

## ADVERSE EFFECTS OF AUTOMOBILES RELATED $Pb^{2+}$ POLLUTION ON PHOTOSYNTHETIC ATTRIBUTES AND WATER RELATIONS OF ROADSIDE VEGETATION

IJAZ AHMAD<sup>1</sup>, MUMTAZ HUSSAIN<sup>1</sup>, MANSOOR HAMEED<sup>1</sup> AND RASHID AHMAD<sup>2</sup>

<sup>1</sup>Department of Botany, University of Agriculture, Faisalabad, Pakistan

<sup>2</sup>Department of Crop Physiology, University of Agriculture, Faisalabad, Pakistan

\*Corresponding author's email: [ijaz.hed@gmail.com](mailto:ijaz.hed@gmail.com)

### Abstract

This research was designed for the phyto-monitoring of  $Pb^{2+}$  pollution emitted from automobiles running along Motorway (M-2) and G.T. road and its effects on photosynthetic attributes and water relations of selected plant species growing along these roads. The data were collected from specified sites at different time intervals during all four seasons of the year. The results revealed significantly ( $p < 0.05$ ) higher  $Pb^{2+}$  content plant leaves growing in the vicinity of roadside (0 m distance) as compared to plant leaves collected from 50 m distance (Control) along both roads (M-2 and G.T. road). The leaves of *Nerium oleander* ( $2.45 \text{ mg kg}^{-1}$  dry wt.) collected from M-2 trapped the higher amount of  $Pb^{2+}$  ( $p < 0.001$ ) at Kalar Kahar in Summer and *Calotropis procera* ( $2.78 \text{ mg kg}^{-1}$  dry wt.) had the highest ( $p < 0.05$ )  $Pb^{2+}$  deposition at Bahyria Town during summer. Photosynthetic rate, transpiration rate and stomatal conductance decreased significantly ( $p < 0.01$ ) in plants along roadsides; whereas, inconsistent results in water use efficiency were perceived in plants at 0 m distance as compared to those collected from 50 m distance. These outcomes are important to identify the existence of roadside vehicular pollutants on plants and to its ecological hazards.

**Key words:** Vehicular,  $Pb^{2+}$  pollution, Seasonal variation, Gas exchange, Photosynthetic rate.

### Introduction

Environmental pollution has become a universal problem due to its adverse effects on biota. The automobile sector contributes especially in this aspect by releasing a number of metals into the environment (Warren & Birch, 1987). The metals commonly released by automobiles during different operations (wear and tear of tyres, brake pads, rusting of body parts, removal of paint, burning of fuels and lubricants, and breakage of batteries) include Cd, Cr, Pb, Zn, Fe and Cu (Akoto *et al.*, 2008, Moreki *et al.*, 2013; Aslam *et al.*, 2013). However, the intensity of metal pollution along any road depends upon the traffic density varying from time to time at different locations (Nawazish *et al.*, 2012), the quality of fuel used and the degree of wear and tear of tyres and other body parts.

High concentrations of metals released by automobile sector into the environment prove very toxic for the biota along roadsides (Sofilic *et al.*, 2013; Mafuyai *et al.*, 2015). Some metabolic processes and plant growth get severely affected in some plant species growing along roadside (Waseem *et al.*, 2014; Gall *et al.*, 2015; Aminiyani *et al.*, 2016) because metals contained in roadside dust can block stomatal gas exchange and reduce photosynthetic activity (Ghosh *et al.*, 2009). They also severely influence plant development and growth (Nawazish *et al.*, 2012; Dezhban *et al.*, 2015) due to cellular damages and metabolic disorders (Gomes-Junior, *et al.*, 2006; Dezhban *et al.*, 2015). Some other disorders caused by high metal contents in plants include change in catalytic function of the enzymes, inhibition of root and shoot development and damage to cellular membranes (Ghosh *et al.*, 2009).

Phyto-monitoring of metals has proved a useful technique for the determination of level of automobiles related pollution and for its proper management. A number of wild and cultivated plant species can be used for this

purpose. However, the selection of appropriate plant species as an ecological indicator looks essential to attain precise facts about the existence of metals in the atmospheres (Wolterbeek, 2002). Plant species capable of detecting automobile related metal pollution include *Sagittaria sagittifolia* (Arindam, 1999), *Nerium oleander* (Dongarrà *et al.*, 2003) and *Rosa rugosa* (Calzoni *et al.*, 2007).

Air pollution influences several physiological as well as biochemical processes in plants and thereby decreases their growth and productivity (Woo *et al.*, 2007). Metals released from the automobiles especially influence roadside plants. This aspect is being monitored extensively worldwide to determine the extent of pollution and chalk out suitable strategies for its control (Celik *et al.*, 2005). Some previous studies have indicated that metals released by automobiles pollutants reduced leaf area and altered surface architecture, photosynthetic pigments, cysteine and protein contents (Miteva & Merakchiyska, 2002). Therefore plants can prove good biomarkers for roadside pollution. The purpose of this study was to examine the extent of metals released by automobiles pollution and their adverse effects on some selected plant species growing along a sections of M-2 (Lillah to Taxila) and G.T. road (Kharian to Taxila).

### Material and Methods

**Study sites:** For this study, two roads viz., M-2 and G.T. road were selected. The Grand Trunk (G.T.) road extending from Peshawar to Kolkata was constructed in 16<sup>th</sup> century for military logistic purposes. It has high traffic density of heavily vehicles and many domestic settlements along both sides. As regards Motorway 2 (M-2) it was constructed under a Motorways Building Project initiated by National Highway Authority (NHA), Pakistan in 1997 for providing a well-organized, safe and widespread network of roads in Pakistan and to overcome heavy traffic load on G.T. It has comparatively lower

traffic density, as well as, domestic settlements on both sides as compared with those at G. T. road. The amount of metals released by automobiles was expected to differ greatly between both roads because of variation in their traffic density, road quality and the nature of vehicles running on both roads, which vary both spatially and temporally and might be posing different effects on the surrounding biota at different sites. Hence five sites on G.T. road i. e. Kharian, Deena, Gujjar Khan, Bahiriya Town (Rawalpindi) and Texila, were selected. Similarly five sites viz., Lillah, KalarKahar, Balkasar, Chakry and Texila were selected along M-2 as given in (Fig. 1.).

**Plants sample collection:** Three leaf samples of five wild plants namely *Calotropis procera*, *Datura mete* L., *Nerium oleander* L., *Ricinus communis* L. and *Cenchrus ciliaris* L. were collected from all sites along both selected roads (both sides) during all four seasons of 2014. Leaf samples were collected from selected sites at a distance of 0-5 m along both sides of the road while plant samples collected from a distance of 50 m acted as control. The collected leaf samples were brought to laboratory in polyethylene zipper locked bags for further analysis.

**Digestion procedure:** Leaf samples were digested with sulphuric acid digestion method. A fresh sample (0.1 g) was measured after digesting in 14 g of  $\text{LiSO}_4 \cdot 2\text{H}_2\text{O}$  and 0.42 g of selenium, mixing it in hydrogen peroxide slowly and slowly adding 420 mL sulphuric acid.

**Elemental determination:** The metallic (cadmium (Cd), nickel (Ni), lead (Pb) and zinc (Zn) contents in plants were measure by Atomic Absorption Spectrophotometer according to Anon., (1990).

