their control, A significant decrease was recorded in the total free amino acids of plants growing at G.T. road. The maximum reduction for total amino acids was noticed in *Cenchrus ciliaris*(0.62 mg/g F.wt) growing on the roadside, while the minimum was in *Nerium oleander* followed by *Ricinus communis* and *Calotropis procera* (Figure 5).

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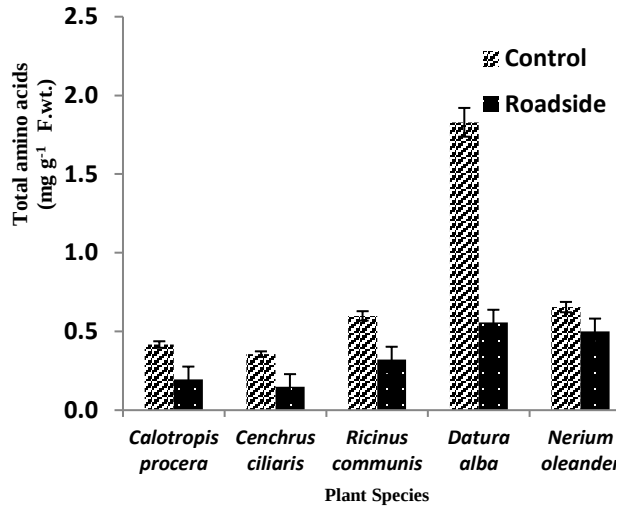

Figure 4. Total free amino acids in all plants species of Motorway (M-2)

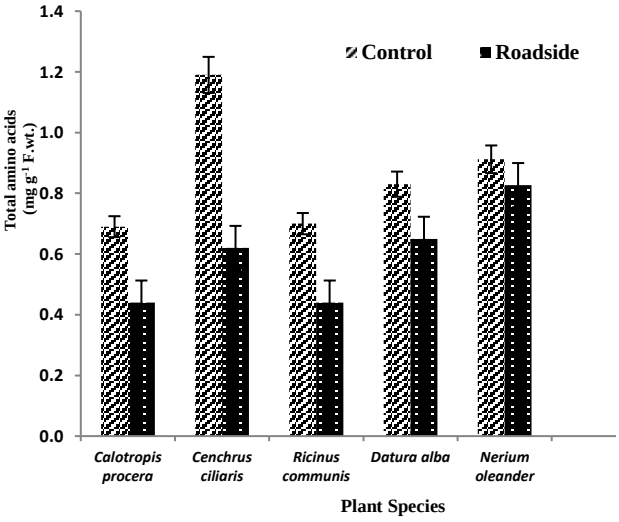

Figure 5. Concentration of total free amino acids in all plant species along G. T. road (N-5)

c) Total soluble proteins

The total soluble proteins decreased in all plants under study growing on the roadside of M-2 and G.T. road (Figuer 6). A prominent decrease in *Cenchrus ciliaris* (3.91 mg/g F.wt) and smaller was recorded in roadside plants, followed by Datura alba and Calotropis procera compared to their controls. The maximum decrease was observed in *Ricinus communis* (0.73 mg/g F.wt) *and Nerium oleander* growing on the roadside of G.T. road. The minimum decrease was found in *Calotropis procera* compared to their controls. *Calotropis procera* seems persistent on M-2 and G.T. roads (Figure 7).

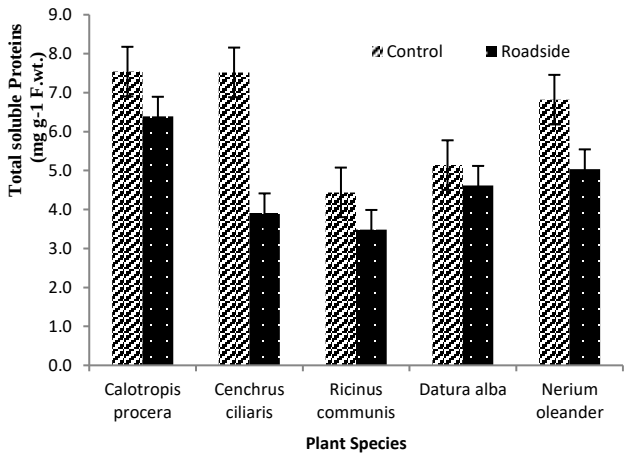

Figure 6. Concentration of total soluble proteins in all plant species of Motorway (M 2)

