

## IMPACT OF AUTOMOBILES AND INDUSTRIAL METAL POLLUTION ON PHOTOSYNTHETIC ATTRIBUTES OF SELECTED WILD AND CROP PLANT SPECIES

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### Abstract

Metal pollution is one of the major environmental hazards contaminating water, soil and air globally. Major sources of metal pollution include industrial emissions, wastes and effluents, automobiles running on roads, application of fertilizers and pesticides, crop harvesting and storage processes etc. A study was conducted to explore comparative responses of eight plants species (four crops and four wild plants) to metal pollution caused by industries and road vehicular traffic. Crop plants species selected for the study were *Zea mays* L., *Sorghum bicolor* L., *Oryza sativa* L. and *Brassica oleracea* var. *botrytis*. While the wild plants species were *Parthenium hysterophorus* L., *Calotropis procera* (Aiton) W.T Aiton, *Achyranthes aspera* L. and *Ricinus communis* L. The area under study was Gujranwala division comprised of six districts namely: Gujranwala, Sialkot, Narowal, Gujrat, Mandi Bahaudin and Hafizabad. Metal pollution load index (PLI) was found in an order of Gujranwala>Gujrat>Sialkot>Hafizabad>MBD>Narowal. Gas exchange attributes of all plants under study were affected by heavy metal pollution with maximum reduction in most polluted district i.e., Gujranwala. Photosynthetic rate (A) and Transpiration (E) were reduced by 69.18% and 53.90%, respectively in *A. aspera* (maximum reduction among all wild and crop plants) at Gujranwala under vehicular metal pollution as compared to control. Water use efficiency and stomatal conductance were reduced maximum i.e. 37.20% in *R. communis* and 58.14% in *Z. mays*, respectively at Gujrat under vehicular metal pollution compared to control. Inter cellular CO<sub>2</sub> concentration (Ci) was decreased in all plants except in *B. oleracea* with maximum value 167.5 μmol/molar Sialkot under roadside metal pollution. The most significant being were *B. oleracea* among crop plants and *C. procera* among wild plants by showing least reduction in photosynthetic attributes.

**Key words:** Heavy metals; Photosynthetic efficiency; Crop plants.

### Introduction

Pollution is one of the key global concerns as the environment continues to be degraded by human activity. Influx of pollutants to natural environment is being considered a big threat to survival of life on earth planet. Humans, animals and plants all are facing adverse effects of environmental pollution. Unplanned urbanization and industrialization are major sources of environmental pollution. Metal pollution is one of the major environmental hazards (Hu *et al.*, 2019). A number of factors are contributing heavy metals to water, soil and air. They include industrial emissions, wastes and effluents, automobiles running on roads, application of fertilizers and pesticides, crop harvesting and storage processes etc. (Lombi *et al.*, 2002, Yang *et al.*, 2002, Bernard, 2008, Alengebawy *et al.*, 2021). Most of the heavy metals are toxic, having no biological function. Due to their toxicity, heavy metals are responsible for persistent environment degradation (Khan *et al.*, 2011, Jessica *et al.*, 2020). With the development of industrialization and urbanization, the abundance of heavy metals in the environment has increased enormously during the past decades, which raised significant concerns throughout the world (Suman *et al.*, 2018; Ashraf *et al.*, 2019). Heavy metals are non-degradable by any biological or physical process and are persistent in the soil for a long period, which poses a long-term threat for the environment (Suman *et al.*, 2018). Environmental pollution is an important problem causing substantial damage to the natural ecosystem (Erdős *et al.*, 2022; Khan *et al.*, 2021). Heavy metals pollution adversely affects flora and fauna (Blaylock, 2020; Kominko *et al.*, 2022). It obstructs enzymes' activity by binding with a sulfhydryl group, oxidative stress cause injuries to cellular structures and affects photosynthesis, transpiration and growth rate (Jadia & Fulekar, 2009; Thakur *et al.*, 2022).

Industrial effluents poisoned by heavy metals pose a serious threat to consumers' i.e. both plants and animals (Khan *et al.*, 2011, Jessica *et al.*, 2020). Vehicular traffic releases a wide range of hazardous pollutants, including heavy metals and a correlation exists between traffic density and heavy metals concentration in roadside soil (Mahbub *et al.*, 2011; Gunawardena *et al.*, 2012). Pb, Cd, Cr, Fe, Zn, and Cu are the metals commonly released by automobiles while running on the road. Wear and tear of tyres, brake pads, rusting of body parts, burning of fuels (petrol and diesel) and lubricants, and breakage of batteries are various automobile operations releasing metals (Ghrefat & Yusuf, 2006; Akoto *et al.*, 2008; Nawazish *et al.*, 2012).

The most widespread effect of toxic heavy metals in plants is their attack on the photosynthetic machinery. This property is common to all heavy metals and isn't specific to a particular metal, which make measuring the photosynthetic activities a good screening method for detecting heavy metal stress (Appenroth, 2010). Some heavy metals such as Cu, Zn, Co and Fe are essential in trace amounts for various metabolic activities in plants. However, plant metabolism is adversely affected by excess of any kind of metal (Hall, 2002). In plants, heavy metals employ their toxic actions mostly by damaging chloroplasts and disturbing photosynthesis. Photosynthesis inhibition is the result of intervention of metals with photosynthetic enzymes and chloroplast membranes (Aggarwal *et al.*, 2012). In higher plants, photosynthesis is secondarily reduced by heavy metals accumulation in leaves which affects the functioning of the stomata and hence upsets photosynthesis and transpiration rates overall. Photosynthetic pigment reduction by heavy metals affects photosynthesis indirectly, hence the use of non-destructive methods and the measurement allows photosynthetic pigments to be frequently used to govern stress for monitoring purposes (Aggarwal *et al.*, 2012).