**Gas exchange characteristics:** Portable Infrared Gas Analyzer (IRGA) (Analytical Development Company, Hoddesdon, England: Model CI-340) was used to measure the stomatal conductance ( $g_s$ ), net  $\text{CO}_2$  assimilation rate (A), transpiration rate (E) and sub-stomatal  $\text{CO}_2$  concentration ( $C_i$ ) of all previously mentioned plant species at all sites along both roads of G.T. road and M-2 during 10.00 a.m. -02.00 p.m. of fully expanded central leaf.

**Statistical analysis and Experimental design of data:** Data were statistically analyzed by COSTAT (Cohort Software, Berkeley, California USA). Two ways complete randomized block design was used for stratified data. Least significance difference test was used to compare mean values according to the method of Steel & Torrie (1980). Data were analyzed for canonical correspondence analysis (CCA) to study the association in different parameters using computer package CANOCO for window (v. 4.5).

## Results

The concentration of  $\text{Pb}^{2+}$  in different plant leaves varied significantly during different seasons and at different sites along M-2 and G.T. road (Fig. 2). The  $\text{Pb}^{2+}$  content differed significantly in plant leaves collected from road side along both roads (M-2 and G.T. road) as compared to control. Along M-2, the maximum  $\text{Pb}^{2+}$  content was accumulated by *Nerium oleander* during winter whereas, the minimum  $\text{Pb}^{2+}$  content was observed in *Ricinus communis*, however *Cenchrus ciliaris*, *Datura alba* and *Calotropis procera*

showed non-significant differences. The maximum  $\text{Pb}^{2+}$  concentration was accumulated by all plant species during summer along G.T. road whereas the minimum  $\text{Pb}^{2+}$  concentration was observed in *Ricinus communis* during winter and autumn. However, *Cenchrus ciliaris*, *Datura alba* and *Calotropis procera* differed non-significantly in  $\text{Pb}^{2+}$  content but sites differed significantly along G.T. road sites.

The *Calotropis procera* showed higher concentration of  $\text{Pb}^{2+}$  throughout the year as compared to control at both M-2 and G.T. road sites. Along M-2 the maximum  $\text{Pb}^{2+}$  concentration was observed in summer at Kalar kahar and Texila where as the minimum concentration was observed during winter and autumn at Lila, Balkasar and Chakry. The maximum  $\text{Pb}^{2+}$  was observed at Bahyria town and Texila (G) along G.T. road where as the minimum concentration was observed during winter and autumn at Kharian and Deena. At all sites  $\text{Pb}^{2+}$  concentration increased in *Cenchrus ciliaris* during all seasons of the year as compared to control at M-2 and G.T. road. The maximum  $\text{Pb}^{2+}$  concentration was observed in summer at Kalar kahar, Chakry and Texila at M-2 whereas the low concentration was observed during winter and autumn along roadside of M-2. Along G.T. road the  $\text{Pb}^{2+}$  concentration increased in *Cenchrus ciliaris* at roadside as compared to control in all seasons of year. The maximum was observed in spring and summer at all sites along G.T. road whereas the low concentration was observed during winter and autumn and it has insignificant difference in these two seasons at G.T. road.

Non-significant  $\text{Pb}^{2+}$  concentration was observed in *Ricinus communis* during winter and autumn at M-2. The higher concentration was observed in spring and summer at kalar kahar and Texila along M-2 sites. Along G.T. road the significant  $\text{Pb}^{2+}$  concentration increase was observed in *Ricinus communis* during spring and summer. The higher concentration was observed in spring and summer at Gujjar Khan, Bahyria town and Texila (G) whereas in spring and autumn it differed non-significantly along G.T. road.

The *Nerium oleander* showed higher concentration of  $\text{Pb}^{2+}$  throughout the year as compared to control at both M-2 and G.T. road sites. There is non-significant difference among the seasons at different sites along M-2. The maximum  $\text{Pb}^{2+}$  concentration was observed in summer at Kalar Kahar and Texila at M-2 whereas the minimum amount was observed during autumn. Along G.T. road the maximum  $\text{Pb}^{2+}$  concentration was observed in summer at Bahyria town where as the minimum concentration was observed during winter and autumn at Deena and Gujjar Khan Sites. The comparative study during four seasons showed the significant increase of  $\text{Pb}^{2+}$  in summer as compared to other seasons.

*Datura alba* collected from roadside had higher  $\text{Pb}^{2+}$  content as compared to that in control plants for both roads. There is non-significant  $\text{Pb}^{2+}$  concentration during autumn along M-2 whereas significantly increased during summer. Along M-2, the maximum  $\text{Pb}^{2+}$  concentration was observed in summer at Kalar Kahar and Texila and minimum concentration, observed during autumn at Chakry. In *Datura alba*, there was non-significant increase, observed during winter and autumn along G.T. road but a significant increase was observed during Spring and Summer both. The maximum  $\text{Pb}^{2+}$  concentration increase was observed in summer at Bahyria town and Texila (G) along G.T. road while a minimum concentration was observed during winter and autumn at different sites.