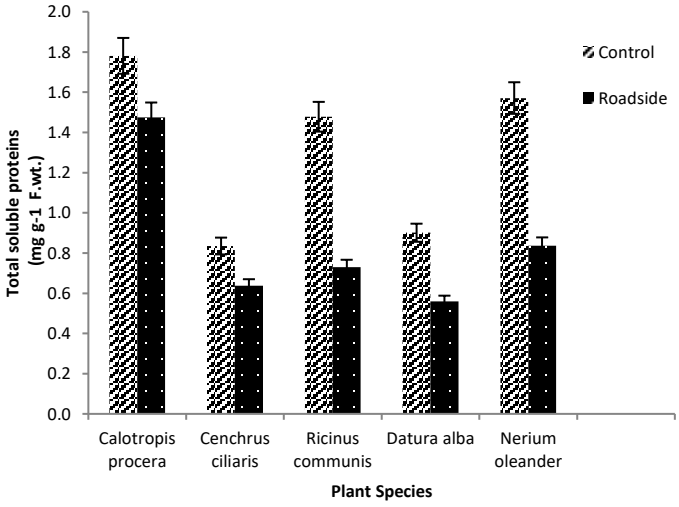

Figure 7. Concentration of total soluble proteins in all plant species of G.T. road

3.3 Spatial distribution of biochemical attributes

(a) Spatial distribution of biochemical attributes along M-2

CCA ordination biplot (Figure 8) shows the effect of the distribution of biochemical attribute concentrations in five different plant species along the Motorway at five different sites along M-2. The *Datura alba* and *Ricinus communis* plants showed association with C-TP and RS-TAA. *Cenchrus ciliaris* has a significant association with C-TAA. It also showed a weak association with C-TSS and RS-TSS. *Nerium oleander* also showed a weak association with C-TAA and RS-TAA. *Calotropis procera* showed an association with RS-TP.

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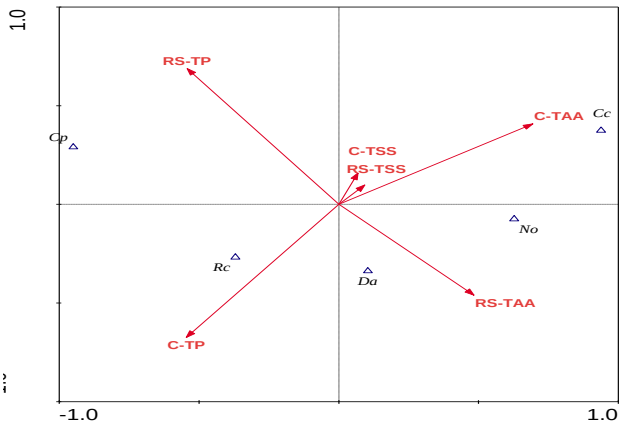

Figure 8. CCA ordination biplot shows metals' effect on plants' biochemical attributes along Motorway (M-2). (Cp: *Calotropis procera*, Cc: *Cenchrus ciliaris*, Rc: *Ricinus communis*, No: *Nerium oleander*, Da: *Datura alba*).
C: control, R: roadside, TSS: Total soluble sugar, TAA: Total aminoacid, TP: Toatal Proteins

(b) Spatial distribution of biochemical attributes along G.T. road (N-5)

CCA ordination biplot (Figure 9) shows the effect of the distribution of biochemical attribute concentrations in five different plant species along G.T. road at five sites. The biochemical attributes showed different associations in all five control species and roadside along G.T. road. The *Datura alba* and *Ricinus communis* plant showed a strong association with control TSS and control TSS. *Cenchrus ciliaris* has an insignificant association with all biochemical attributes except with C-TP. *Nerium oleander* also showed a weak association with R-TAA and C-TAA. *Calotropis procera* showed weak association with R-TSS, C-TSS and RS-TP.

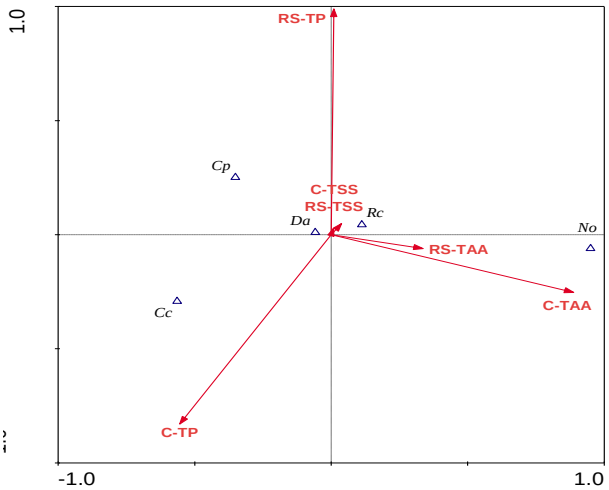

Figure 9. CCA ordination biplot showing metals' effect on plants' biochemical attributes along G. T. road. (Cp: *Calotropis procera*, Cc: *Cenchrus ciliaris*, Rc: *Ricinus communis*, No: *Nerium oleander*, Da: *Datura alba*).
C: control, R: roadside, TSS: Total soluble sugar, TAA: Total aminoacid, TP: Toatal Proteins