Photosynthetic machinery is directly affected by environmental stresses, mainly by disrupting all major components of photosynthesis including carbon reduction cycle and the stomatal control of the CO<sub>2</sub> supply, it also increases accumulation of carbohydrates, disturbance of water balance and peroxidative destruction of lipids (Allen & Ort, 2001). Metal pollution reduces chlorophyll content and the enzymatic activity involved in CO<sub>2</sub> fixation leading to decrease in photosynthetic rate (Greger *et al.*, 1991). Metal stress induces disturbance in the uptake and distribution of mineral nutrients in plants that also affect the photosynthesis (Gussarson *et al.*, 1996).

Gujranwala Division (area under study) is one of the thickest populated regions of the Punjab, Pakistan. Gujranwala division is consists of leading industrial cities of the country. Likewise, all cities are connected with each other with good road infrastructure varying in traffic density and vehicular classes. Consequently, the intensity of metal pollution caused by the industrial and transport sectors varies largely from city to city. The toxic effects of said metal pollution on the surrounding vegetation are yet to be investigated. Therefore, this study was conducted with objectives to find out photosynthetic attributes of wild

and crop plants under industrial and automobile metal pollution in four districts of Gujranwala division.

### Material and Methods

The area selected for this study was Gujranwala division consisting of six districts namely Gujranwala, Gujrat, Sialkot, Hafizabad, Mandi Bahaudin (MBD) and Narowal. Gujranwala division includes leading industrial cities of Pakistan. Diverse kinds of industries including steel, textile, food, marble and ceramics, sanitary wares and sanitary fittings, plastic furniture, electrical home appliances, metal and melamine utensils, cutlery and kitchen wares, furniture, pottery, sports, leather, surgical and rice processing industries are present here. Roads selected for sampling were busiest and vary in traffic volumes and vehicular categories. Traffic volume wise ranking of selected roads was Gujranwala>Gujrat>Sialkot>Hafizabad>MBD>Narowal.

**Sampling locations:** On both industrial and roadside locations following sites were selected.

Sampling locations			
Sr. No.	District	Industrial Site	Roadside
1.	Gujranwala	Industrial Estate # 2	Gujranwala-Lahore Road
2.	Gujrat	Gujrat Industrial Estate	Gujrat Bypass Road
3.	Sialkot	Allama Iqbal Industrial Estate	Sialkot-Gujranwala Road
4.	Hafizabad	Hafizabad Industrial Estate	Hafizabad-Gujranwala Road
5.	MBD	MBD Industrial Estate	MBD-Gujrat Road
6.	Narowal	Narowal Industrial Estate	Narowal-Lahore Road

Vehicular traffic density			
Sr. No.	District	Roadside Location	Number of vehicles per day
1.	Gujranwala	Gujranwala-Lahore Road	1,25,000
2.	Gujrat	Gujrat Bypass Road	92,000
3.	Sialkot	Sialkot-Gujranwala Road	56,000
4.	Hafizabad	Hafizabad-Gujranwala Road	34,000
5.	MBD	MBD-Gujrat Road	29,000
6.	Narowal	Narowal-Lahore Road	24,000

Soil and leaves samples were collected from the above selected sites. Samples collected from roadside locations within 10 to 15 meters area from the carriageway whereas industrial sites samples were collected from the fields alongside the industrial estates, irrigated through drains carrying industrial wastewater. Control samples were collected 1 kilometer away from road and industrial sites. Vehicular traffic density was noted for each road.

**Soil heavy metal pollution assessment:** The Pollution Load Index (PLI) evaluates the degree to which the metals associated with the soil might impact the vegetation on that particular soil. PLI was measured for all the locations under study (Table 1).

### Calculation of pollution load index (PLI)

**Contamination factor (CF):** It is used to illustrate the contamination of a given metal and assess the soil contamination (Hakanson 1980; Liu *et al.*, 2005).

$$CF = C_s / C_b$$

where C<sub>s</sub> is the concentration of metal in the soil study samples and C<sub>b</sub> is baseline concentration as in index of geoaccumulation. Hakanson (1980) classified the concentration factor as the following: CF<1 low; 1<CF<3 moderate; 3<CF<6 considerable, and CF>6 as high contamination.

**Pollution load index (PLI):** The pollution load index (PLI) proposed by Tomlinson *et al.*, (1980), a simple and proportional means for assessing the level of heavy metal pollution. Thus, it is a distinctive index in the comparison of pollution rank in different localities (Abou El-Anwar *et al.*, 2018; Mekky *et al.*, 2019):

$$PLI = \sqrt[n]{(CF_1 \times CF_2 \times CF_3 \times \dots \times CF_n)}$$

where n is the number of metals and CF is the contamination factors. PLI > 1 indicates pollution exists; PLI<1 indicates no metal pollution (Chakravarty & Patgiri, 2009); and PLI = 1 indicates heavy metal loads close to the baseline level (Cabrera *et al.*, 1999).