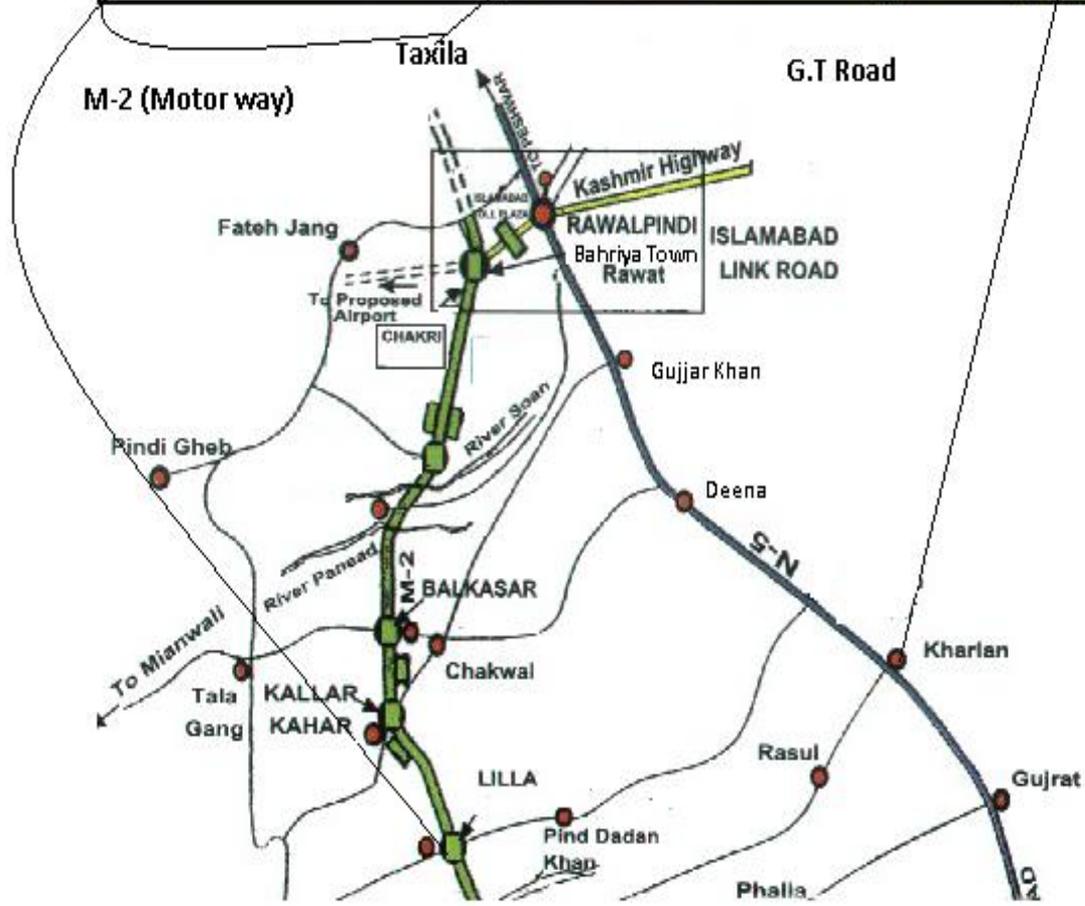
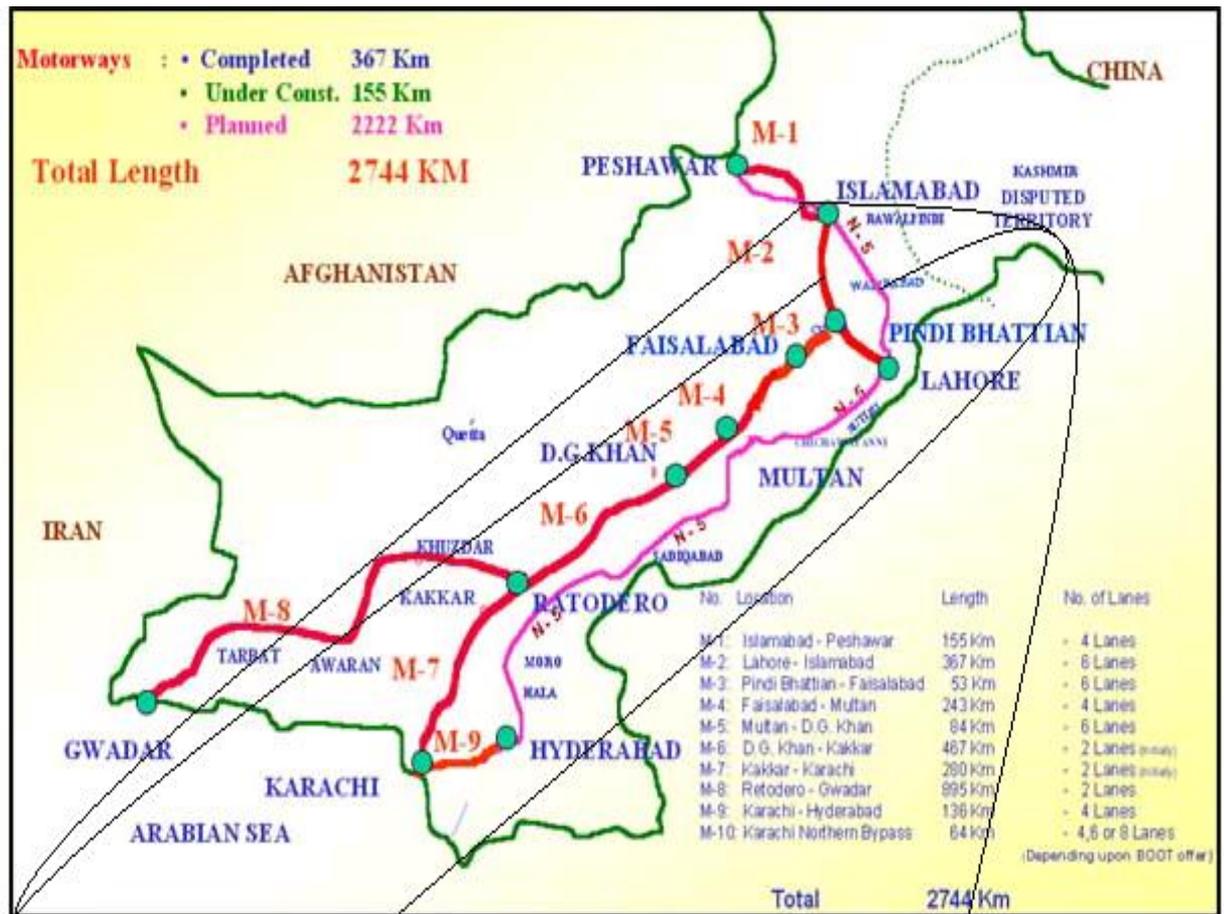


Fig. 1. Site Map motorway (M-2) and G.T. road (N-5).

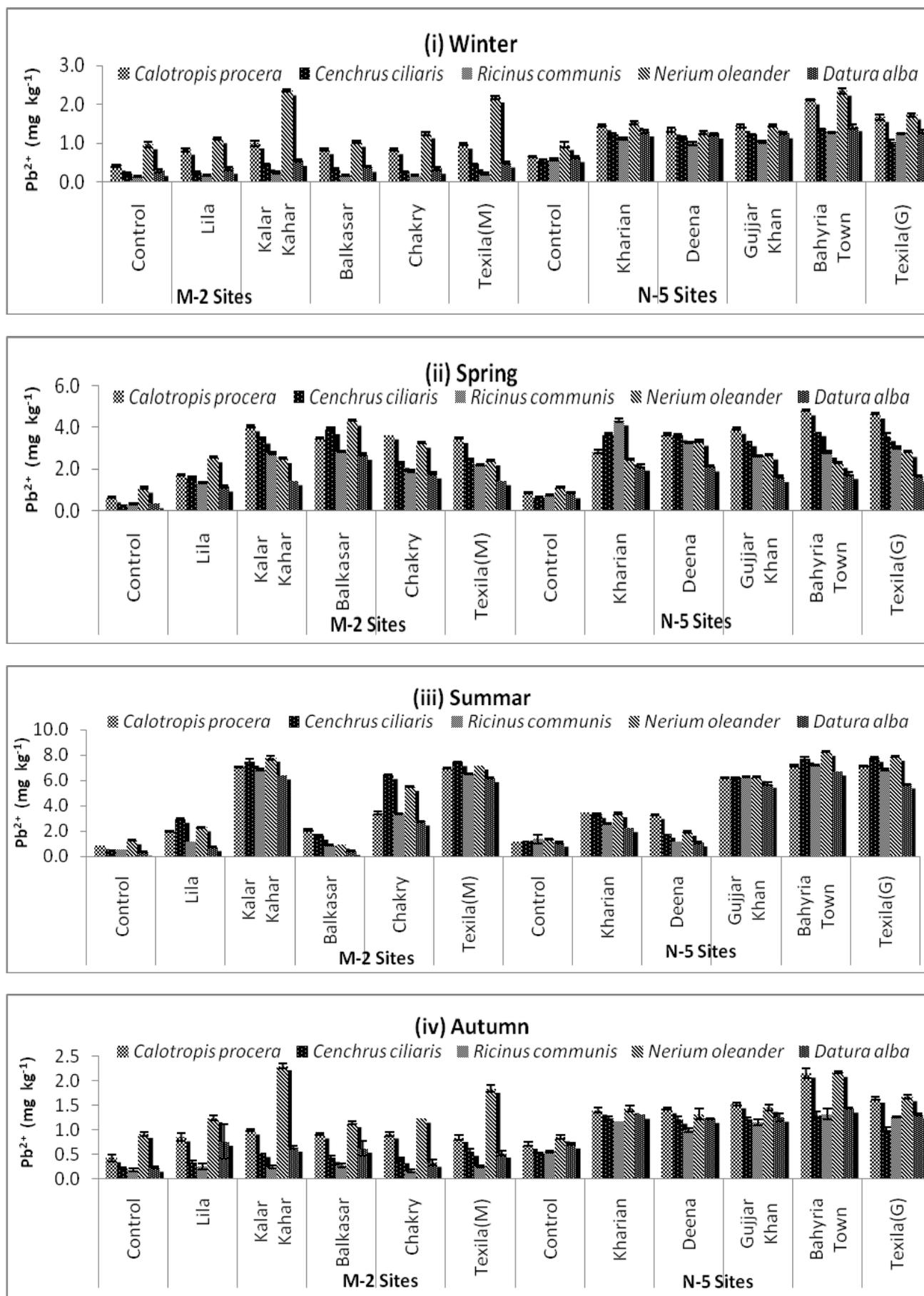


Fig. 2 Pb<sup>2+</sup> concentrations in the plant species during different seasons along Motorway (M-2) and G.T. road (N-5) in the Punjab, Pakistan during the year 2013-14.

