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

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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 Pb2+ 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 calmodulensis 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.

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>G.T. Road</th>
<th>Motorway (M-2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Kharian</td>
<td>Lilla</td>
</tr>
<tr>
<td>2.</td>
<td>Deena</td>
<td>Kalarkahar</td>
</tr>
</tbody>
</table>

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 LiSO₄·2H₂O by slowly adding hydrogen peroxide (H₂O₂) mixed well, and finally added 420 mL of sulphuric acid and made the volume up to 50 mL.

**Pb²⁺ determination**

Pb²⁺ concentration was determined by Flame-Atomic Absorption Spectrophotometer ( Analyst 300 Perkin-Elmer Germany). A calibration curve was prepared using various concentrations of Pb²⁺ 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),

\[
\text{Total free amino acids} = \frac{\text{Sample Readings} \times \text{Sample Volume} \times \text{dilution factor}}{\text{Fresh Leaf Weight} \times 1000}
\]

**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**

**Pb²⁺ concentration in plant leaves**

It is evident from Figure 1 that the concentration of Pb²⁺ 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 Pb²⁺ concentration in plants varied significantly along the M-2 and G.T. road sites compared to the control. Maximum Pb²⁺ concentration (2.9571 mg kg⁻¹ dry wt.) was observed at Bahyria town, whereas the minimum Pb²⁺ 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 Gujar khan, Kharian whereas the minimum Pb²⁺ concentration was found at Deena. The higher concentration was found during the summer, followed by that recorded during spring, while the minimum Pb²⁺ concentration was recorded during winter.

The Pb²⁺ 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 Pb²⁺ in different plants was found from 2.04501-1.3233 mg kg⁻¹ dry wt. This level of Pb²⁺ concentration is higher than the permissible limit according to WHO (Alexander, 2015). The maximum Pb²⁺ concentration in soil dust was 0.37 mg kg⁻¹ along M-2 and G.T. road respectively. The level of Pb²⁺ 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 Pb²⁺ concentrations around both roads. D’Souza et al., (2010) and Barthwal et al. (2008) recorded high Pb²⁺ concentrations in *Calotropis*...
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
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). Ledged gasoline is the main source of ledged 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 Ricinus 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)**

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).

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

**3.3 Spatial distribution of biochemical attributes**

**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 (Figure 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).
The proteins of Calotropis procera, Cenchrus ciliaris, Ricinus communis, and Datura alba showed a strong association with R-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-TAA and RS-TP.

### Figure 9. CCA ordination biplot showing metals' effect on plants' 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-TAA and RS-TP.

<table>
<thead>
<tr>
<th>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)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-TP: Total free amino acids, C-TSS: Total soluble sugars, C-TAA: Total amino acids, RS-TSS: Total soluble sugars along roadside, RS-TAA: Total amino acids along roadside, RS-TP: Total proteins along roadside</td>
</tr>
</tbody>
</table>

### 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, 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 Pb²⁺ and Cd²⁺ 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 Pb²⁺, Ni²⁺ or Cd²⁺ replaces its co-factors. The replacement of co-factor blocks the active site of enzymes; thus a reduction in ion uptake occurs (Salsbury 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-Ciupea 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 sulphahydryl (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 Cd²⁺ 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.

**References**


of heavy metal accumulation and antioxidant responses in Vaccinium myrtillus L. leaves in polluted and non-polluted areas. *Environmental Science and Pollution Research*, 20, 4920-4932.


Declarations

Data Availability statement

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

Ethics approval and consent to participate
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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Conflict of Interest
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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