**Table 1. Heavy metals contamination factor (CF) and Pollution load index (PLI) of soils under study.**

District	Location	Contamination factor (CF)						PLI
		Cd	Pb	Cu	Cr	Zn	Ni	
Gujranwala	Control	0.110	0.4	0.621	0.286	0.322	1.194	0.329
	Roadside	0.464	1.62	2.47	0.616	2.903	3.888	21.557
	Industrial site	1.636	2.143	3.453	26.404	2.096	19.44	684.748
Gujrat	Control	0.0819	0.48	0.646	0.309	0.370	1.058	0.332
	Roadside	0.363	1.216	1.846	0.476	2.258	3.277	10.165
	Industrial site	1	1.007	1.303	21.785	1.322	9.306	112.516
Sialkot	Control	0.1	0.52	0.576	0.310	0.467	1.376	0.463
	Roadside	0.318	1.14	1.597	0.452	1.951	3.111	7.562
	Industrial Site	1.181	1.415	2.119	23.928	1.629	11.98	243.984
Hafizabad	Control	0.1	0.44	0.647	0.238	0.306	1.692	0.355
	Roadside	0.382	1.289	1.955	0.490	2.129	3.167	10.700
	Industrial Site	0.454	0.618	0.962	2.5	0.968	4.770	10.591
MBD	Control	0.082	0.4	0.598	0.238	0.354	1.753	0.323
	Roadside	0.318	1.118	1.578	0.387	1.806	2.111	5.458
	Industrial Site	0.364	0.586	0.911	2.762	0.790	4.259	8.063
Narowal	Control	0.091	0.48	0.691	0.214	0.419	1.580	0.392
	Roadside	0.273	0.84	1.259	0.331	1.322	1.888	2.930
	Industrial Site	0.273	0.56	0.884	2.262	0.725	3.309	5.138

The baseline (BL) measurement represents the natural concentration of an element in soil with no human influence (Ramos-Miras *et al.*, 2011).

**Plants under study:** The following 8 plant species (4 crop plants and 4 wild plants) were selected for this study.

Crop plants	Wild plants
<i>Zea mays</i> L.	<i>Parthenium hysterophorus</i> L.
<i>Sorghum bicolor</i> L.	<i>Calotropis procera</i> (Aiton)
<i>Oryza sativa</i> L.	W.T Aiton
<i>Brassica oleracea</i> var. <i>botrytis</i>	<i>Achyranthes aspera</i> L.
	<i>Ricinus communis</i> L.

**Measurement of gas exchange parameters:** Infra-red Gas Analyser (IRGA) (Analytical Production Firm, Hoddeson, England. Model (C1-340) was used to measure the gas exchange parameters including stomatal conductance, sub stomatal carbon dioxide concentration, transpiration, photosynthetic rates and water use efficiency. As explained by Khalid *et al.*, (2017), readings were noted between 11:00am and 02:00pm. The following adjustments of IRGA were made: leaf surface area (11.35 cm<sup>2</sup>), leaf chamber temperature (Tch) which may varied from 31-36°C, ambient CO<sub>2</sub> concentration (349.12 µmolmol<sup>-1</sup>), ambient temperature 29.2-33°C, gas flow rate of leaf chamber (397 ml min<sup>-1</sup>), water vaporising pressure in chamber 6-9.0 m bar, molar flow of air per unit leaf area (401.06 molm<sup>-2</sup>sec<sup>-1</sup>), ambient air pressure (99.95) KPa, PAR at leaf surface was up to (1625 µmolm<sup>-2</sup>).

## Results

### Gas exchange characteristics

**1. Photosynthetic rate (A) (µmole CO<sub>2</sub> m<sup>-2</sup>s<sup>-1</sup>):** Photosynthetic rate was considerably reduced (30.80%) in *Z. mays* growing along the roadside compared to control in Gujranwala district. Metal pollution at roadside did not affect the photosynthetic rate significantly in other crop plants. However, *B. oleracea* showed maximum photosynthetic rate (17.68 µmole CO<sub>2</sub> m<sup>-2</sup>s<sup>-1</sup>) compared to other crop plants growing under same conditions. The

photosynthetic performance of *Z. mays* and *O. sativa* was maximum (19.04 µmole CO<sub>2</sub> m<sup>-2</sup>s<sup>-1</sup>) and minimum (9.08 µmole CO<sub>2</sub> m<sup>-2</sup>s<sup>-1</sup>), respectively at control site. Industrial metal pollution also reduced photosynthetic rate significantly (21.44%) in *Z. mays* followed by *O. sativa* (14.71%) whereas other two crop plants showed non-significant variation in photosynthetic rate (Fig. 1a).

Maximum reduction (55.67%) in photosynthetic rate was observed in *A. aspera* followed by *P. hysterophorus* (31.87%) and *R. communis* (27.47%) growing along the roadside compared to control but the conditions did not affect significantly photosynthetic rate of *C. procera*. Similarly, photosynthetic rate of all wild plants except *R. communis* growing at industrial site was reduced with maximum reduction in *A. aspera* (69.18%). Among all species of wild and crop plant, heavy metal pollution proved most detrimental for *A. aspera* species wherein it reduced photosynthetic rate from 12.8 µmole CO<sub>2</sub> m<sup>-2</sup>s<sup>-1</sup> (control) to 3.97 µmole CO<sub>2</sub> m<sup>-2</sup>s<sup>-1</sup> (industrial site).

In Gujrat district *Z. mays* and *B. oleracea* growing along the roadside showed significant reduction (26.33% and 21.33% respectively) in photosynthetic rate compared to control conditions. The other two crop plants displayed slight reduction in said parameter. Conversely, photosynthetic rate was remarkably increased in all four crop plants irrigated with industrial wastewater with maximum increase (22.41%) in *Z. mays*. Photosynthetic rate of wild plants at the roadside drastically affected whereas it remained almost unchanged for plants on industrial site. Maximum reduction (9.47 µmole CO<sub>2</sub> m<sup>-2</sup>s<sup>-1</sup>) in photosynthesis rate was observed in *P. hysterophorus* growing under influence of the vehicular metal pollution which was the most severe among all crop and wild plant species (Fig. 1b).