Overall, the results of four seasons showed that the  $Pb^{2+}$  concentration varied significantly during all seasons, however, maximum values were observed during summer. It increased non-significantly during autumn. All plant species showed significant spatial variations regarding  $Pb^{2+}$  concentrations at different sites. All plant species have more  $Pb^{2+}$  concentration along G.T. road as compared to plant samples collected from M-2 sites.

#### Photosynthetic attributes and water relation parameters

**Photosynthetic rate (A):** Photosynthesis is regarded a vital physiological process for plants which largely gets affected by environmental changes. When photosynthesis becomes badly affected, the growth and development of plants decreased. The photosynthetic activity was significantly ( $p \geq 0.05$ ) higher in plants growing away from the roadside at M-2 and G.T. road. *Calotropis procera*, *Ricinus communis* and *Datura alba* maintained maximum rate of photosynthesis at M-2. The lowest photosynthetic rate was recorded in *Cenchrus ciliaris*, however the comparison for photosynthetic rate showed marked reduction in *Cenchrus ciliaris* and *Nerium oleander* (Fig. 4).

The rate of photosynthesis in plants growing along G.T. road was significantly ( $p \geq 0.05$ ) reduced as compared to control, however *Nerium oleander*, *Datura alba*, *Calotropis procera* retained maximum photosynthetic rate with roadside as well as, away from the roadside. The highest photosynthetic rate was observed in *Ricinus communis* followed by that recorded for *Cenchrus ciliaris* and *Nerium oleander*. It can be observed that this reduction in photosynthetic rate based on plant morphology and its response to the environment. The results clearly indicated that plants (*Calotropis procera* and *Nerium oleander*) found in varying environments had higher photosynthetic rate probably due to their adaptation to face adverse environment.

**Transpiration rate (E):** The transpiration rate was lower in all plants species along roadside of G.T. road and M-2 motorway as compared to control. In present study results showed that the plants along roadside were badly affected due to exposure of metals as compared to control plants (Fig.4). The significant decrease in transpiration rates was measured in *Nerium oleander* at M-2 and for *Cenchrus ciliaris* at G.T. road (Fig. 4).

**Stomatal conductance ( $g_s$ ):** Stomatal conductance was lowered at both roads as compared to control (Fig. 4). The *Cenchrus ciliaris* showed minor variation in stomatal conductance (1.31 and 0.91  $mmol\ m^{-2}s^{-1}$ ) at G.T. road (Fig. 4) while *Ricinus communis* and *Nerium oleander* had non-significant effect at M-2. *Ricinus communis* and *Datura alba* showed minimum value for stomatal conductance (0.91 & 1.25  $mmol\ m^{-2}s^{-1}$ , respectively) along G.T. road.

**Substomatal  $CO_2$  concentration ( $C_i$ ):** A significant effect of  $Pb^{2+}$  on internal  $CO_2$  concentration was observed in all plant species along M-2 and G.T. road but minimum value of  $C_i$  was observed in *Cenchrus ciliaris* (182.1  $mmol\ m^{-2}s^{-1}$ ). It was also observed that  $C_i$  value also

lowered in *Nerium oleander* as compared to control (247.69 and 227.29  $mmol\ m^{-2}s^{-1}$ ). *Nerium oleander* and *Cenchrus ciliaris* had similar  $C_i$  on control and roadside at G.T. road but prominent effect of  $Pb^{2+}$  was found in *Calotropis procera* (309.7  $mmol\ m^{-2}s^{-1}$ ) and least in *Cenchrus ciliaris* at G.T. road (Fig. 4).

**Water use efficiency (WUE):** Water use efficiency was clearly affected by alterations in environmental conditions at M-2 and G.T. road. The highest value of water use efficiency was observed in *Calotropis procera* and *Ricinus communis* (9.54 and 9.87) on roadside with respect to their controls (9.43 and 9.57) but lowest value of water use efficiency was observed in *Datura alba* along roadside (3.69) as compared to control (3.01) at M-2 (Fig. 4). Uneven water use efficiency was observed mostly in the plants growing around the G.T. road. The trend in water use efficiency for various plant species was in order of *Datura alba* followed by *Nerium oleander* (5.03, 3.83 and 3.18 respectively) while minimum WUE was observed in *Ricinus communis* (1.02) (Fig. 4).

#### Discussion

The  $Pb^{2+}$  concentration was significantly higher in roadside (M-2 and G.T. road) plant leaves than control plants. All plant species showed significant difference of  $Pb^{2+}$  at different sites in different seasons. Accumulation of metals in various plant species depends on their leaf anatomy and morphology.  $Pb^{2+}$  is a metal of highly environmental concern (Srivastava *et al.*, 2015). In this study,  $Pb^{2+}$  concentration in plants ranged from 2.045 to 1.323  $mg\ kg^{-1}$  dry wt. This  $Pb^{2+}$  concentration level was above the permissible limit devised by WHO is 0.12  $mg\ kg^{-1}$  (Alexander, 2015). Highest  $Pb^{2+}$  concentration was found in soil dust 0.37  $mg\ kg^{-1}$  around M-2 and G.T. road, respectively. The value of  $Pb^{2+}$  in soil dust was higher than permissible limit as observed by WHO (0.015  $mg\ kg^{-1}$ ).

Along M-2, the maximum value of  $Pb^{2+}$  was observed at Kalar Kahar and Texila due to more wear and tear and high fuel consumption in hilly area. Similarly, at Bahyria town and Texila, high  $Pb^{2+}$  concentration was observed due to high traffic density and use of heavy traffic. Begum *et al.* (2009) observed that concentration of  $Fe^{2+}$ ,  $Pb^{2+}$  and  $Ni^{2+}$  was much higher in the plants along the roadside than away from roadside. Sharma & Prasad, (2010) also found that  $Pb^{2+}$  and  $Cd^{2+}$  deposition varies plant to plant and the intensity and trapping of  $Pb^{2+}$  and  $Cd^{2+}$  deposition may be affected by the adaptability mechanism and fundamental pathways of plants under stress environment. The speed of automobiles remains very high at Kalar Kahar and Taxila along M-2 which strongly contribute metals contamination. The M-2 made from concrete which have higher  $Pb^{2+}$  contamination (Duong & Lee, 2011).

The highest  $Pb^{2+}$  contamination were observed at Bahyria Town and Taxila along G.T. road due to high traffic density. Amusan *et al.* (2003) reported that plants showed positive correlation with traffic density and also depicted that  $Pb^{2+}$  concentration at 1 m distance was 0.050  $mg\ g^{-1}$  and at 50 m distance it was 0.003  $mg\ g^{-1}$ .