The metal stress disrupts the enzymes involved in photosynthesis by replacing its prosthetic group, which affects its compatibility with a particular substrate, indirectly affecting the carbon dioxide assimilation and resulting in low production of total soluble sugars. The previous findings also indicated that total soluble sugars decreased in *Laguncularia racemosa* L. when subjected to chromium stress (Rocha *et al*., 2009). An increase was observed in soluble carbohydrates in leaves collected from different polluted sites in Iran. The decrease in total soluble sugars and proteins on increasing metal concentration was reported by Costa and Spitz, 1997 and Bhardwaj *et al*., 2009. Xie *et al*., 2013 and Zemanova *et al*., 2013 also observed increased total free amino acids under heavy metal stress. Heavy metals, particularly Pb^{2+} and Cd^{2+} targeted metalloenzymes, are involved in the plants' various metabolic activities. The restricted binding sites of enzymes like nitrate reductase, a key enzyme in the nitrogen assimilation pathway whose activity is retarded when Pb^{2+}, Ni^{2+} or Cd^{2+} replaces its co-factors. The replacement of co-factor blocks the active site of enzymes; thus a reduction in ion uptake occurs (Salsburry and Ross, 2003). A significant reduction in total soluble protein contents was observed along both roads compared to their control. Panday and Tripathi, 2011 also reported the reduction of protein content under heavy metals and other abiotic stress, which could be due to decreased photosynthesis(Khosravinejad *et al*., 2009). Heavy metals negatively affect These protein contents (Kastori *et al*., 1992).The proteins and changes in GPX activities were sensitive, universal and well-correlated with heavy metals (Kandziora-Ciupa *et al*., 2013). Results of this present study indicated that total free amino acids, total soluble sugars and total proteins decreased in roadside plants compared to control plants. The production of low nitrogenous compounds might be due to metal exposure may be retarding the nitrogen uptake. Sharma and Prasad (2010) also documented that the metals bind with the sulpha-hydryl (SH) links or coupled with active enzyme sites, ultimately slowing down the nitrogen metabolism. Soluble proteins and nitrate reductase activity were decreased in *Vigna radiata* by increasing the Cd^{2+} stress (Muneer *et al*., 2011). Plant pigments and total soluble proteins decreased but antioxidant activity increased at industrial and urban street sites (Doganlar and Atmaca, 2011). Toxic effects on proteins contents were also reported in *Azolla pinnata* (Masood and Abraham, 2006), *Brassica oleracea* (Chatterjee and Chatterjee, 2000),

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Brassica juncea (John *et al*., 2009). While no change was reported in protein contents and metal stress retarded nitrogen uptake and resulted in too low proteins in the *Pisum sativum* (Parys *et al*., 1998) and the same results were obtained for *Paspalum distichum* (Bhattacharya *et al*., 2010). Amonia and nitrogen fixation conversion reduced in soybean nodules when subjected to Cd^{2+} stress (Balestrasse *et al*., 2003; Jalil et al., 2023). Nitrate reductase and glutamate synthase activities were also inhibited in beans at long term exposure of Cd^{2+} (Gouiaa *et al*., 2000; Raza et al., 2023; Zubair et al., 2016). The variation between amino acids and soluble proteins in leaves of plants may be due to the differences in metabolism rates, and catabolism of proteins. As Noctor *et al*. (2002) reported, variation is due to the difference in metabolism of proteins that may contribute to the coordination of minor amino acids.

Conclusion

The plants along roadsides are directly exposed to traffic-related emissions, ultimately affecting their biochemical attributes. Although pollution due to metal is in permissive limits along Motorway (M-2) and G.T. road, the concentration of Pb^{2+} rapidly adds to the food chain due to heavy traffic pollution. If their concentration is not properly monitored for couple of years, It poses a significant health risk to all living beings, including plants and animals.

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Declarations

Data Availability statement

All data generated or analyzed during the study are included in the manuscript.

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Ethical approval was given from Ethical Review committee of department.

Consent for publication

The consent form was approved from Ethical Review committee of department.

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Regarding conflicts of interest, the authors state that their research was carried out independently without any affiliations or financial ties that could raise concerns about biases.

Author Contribution

RR conducted research and wrote up initial draft of manuscript. MAM, AH, SA and MAS provided resources. SA, MAF, MWA an SA made final editing in the manuscript. All authors approved final version of manuscript.

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