Vehicular metal pollution in Sialkot district affected the rate of photosynthesis in *Z. mays* and *O. sativa* (24.64% and 13.76% decreased respectively) whereas other two crop plants remained unaffected in lieu of this parameter when compared with control conditions. Similarly, the photosynthetic rate of plants growing at industrial sites of the district was not affected significantly except in *S. bicolor* where it was increased 15.66% compared to the control. Wild plants

growing in district Sialkot showed intolerance in respect of photosynthetic rate because the characteristics decreased in all four wild plants measured from roadside. Maximum (49.64%) and minimum (43.46%) reduction was expressed by *P. hysterophorus* and *A. aspera*, respectively. Photosynthetic rates of *P. hysterophorus* and *R. communis* increased (11.34% and 6.46% respectively) whereas the rate of *C. procera* and *A. aspera* was decreased slightly under industrial metal pollution of the said district. *A. aspera* and *O. sativa* growing on roadside showed minimum rate of photosynthesis amongst wild and crop plants, respectively (Fig. 1c).

Crop plants growing on roadside of Hafizabad exhibited slight reduction in photosynthetic rate (maximum in *Z. mays* i.e. 4.06 9.47  $\mu\text{mole CO}_2 \text{ m}^{-2}\text{s}^{-1}$ ) in all the studied plants. On the other hand, the photosynthetic rate of two crop plants (*B. oleracea* and *O. sativa*) from industrial site showed a slight increase whereas parameter remained unchanged in *S. bicolor*. Similarly, in wild plants (*C. procera*, *A. aspera* and *R. communis*) growing under vehicular metal pollution photosynthetic rate remained almost unchanged but in *P. hysterophorus* results with the increase of 3.0  $\mu\text{mole CO}_2 \text{ m}^{-2}\text{s}^{-1}$  in photosynthesis (Fig. 1d).

Rate of photosynthesis decreased in all four crop plants studied on roadside of district MBD in comparison with control plants with maximum reduction in *B. oleracea*

(31.03%) followed by *Z. mays* (28.83%). This photosynthetic characteristic showed increase in *O. sativa*, *Z. mays* and *B. oleracea* (20.33%, 11.13% and 8.98%, respectively) growing at industrial site of district MBD. As for as wild plants are concerned, vehicular metal pollution affected negatively on photosynthetic rate with maximum decrease of 4.65  $\mu\text{mole CO}_2 \text{ m}^{-2}\text{s}^{-1}$  in *C. procera*. Industrial metal pollution of district MBD enhanced photosynthetic rate in *R. communis* and *P. hysterophorus* @ 22.54% and 19.88%, respectively. *C. procera* and *A. aspera* almost remained unaffected (Fig. 1e).

In district Narowal, maximum reduction (7.29  $\mu\text{mole CO}_2 \text{ m}^{-2}\text{s}^{-1}$ ) in photosynthesis rate was observed in *S. bicolor* and *B. oleracea* followed by *Z. mays* and *O. sativa* growing on roadside. As compared to control, there was insignificant variation in photosynthetic rate of crop plants sampled from industrial site of the district. Among wild plants collected from roadside *R. communis* showed maximum reduction (29.80%) in photosynthetic rate. At industrial site of the district, photosynthetic rate of *P. hysterophorus* was increased (18.34%) whereas other three wild plants showed negligible reduction (Fig. 1f). ANOVA of photosynthetic rate indicated that all crop and wild plants growing on all three sites in Gujranwala division differed significantly and difference among sites was also highly significant ( $p \leq 0.001$ ) (Table 2).

**Table 2. Mean squares from analyses of variance of data for Gas Exchange attributes of some wild and crop plant species growing under industrial and automobiles metal pollution in Gujranwala division.**

a. District Gujranwala						
SOV	df	A	E	A/E	g <sub>s</sub>	Ci
Species (S)	7	153.87***	6.300***	18.183***	0.01094***	339.0*
Sites (S)	2	81.88***	1.297*	4.589*	0.00415***	3365.8***
S x S	14	17.32***	1.302***	2.716*	0.00221***	143.5ns
Error	72	2.40	0.313	1.349	0.00021	194.8
b. District Gujrat						
Species (S)	7	186.95***	5.367***	11.914***	0.00590***	724.1***
Sites (S)	2	283.55***	1.895***	15.296***	0.00789***	3047.7***
S x S	14	13.45***	1.056***	1.993***	0.00174***	232.6*
Error	72	2.12	0.207	0.640	0.00026	114.5
c. District Sialkot						
Species (S)	7	192.92***	4.724***	7.8714***	0.0041***	1236.7***
Sites (S)	2	154.02***	2.689**	3.7000***	0.0020***	2926.7***
S x S	14	11.63***	1.183*	0.8591**	0.0008***	150.92*
Error	72	2.48	0.526	0.3339	0.00022	81.34
d. District Hafizabad						
Species (S)	7	77.588***	2.238***	4.5406***	0.00068***	299.02***
Sites (S)	2	67.954***	1.976**	0.5317ns	0.00110***	75.41ns
S x S	14	3.201***	0.662ns	0.5103**	0.00020**	89.97ns
Error	72	2.40	0.395	0.1880	0.00007	59.68
e. District MBD						
Species (S)	7	108.53***	4.870***	6.9843***	0.00093***	218.14***
Sites (S)	2	209.60***	4.497***	4.4230***	0.00317***	895.20***
S x S	14	5.37***	1.257***	0.9463***	0.00011ns	84.72*
Error	72	1.56	0.222	0.2092	0.00007	45.05
f. District Narowal						
Species (S)	7	119.67***	0.960**	3.0314***	0.0002***	148.12*
Sites (S)	2	131.81***	8.681***	0.3164ns	0.0017***	293.66ns
S x S	14	5.00***	0.350ns	0.2588ns	0.00006ns	76.47ns
Error	72	1.46	0.275	0.1493	0.00005	45.86