A comparison between both roads revealed that  $Pb^{2+}$  concentrations was higher along G.T. road than control plants which might be due to greater traffic load. Salam *et al.* (2015) found that concentration of  $Pb^{2+}$  in the plants along roadside depended upon traffic density. This traffic density contributes to  $Pb^{2+}$  concentrations in plants (Van Der Gon & Appelman, 2009). Among plants, *Calotropis procera* and *Nerium oleander* accumulated more  $Pb^{2+}$  concentrations along both roads as observed by D'Souza *et al.* (2010). It was further proved by the results of Barthwal *et al.* (2008). Jaradat *et al.* (2004) that plant species growing along roadsides contained more concentration of toxic metals mostly  $Pb^{2+}$ . Same results were observed by Munendra *et al.* (2004).

Among seasons, higher concentration of  $Pb^{2+}$  value was observed during summer in plants along the both roads. The concentrations of  $Pb^{2+}$  observed in current study were lesser than those observed for various other sites of word (Silva *et al.*, 2016). Adedeji *et al.* (2013) found 26.7 mg  $kg^{-1}$  level of  $Pb^{2+}$  in the soil of Nigeria along roadside. High traffic density was main source of Pb in the plants. Bhowmik *et al.* (2015) reported that  $Pb^{2+}$  gasoline used in automobiles was main source of  $Pb^{2+}$  pollution. Some other studies also documented that higher contents of  $Pb^{2+}$  found in petrol and diesel. The existence of  $Pb^{2+}$  fuel was main reason for high concentration of Pb contamination in these observations along both roads (Teju *et al.*, 2012; Bhowmik *et al.*, 2015).

Photosynthetic attributes and water relation parameters have high importance among physiological parameters of plants (Ashraf, 2009). In present study, a decrease in transpiration rate, photosynthetic rate and stomatal conductance was noted but water use efficiency and internal  $CO_2$  concentration were maintained or increased to some extent under stress condition of metals and some other pollutants along both M-2 and N-5 roads. The net reduction in stomatal conductance and photosynthetic rate was also reported by Hassan & Basahi (2013) and Moradi & Ehsanzadeh (2015).

The photosynthetic attributes were decreased under roadside pollution. The high concentration of metals along both M-2 and N-5 might be involved in suppression of stomatal conductance which increase stomatal  $CO_2$  concentration and reduced net photosynthetic rate as reported by Bhardwaj *et al.* (2009) and Qadir *et al.* (2016). Nawazish *et al.* (2012) also reported reduction in photosynthetic activity under Cd and Pb deposition which might block stomatal aperture. Plants may adjust their internal mechanisms to tolerate high metal stress by increasing or decreasing their gas exchange characteristics. Toxic effects of metals did not reveal any variation in stomatal conductance and transpiration rate of rice (Llamas *et al.*, 2008). Kaznina *et al.* (2005) worked on effect of various levels of  $Pb^{2+}$  (0.02-0.008 mg  $kg^{-1}$ ) on gas exchange characteristics of plants such as barley and oat. Similarly Cr and Pb stress also reduced the efficiency of photosynthetic pigments in mash bean (Hussain *et al.*, 2006) whereas no variation was found in photosynthetic pigments of *Pisum sativum* with higher levels of  $Pb_2(NO_3)_2$  (Parys *et al.*, 1998). Similar results were found in *Paspalum distichum* under  $Zn^{2+}$ ,  $Pb^{2+}$  and Mn stress (Bhattacharya *et al.*, 2010).  $Pb^{2+}$  and Cd also decreased the photosynthetic rate and chlorophyll contents in two varieties of wheat (Oencil *et al.*, 2000). Plant species react differently under same environmental conditions due to changes in their

physiological, anatomical and genetic attributes. The deposition of smoke of motor vehicles and industries might have slowed down the photosynthetic rate and finally decreased the degree of growth in numerous species of plants (Gupta & Iqbal, 2005; Maruthi *et al.*, 2007).

The current study indicated that photosynthetic rate was decreased in plants growing along roadside as compared to control plants collected from 50 m away from road. Hence plant metabolic processes were severely affected by metal pollution released from vehicles. The current findings resembled with some previous studies, conducted by Awasthi & Das, (2005) who reported that photosynthetic rate and plant growth were due to *Chlorella vulgaris* (Awasthi & Das, 2005). Metal pollution also decreased the photosynthetic activity and plant pigments in mustard and wheat plants (Chauhan & Joshi, 2010). Due to chromium, cobalt and copper stress, leaves of *Brassica oleraceavar. botrytis* cv. Maghi showed low water potential and significant reduction in transpiration rate (Chatterjee & Chatterjee, 2000). Parys *et al.* (1998) reported that decrease in photosynthetic activity and rise of transpiration rate in *Pisum sativum* leaves under Pb stress. Cadmium stress also influenced gas exchange attributes (water relations and stomatal conductance (Hassan & Basahi, 2013).

The dust accumulated on the leaves blocks the stomatal opening and as a result photosynthetic activity (Taiz & Zeiger, 2010). Jing *et al.* (2005) also reported that photosynthetic rate significantly reduced but intracellular  $CO_2$  concentration was increased in tomato cultivars under  $Cd^{2+}$  stress.

Closing of stomata also stops uptake of  $CO_2$  and photosynthesis affects plant growth. Burzynski & Żurek, (2007) reported that  $Cr^{2+}$  and  $Cd^{2+}$  (50 and 20  $\mu M$  respectively) decreased the photosynthetic activity but increased the internal  $CO_2$  level under metal stress. A significant reduction was found in water use efficiency of barley and pea under  $Cd^{2+}$  stress (Januskaitiene, 2010). A contrasting finding indicated high water use efficiency of four *Datura* species under  $Zn^{2+}$  stress (Valliant *et al.*, 2005). These findings are related to present findings.

**Seasonal variation in  $Pb^{2+}$  concentration of plants along M-2 and G.T. road:** The canonical correspondence analysis (CCA) biplot (Fig. 3a & b) showed that different species had different  $Pb^{2+}$  values at different sites along M-2. *Nerium oleander* had more  $Pb^{2+}$  concentration at Texila and Kalar kahar sites in winter. It showed weak association at Lila in spring, at chakry in summer, and during autumn for Kalar Kahar and Texila. However, the concentration of  $Pb^{2+}$  in *Calotropis procera* was high at Texila in winter and Balkasar site in summer. It was weakly associated with Lila and Chakry in autumn. The concentration of  $Pb^{2+}$  in *Cenchrus ciliaris* was weakly associated with Balkasar site in winter, less  $Pb^{2+}$  concentration at Kalar Kahar and Balkasar in spring. It had high concentration of  $Pb^{2+}$  at Lila in summer and at Chakry in autumn. The concentration of  $Pb^{2+}$  in *Datura alba* was high at Balkasar site in winter, Lila in spring, Kalar Khar in summer, and weakly associated with Lila and Balkasar in autumn whereas the concentration of  $Pb^{2+}$  in *Ricinus communis* was strongly associated with Balkasar in winter, at Kalar Kahar in spring. It had high  $Pb^{2+}$  concentration at Kalar Kahar and Texila in summer and weakly associated with Lila and Balkasar in autumn.