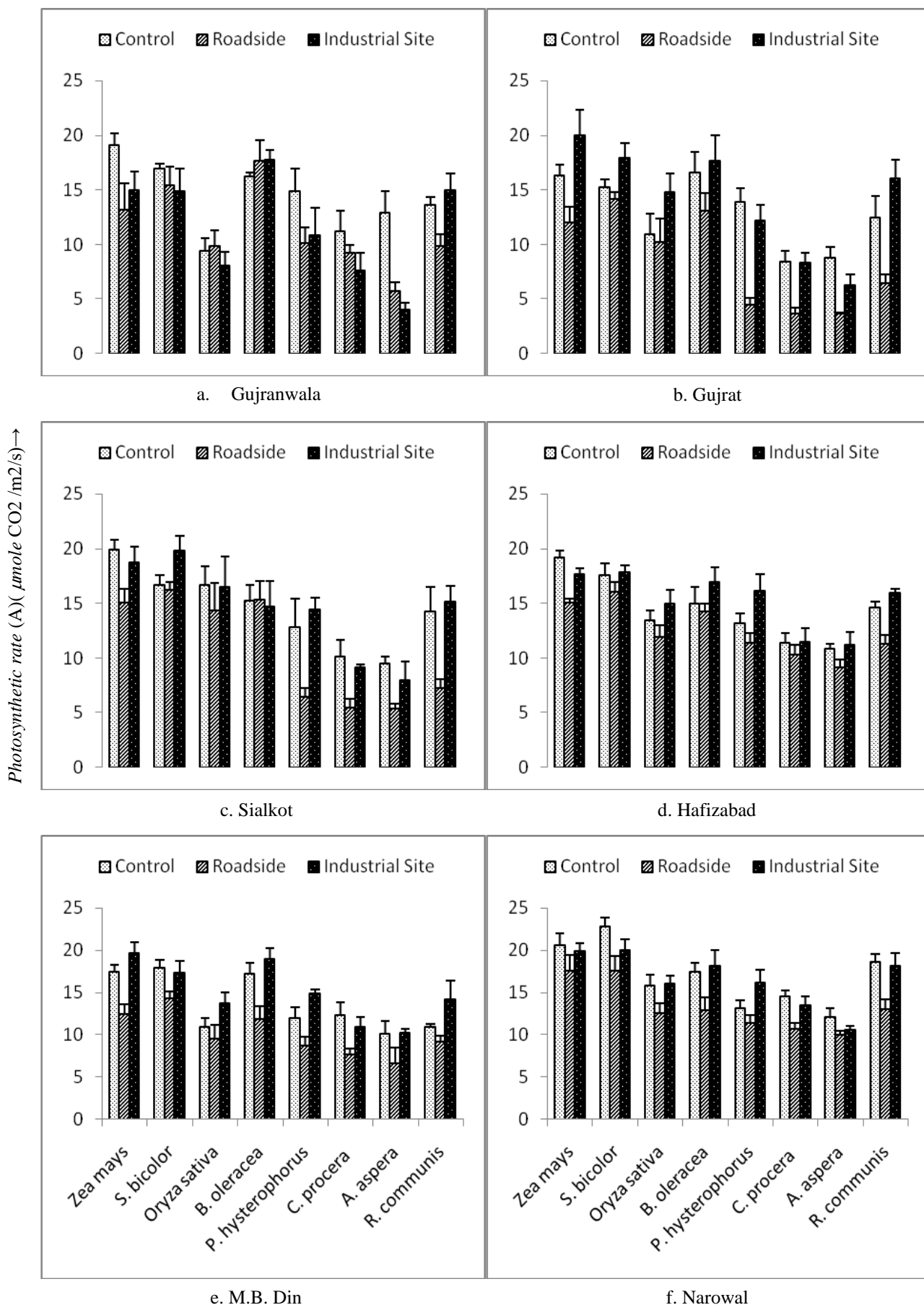


Fig. 1. Photosynthetic rate (A) ( $\mu\text{mole CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) of some wild and crop plant species growing under industrial and automobiles metal pollution in Gujranwala division.

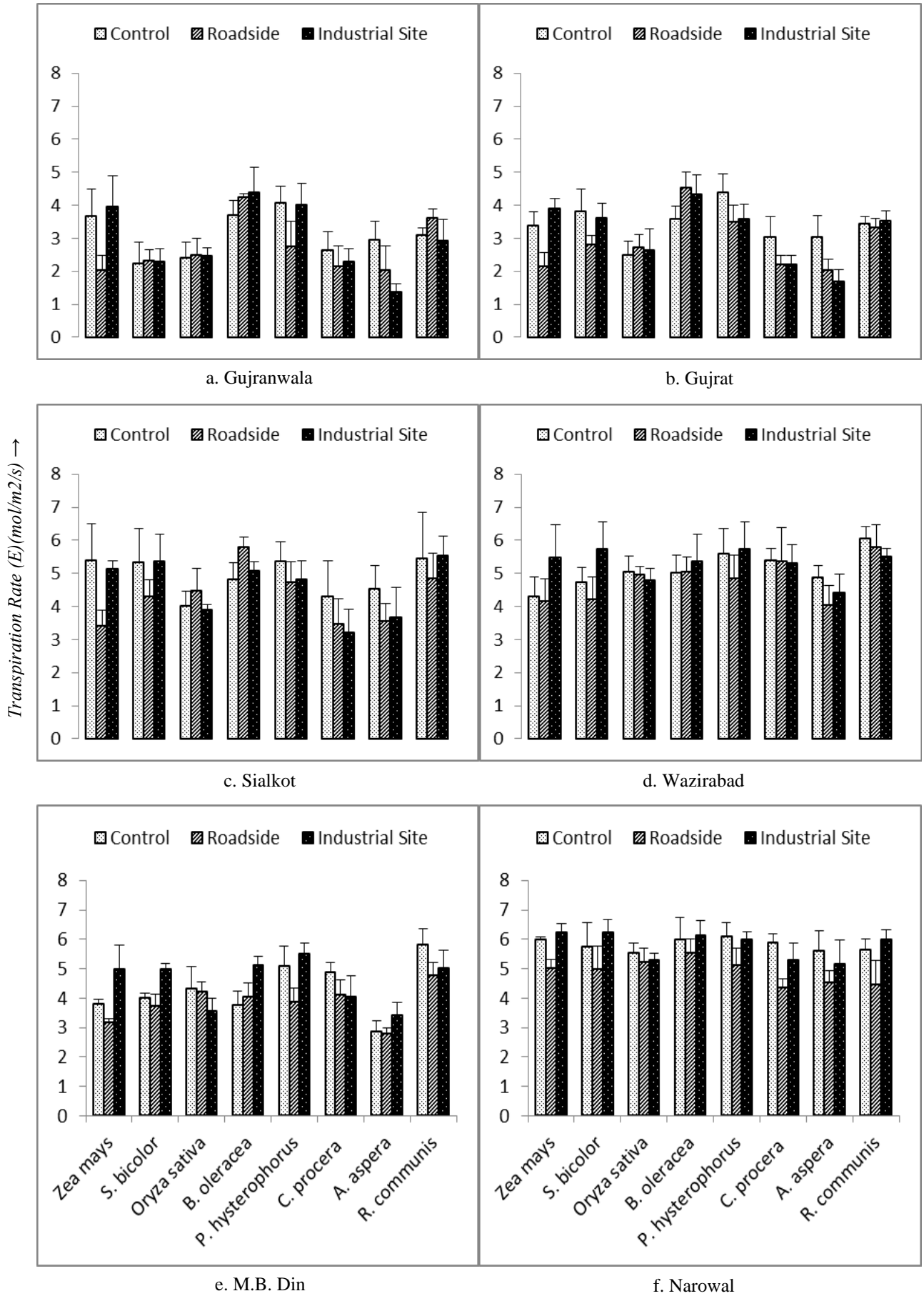


Fig. 2. Transpiration rate (E) of some wild and crop plant species growing under industrial and automobiles metal pollution in Gujranwala division.

**2. Transpiration rate (E) (mole CO<sub>2</sub> m<sup>-2</sup>s<sup>-1</sup>):** Rate of transpiration in all crop plants in Gujranwala district was increased under vehicular metal pollution except *Z. mays* where it was decreased by 44.66% whereas it was slightly enhanced under industrial metal pollution. In wild plants the rate was also reduced at roadside and industrial site but the reduction at roadside was slightly greater than industrial site. *A. aspera* showed maximum reduction among crop and wild plants at both roadside and industrial sites (31.52% and 53.90%, respectively) (Fig. 2a). In Gujrat district, *B. oleracea* and *O. sativa* responded with enhanced transpiration rate (21.14% and 8.11%, respectively) at roadside whereas it was decreased in other two crop plants i.e. *S. bicolor* (26.70%) and *Z. mays* (36.68%). Conversely, the rate was slightly increased by crop plants except *S. bicolor* (increased 5.50%) under the influence of industrial pollution. Transpiration rate of wild plants at roadside was slightly greater than industrial site. *A. aspera* showed maximum reduction among crop and wild plants at both roadside and industrial sites (31.52% and 53.90%, respectively) (Fig. 2a). In Gujrat district, *B. oleracea* and *O. sativa* responded with enhanced transpiration rate (21.14% and 8.11%, respectively) at roadside whereas it was decreased in other two crop plants i.e. *S. bicolor* (26.70%) and *Z. mays* (36.68%). Conversely, the rate was slightly increased by crop plants except *S. bicolor* (increased 5.50%) under the influence of industrial pollution. Transpiration rate of wild plants was considerably reduced at roadside and industrial site except *R. communis* where it remained almost unaffected. *O. sativa* and *R. communis* were more resistant against reduction in transpiration rate under vehicular as well as industrial metal pollution (Fig. 2b).

Under vehicular metal pollution in Sialkot district, transpiration rate of *O. sativa* and *B. oleracea* was increased (10.54% and 16.78%, respectively) whereas the same was decreased in *Z. mays* (36.99%) and *S. bicolor* (19.21%). The transpiration rate was not changed notably in all four crop plants under industrial metal pollution. Wild plants under vehicular and industrial metal pollution showed reduction in transpiration rate except in *R. communis* growing under industrial metal pollution where it increased by 1.45% (Fig. 2c).