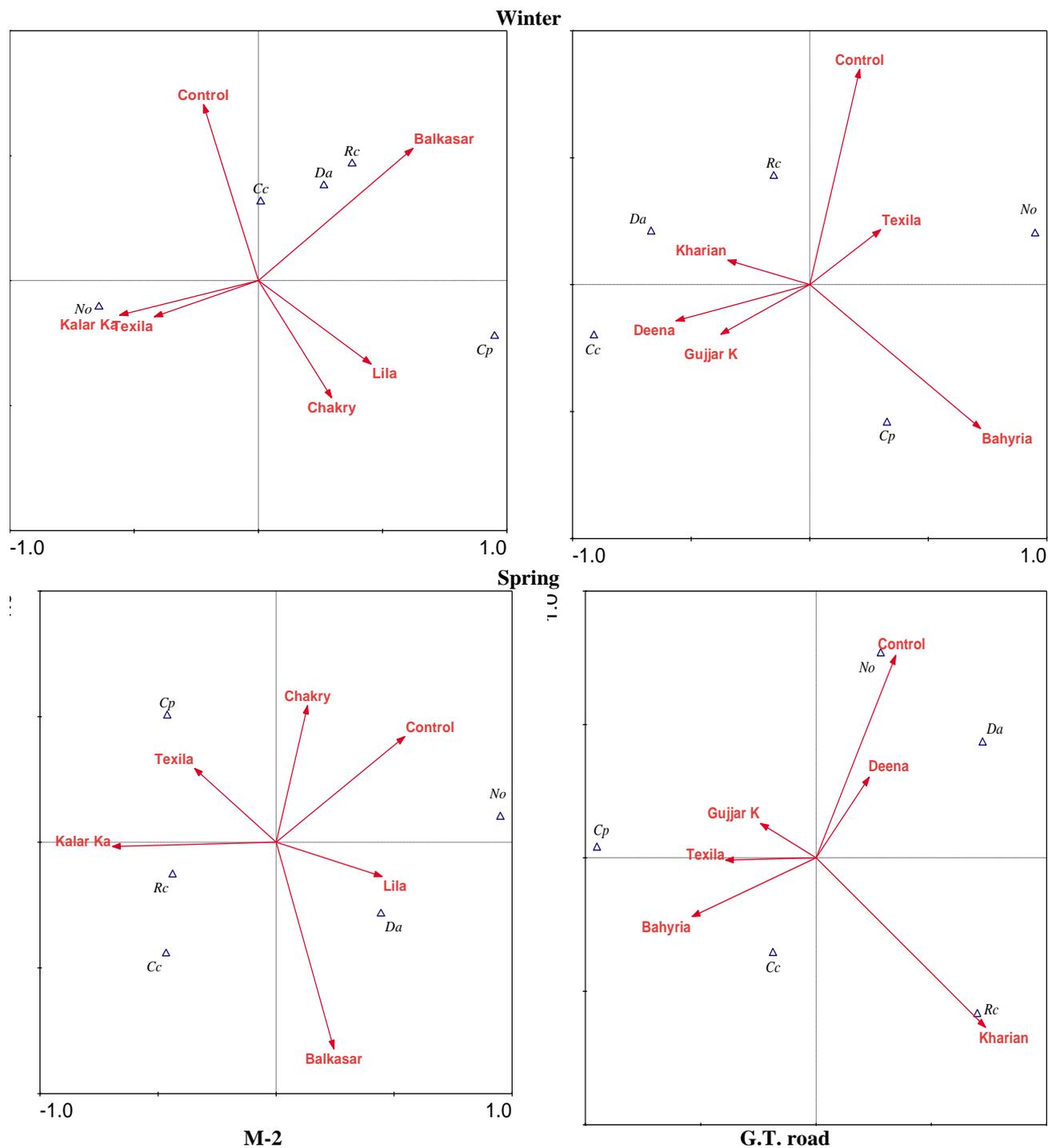


Fig. 3(a). CCA ordination biplot showing the effect of lead concentration in plant species along M-2 and G.T. road in winter and spring seasons. *Calotropis procera* (Cp), *Cenchrus ciliaris* (Cc), *Ricinus communis* (Rc), *Nerium oleander* L. (No), *Datura alba* Linn. (Da).

The canonical correspondence analysis (CCA) biplot (Fig. 3a & b) of plant species along G.T. road showed that *Nerium oleander* had high concentration of  $Pb^{2+}$  at Texila site in winter, at Deena in spring, at Texila in summer, at Bahyria town and Texila in autumn. However, the concentration of  $Pb^{2+}$  in *Calotropis procera* was closely associated with Bahyria town site in winter. It had high concentration of  $Pb^{2+}$  at Texila in spring, at Deena in summer, at Bahyria town in autumn. The concentration of  $Pb^{2+}$  in *Cenchrus ciliaris* was high at Gujjar khan and Deena sites in winter, weakly associated with Bahyria town in spring, showed strong association with Texila in summer and at Deena in autumn.

*Datura alba* had more  $Pb^{2+}$  concentrations at Kharian site in winter, Deena in spring, Bahyria town in summer, and Kharian in autumn whereas the concentration of  $Pb^{2+}$  in *Ricinus communis* was weakly associated with Kharian and Texila in winter, at Kharian in spring, Bahyria town in summer and Texila in autumn.

The canonical correspondence analysis (CCA) of  $Pb^{2+}$  concentration with respect to seasons as variables and sites as co-variables showed highly significant variations of different plant species along G.T. road and M-2. Most of the species had maximum  $Pb^{2+}$  concentration during summer. Some species had more  $Pb^{2+}$  concentration associated with spring.