*O. sativa* and *B. oleracea* did not show any change in transpiration rate on roadside in Hafizabad district whereas it was decreased in other two crop plants slightly. On the other hand, the rate was increased significantly in crop plants (*Z. mays* 21.53%, *S. bicolor* 17.42% and *B. oleracea* 6.34%) growing under industrial metal pollution except *O. sativa* (decreased 4.95%). Similarly, *C. procera* on roadside remained unaffected in terms of transpiration rate whereas it was decreased in other three wild plants @ *P. hysterophorus* 12.90%, *A. aspera* 17.28% and *R. communis* 4.62% (Fig. 2d).

Transpiration rate of three crop plants at roadside in MBD district was decreased (*Z. mays* 17.10%, *S. bicolor* 6.78% and *O. sativa* 2.09%) whereas it was increased in *B. oleracea* by 6.68%. Contrary, transpiration rate of three crop plants (*Z. mays*, *S. bicolor* and *B. oleracea*) growing under industrial metal pollution was increased (23.69%, 20.24% and 26.22%, respectively) except *O. sativa* where

it was decreased by 17.67%. Wild plants at roadside of this district also showed reduction in transpiration rate. Similarly, the rate was increased in three wild plants under industrial metal pollution except *C. procera* where it was decreased by 14.11% (Fig. 2e).

Reduction in transpiration was observed in all crop plants on roadside of Narowal district whereas it almost remained unchanged at industrial site of the district. The pattern of reduction in transpiration rate in wild plants of roadside was also similar to that of crop plants whereas *R. communis* sampled from industrial site showed slight increase and other three wild plants of same site showed slight decrease i.e. 5.84% (Fig. 2f). The difference in transpiration rate of all plants species under study in 5 districts Gujranwala division was highly significant (P=0.001) except in Narowal district where it was significant at P=0.01. Difference in transpiration rate among sites of all districts also significantly varied i.e. Gujrat, MBD & Narowal (p<0.001), Sialkot & Hafizabad (p<0.01) and Gujranwala (p<0.05) (Table 2).

### 3. Water use efficiency (A/E) (μmolCO<sub>2</sub>/μmolH<sub>2</sub>O):

Water use efficiency of two crop plants (*Z. mays* 6.62 μmolCO<sub>2</sub>μmol<sup>-1</sup>H<sub>2</sub>O and *O. sativa* 4.09 μmolCO<sub>2</sub>μmol<sup>-1</sup>H<sub>2</sub>O) at roadside of Gujranwala district was increased with reference to control i.e. 5.40 μmolCO<sub>2</sub>μmol<sup>-1</sup>H<sub>2</sub>O and 3.97 μmolCO<sub>2</sub>μmol<sup>-1</sup>H<sub>2</sub>O, respectively. Whereas it was decreased in other two crop plants with reference to control. All crop plants from industrial site showed reduction in water use efficiency. Similarly, WUE of *P. hysterophorus* and *C. procera* showed improvement (5.1% and 5.37%, respectively) on roadside whereas it was declined (30.44% and 37.72%, respectively) in *A. aspera* and *R. communis*. This important characteristic was also decreased in three wild plants under industrial metal pollution except in *R. communis* where it was improved 19.15% in comparison to control (Fig. 3a). In district Gujrat, *O. sativa* and *B. oleracea* were at disadvantages in term of WUE while growing on roadside with respect to other two crop plants (*S. bicolor* and *Z. mays*) where it was increased 20% and 15.25%, respectively. Under industrial metal pollution, WUE of three crop plants was increased except *B. oleracea* where it was decreased 12.13%. Conversely, WUE of all wild plants on roadside was decreased whereas at industrial site it was increased (Fig. 3b). WUE in *Z. mays* and *S. bicolor* was increased (14.92% and 14.77%, respectively) on roadside in Sialkot district while it was decreased in *O. sativa* and *B. oleracea*. On industrial site of the district, WUE remained almost unaffected except a slight increase was found in *S. bicolor* i.e. 14.55%. WUE of all wild plants was drastically affected on roadside whereas it did not show noticeable change in crop plants growing under industrial metal pollution (Fig. 3c).

WUE of crop plants on roadside of Hafizabad district was decreased except *S. bicolor*. Under industrial metal pollution it was increased in *B. oleracea* (6.19%) and *A. sativa* (14.96%) whereas it was decreased in *S. bicolor* and *Z. mays*. Wild plants of the district remained unaffected on roadside but an improvement in WUE found in all four wild plants under industrial metal pollution (Fig. 3d).