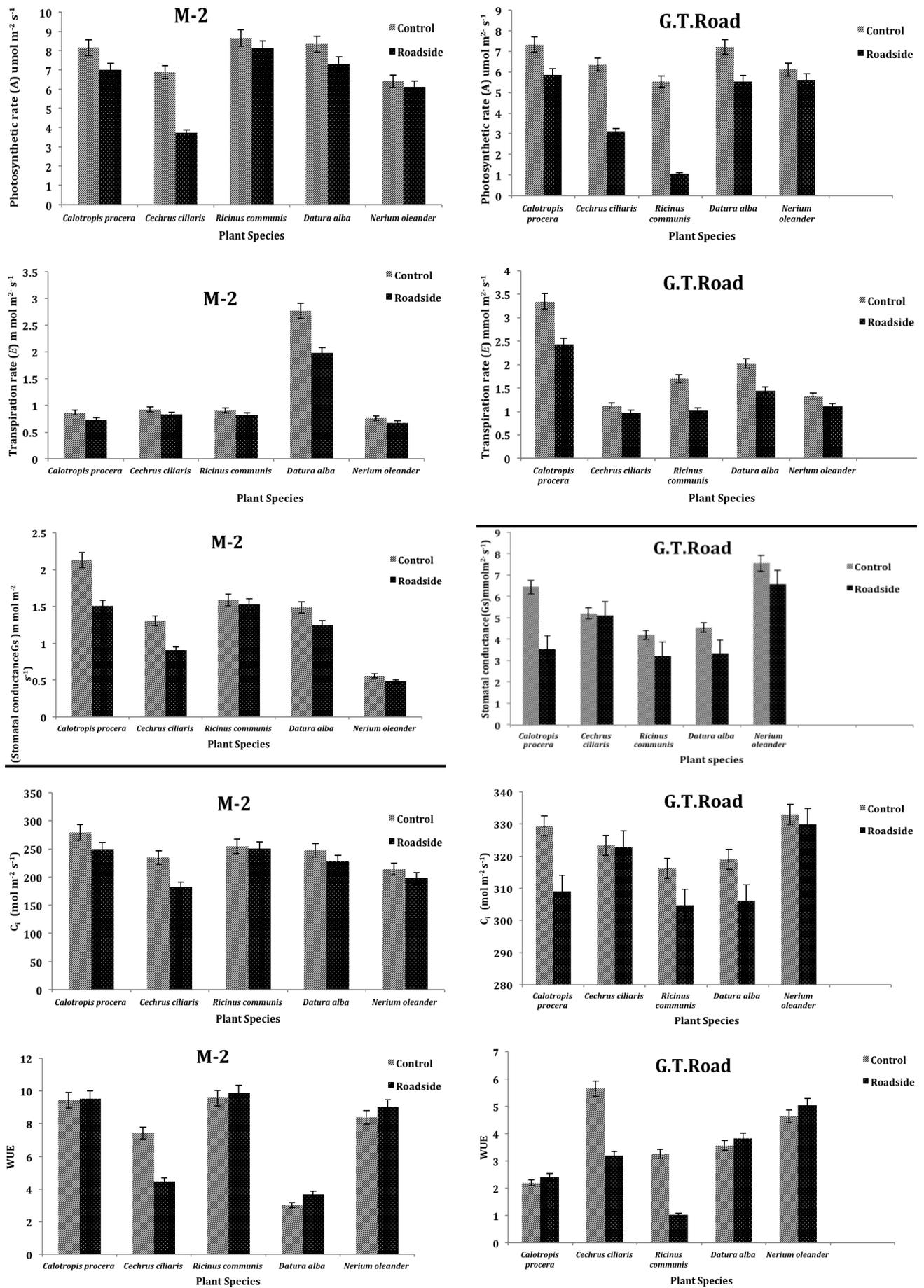


Fig. 4. Photosynthetic attributes along M-2 and G.T. road.

## Acknowledgment

The financial assistance of Higher Education Commission (HEC), Islamabad is highly acknowledged. The authors would like to present special thanks to the Incharge, Central Hi-Tech Laboratory, University of Agriculture, Faisalabad for the provision of analytical facilities.

## References

- Adedeji, O.H., O.O. Olayinka and F.F. Oyebanji. 2013. Assessment of traffic related heavy metals pollution of roadside soils in emerging urban centres in Ijebu-North Area of Ogun State, Nigeria. *J. Appl. Sci. Environ. Manag.*, 17: 509-514.
- Akoto, O., J.H. Ephraim and G. Darko. 2008. Heavy metal pollution in surface soils in the vicinity of abundant railway servicing workshop in Kumasi, Ghana. *Int. J. Environ. Res.*, 2: 359-364.
- Alexander, P. 2015. Assessment of heavy metals in roadside surface soil and vegetation along Mubi–Michika Major Road in Adamawa State, Nigeria. *Int. J. Appl. Sci. Biotechnol.*, 3: 545-551.
- Aminiyani, M.M., F.M. Aminiyani, R. Mousavi and A. Heydariyan. 2016. Heavy metal pollution affected by human activities and different land-use in urban topsoil: A case study in Rafsanjan city, Kerman province, Iran. *Eurasian J. Soil Sci.*, 5: 97-104.
- Amusan, A.A., S.B. Bada and A.T. Salami. 2003. Effect of traffic density on heavy metal contents of soil and vegetation along roadside of Osun State, Nigeria. *West Afr. J. Appl. Ecol.*, 4: 107-112.
- Arindam, K. 1999. *Sagittaria sagittifolia*: Bioaccumulator of industrial pollutants. *Adv. Plant Sci.*, 12: 267-270.
- Ashraf, M. 2009. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biot. Adv.*, 27: 84-93.
- Aslam, J., S.A. Khan and S.H. Khan. 2013. Heavy metals contamination in roadside soil near different traffic signals in Dubai, United Arab Emirates. *J. Saudi Chem. Soc.*, 17: 315-319.
- Awasthi, M. and D.N. Das. 2005. Heavy metal stress on growth, photosynthesis and enzyme activities of free and immobilized *Chlorella vulgaris*. *Ann. Micro Biol.*, 55: 1-7.
- Barthwal, J., N.A. Smitha and P. Kakkar. 2008. Heavy metal accumulation in medicinal plants collected from environmentally different sites. *Biomed. Environ. Sci.*, 21: 319-324.
- Begum, A., M. Ramaiah, Harikrishna, I. Khan and K.Veena. 2009. Analysis of heavy metals concentration in soil and lichens from various localities of Hosur road, Bangalore, India. *E- J. Chem.*, 6: 13-22.
- Bhardwaj, P., K.A. Chaturvedi and P. Prasad. 2009. Effect of enhanced Pb<sup>2+</sup> and cadmium in soil on physiological and biochemical attributes of *Phaseolus vulgaris* L. *Nature Sci.*, 7: 1-13.
- Bhattacharya, T., S. Chakraborty and D.K. Banerjee. 2010. Heavy metal uptake and its effect on macronutrients, chlorophyll, protein, and peroxidase activity of *Paspalum distichum* grown on sludge-dosed soils. *Environ. Monit. Assess.*, 169: 15-26.
- Bhowmik, A.V., R. Schafer, A. Alamdar, I. Katsoyiannis, M. Ali, N. Ali, H. Bokhari and S.A.M.A.S. Eqani. 2015. Predictive risk mapping of drinking water resources in the Indus delta floodplains: Exposure estimation of arsenic and other trace metals pose serious health risks to population. *Sci. Total Environ.*, 538: 306e316.
- Burzynski, M. and A. Zurek. 2007. Effects of copper and cadmium on photosynthesis in cucumber cotyledons. *Photosynthetica*, 45: 239-244.
- Calzoni, G.L., F. Antognoni, E. Pari, P. Fonti, A. Gnes and A. Speranza. 2007. Active biomonitoring of heavy metal pollution using *Rosa rugosa* plants. *Environ. Pollut.*, 149: 239-245.
- Celik, A., A. Kartal, A. Akdogan and Y. Kaska. 2005. Determination of heavy metal pollution in Denizli (Turkey) by using *Robinia pseudo-Achellia* L. *Environ. Int.*, 31: 105-112.
- Chatterjee, J. and C. Chatterjee. 2000. Phytotoxicity of cobalt, chromium and copper in cauliflower. *Environ. Pollut.*, 109: 69-74.
- Chauhan, A. and P.C. Joshi. 2010. Effect of ambient air pollution on wheat and mustard crops growing in the vicinity of urban and industrial areas. *New York Sci. J.*, 3: 1-9.
- D'Souza, R.J., M. Varun, J. Masih and M.S. Paul. 2010. Identification of *Calotropis procera* L. as a potential phytoaccumulator of heavy metals from contaminated soils in Urban North Central India. *J. Hazard. Mater.*, 184: 1-8.
- Dezhban, A., A. Shirvany, P. Attarod, M. Delshad, M. Matinizadaeh and M. Khoshnevis. 2015. Cadmium and Pb<sup>2+</sup> effects on chlorophyll fluorescence, chlorophyll pigments and proline of *Robinia pseudoacacia*. *J. For. Res.*, 26: 323-329.
- Dongarrà, G., G. Sabatino, M. Triscari and D. Varrica. 2003. The effects of anthropogenic particulate emissions on roadway dust and *Nerium oleander* leaves in Messina (Sicily, Italy). *J. Environ. Monit.*, 5: 766-773.
- Duong, T.T. and B.K. Lee. 2011. Determining contamination level of heavy metals in road dust from busy traffic areas with different characteristics. *J. Environ. Manag.*, 92: 554-562.
- Gall, J.E., R.S. Boyd and N. Rajakaruna. 2015. Transfer of heavy metals through terrestrial food webs: A review. *Environ. Monit. Assess.*, 187: 201-216.
- Ghosh, S.P., S.K. Maiti and G. Singh. 2009. Heavy metal contamination in roadside soil and vegetation: A review. *Indian J. Environ. Protec.*, 29: 334-340.
- Gomes-Junior, R.A., C.A. Moldes, F.S. Delite, G.B. Pompeu, P.L. Gratao, P. Mazzafera, P.J. Lea and R.A. Azevedo. 2006. Antioxidant metabolism of coffee cell suspension cultures in response to cadmium. *Chemosphere*, 65: 1330-1337.
- Gupta, M.C. and M. Iqbal. 2005. Ontogenetic histological changes in the wood of mango (*Mangifera indica* L. cv. Deshi) exposed to coal-smoke pollution. *Environ. Exp. Bot.*, 54: 248-255.
- Hassan, I.A. and J.M. Basahi. 2013. Assessing roadside conditions and vehicular emissions using roadside lettuce plants. *Polish J. Environ. Stud.*, 22: 387-393.
- Hussain, A., G. Murtaza, A. Ghafoor, S. M. Basra, M. Qadir and M. Sabir. 2006. Cadmium contamination of soils and crops by long term use of raw effluent, ground and canal waters in agricultural lands. *Int. J. Agric. Biol.*, 12: 851-856.
- Januskaitiene, I. 2010. Impact of low concentration of cadmium on photosynthesis and growth of pea and barley. *Environ. Res. Eng. Manag.*, 3: 24-29.
- Jaradat, Q.M., K.A. Momani, A.Q. Jbarah and A. Massade. 2004. Inorganic analysis of dust fall and office dust in an industrial area of Jordan. *Environ. Res.*, 96: 139-144.
- Jing, D., W. Fei-bo and Z. Guoping. 2005. Effect of cadmium on growth and photosynthesis of tomato seedlings. *J. Zhejiang Univ. Sci.*, 6: 974-980.
- Kaznina, N.M., G.F. Laidinen, A.F. Titov and A.V. Talanov. 2005. Effect of Pb<sup>2+</sup> on the photosynthetic apparatus of annual grasses. *Biol. Bull.*, 32: 147-150.
- Llamas, A., U. Cl and A. Sanz. 2008. Ni<sup>2+</sup> toxicity in rice: effect on membrane functionality and plant water content. *Plant Physiol. Biochem.*, 46: 905-910.

- Mafuyai, G.M., N.M. Kamoh, N.S. Kangpe, S.M. Ayuba and I.S. Eneji. 2015. Heavy metals contamination in roadside dust along major traffic roads in Jos Metropolitan area, Nigeria. *Eur. J. Earth Environ.*, 2: 1-14.
- Maruthi S., B.B., F.X. Han, S.V. Diehl, D.L. Monts and Y. Su. 2007. Effects of Zn and Cd accumulation on structural and physiological characteristics of barley plants. *Braz. J. Plant Physiol.*, 19: 15-22.
- Miteva, E. and M. Merakchiyska. 2002. Response of chloroplasts and photosynthetic mechanism of bean plants to excess arsenic in soil. *Bulg. J. Agric. Sci.*, 8: 151-156.
- Moradi, L. And P. Ehsanzadeh. 2015. Effects of Cd on photosynthesis and growth of safflower (*Carthamus tinctorius* L.) genotypes. *Photosynthetica*, 53: 506-518.
- Moreki, J.C., T.O. Woods and P.G. Nthoiwa. 2013. Estimation of concentration of heavy metals in forages harvested around dibete area, Botswana. *Int. J. Innov. Res. Sci. Eng. Technol.*, 2: 4060-4071.
- Munendra, S., P. Goel and A.K. Singh. 2004. Biomonitoring of Pb<sup>2+</sup> in atmospheric environment of urban center of the Ganga plain, India. *Environ. Monit. Assess.*, 107: 101-114.
- Nawazish, S., M. Hussain, M. Ashraf, M.Y. Ashraf and A. Jamil. 2012. Effect of automobile related metal pollution (Pb<sup>2+</sup> & Cd<sup>2+</sup>) on some physiological attributes of wild plants. *Int. J. Agric. Biol.*, 14: 953-958.
- Oencil, I., Y. Kelese and A.S. Uestuen. 2000. Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings. *Environ. Pollut.*, 107: 315-320.
- Parys, E., E. Romanowska, M. Siedlecka and J. W. Poskuta. 1998. The effect of Pb<sup>2+</sup> on photosynthesis and respiration in detached leaves and in mesophyll protoplasts of *Pisum sativum*. *Acta Physiol. Plant.*, 20: 313-322.
- Qadir, S.U., V. Raja and W.A. Saddique. 2016. Morphological and biochemical changes in *Azadirachta indica* from coal combustion fly ash dumpsite from a thermal power plant in Dehli, India. *Ecotoxicol. Environ. Saf.*, 129: 320-328.
- Salam, M., F. Mohsin, F. Mahmood, I.U. Rahman, A. Afzal and Z. Iqbal. 2015. Pb<sup>2+</sup> and manganese accumulation on leaves of road side plants from mauripur to hawks bay road, Karachi, Pakistan. *Bangladesh J. Bot.*, 44: 665-668.
- Sharma, S., and F.M. Prasad. 2010. Accumulation of Lead and Cadmium in soil and vegetable crops along major highways in Agra (India). *Electronic. J. Chem.*, 7: 1174-1183.
- Silva, S., A.S. Ball, T. Huynh and S.M. Reichman. 2016. Metal accumulation in roadside soil in Melbourne, Australia: Effect of road age, traffic density and vehicular speed. *Environ. Pollut.*, 208: 102-109.
- Sofilic, T., B. Bertic, V. Simunic-Menaric and I. Brnardic. 2013. Soil pollution as a result of temporary steel scrap storage at the melt shop. *Ecol. Balkanica*, 5: 21-30.
- Srivastava, D., A. Singh and M. Baunthiyal. 2015. Lead toxicity and tolerance in plants. *J. Plant Sci. Res.*, 2: 1-5.
- Steel, R.G.D. and J.H. Torrie. 1980. Principles and procedure of Statistics (1st Ed.). McGraw Hill Book Co. Inc., New York. pp. 336-354.
- Taiz, L. and E. Zeiger. 2010. Plant Physiology (5<sup>th</sup> Edn.). Sinauer Associates Inc., Sunderland.
- Teju, E., N. Megersa, B.S. Chandravanshi and F. Zewge. 2012. Determination of the levels of lead in the roadside soils of Addis Ababa, Ethiopia. *SINET: Ethiopian J. Sci.*, 35: 81-94.
- Valliant, N., F. Monnet, A. Hitmi, H. Sallaman and A. Coudret. 2005. Comparative study of response in four datura species to zinc stress. *Chemosphere*, 59: 1005-1013.
- van derGon, H.D. and W. Appelman. 2009. Lead emissions from road transport in Europe: A revision of current estimates using various estimation methodologies. *Sci. Total Environ.*, 407: 5367-5372.
- Warren R.S. and P. Birch. 1987. Heavy metal levels in atmospheric particulates, roadside dust and soil along a major urban highway. *Sci. Total Environ.*, 59: 233-256.
- Waseem, A., J. Arshad, F. Iqbal, A. Sajjad, Z. Mehmood and G. Murtaza. 2014. Pollution status of Pakistan: a retrospective review on heavy metal contamination of water, soil, and vegetables. *BioMed Res. Int.*, 2014: 21-29.
- Wolterbeek, B. 2002. Biomonitoring of trace element air pollution: principles, possibilities and perspectives. *Environ. Pollut.*, 120: 11-21.
- Woo, S.Y., D.K. Lee and Y.K. Lee. 2007. Net photosynthetic rate, ascorbate peroxidase and glutathione reductase activities of *Erythrina orientalis* in polluted and non-polluted areas. *Photosynthetica*, 45: 293-295.

(Received for publication 14 April 2017)