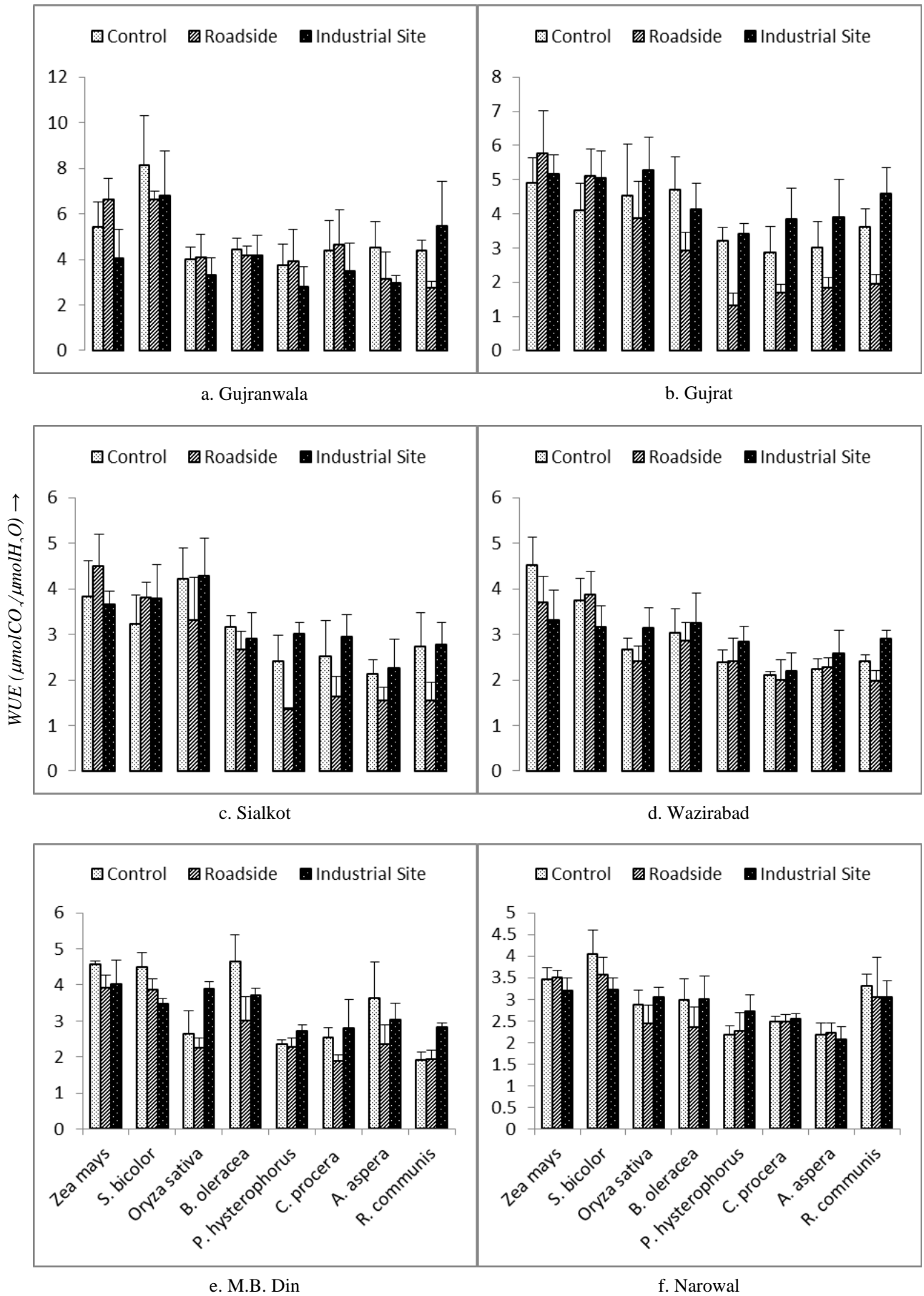


Fig. 3. Water use efficiency (A/E) of some wild and crop plant species growing under industrial and automobiles metal pollution in Gujranwala division.



Crop plants growing under vehicular and industrial metal pollution in MDB district showed reduction in WUE except in *O. sativa* from industrial site where the parameter was increased by 32.65%. Similarly, reduction trend in WUE in wild plants on roadside was only obvious (35.08%) in *A. aspera* whereas it was improved in three wild plants under industrial metal pollution except *A. aspera* where it decreased 16.02% (Fig. 3e). Vehicular metal pollution of Narowal district impacted negatively WUE of crop plant except *Z. mays* where it was increased slightly. Similarly, it was decreased in *Z. mays* (7.24%) and *S. bicolor* (22.30%) on industrial site and remained unaffected in *O. sativa* and *B. oleracea*. WUE of wild plants under both kinds of metal pollution remained almost unchanged except *P. hysterophorus* growing on industrial site where it was increased 19.92% (Fig. 3f).

ANOVA revealed that value of WUE among all crop plants differed highly significantly ( $p \leq 0.001$ ) in all six districts of Gujranwala division. Difference in value of WUE with respects to three sites was significant in Gujranwala ( $p \leq 0.05$ ), Gujrat, Sialkot and MBD ( $p \leq 0.001$ ) and non-significant in Hafizabad and Narowal districts.

**4. Stomatal conductance ( $g_s$ )( $\text{mol m}^{-2} \text{s}^{-1}$ ):** In district Gujranwala, three crop plants i.e. *Z. mays*, *O. sativa* and *B. oleracea*, on roadside showed reduction ( $0.0925 \text{ mol m}^{-2} \text{ s}^{-1}$ ,  $0.0775 \text{ mol m}^{-2} \text{ s}^{-1}$  and  $0.12 \text{ mol m}^{-2} \text{ s}^{-1}$ ) in stomatal conductance compared to control ( $0.0624 \text{ mol m}^{-2} \text{ s}^{-1}$ ,  $0.0725 \text{ mol m}^{-2} \text{ s}^{-1}$  and  $0.11 \text{ mol m}^{-2} \text{ s}^{-1}$ , respectively) except *S. bicolor* where an increase of 3.57% was found. This parameter also exhibited increasing trend under industrial metal pollution in all crop plants except *O. sativa*. Similarly, the trend of reduction in  $g_s$  continued in wild plants on roadside except in *R. communis*. *P. hysterophorus* and *R. communis* increased their efficiency in term of stomatal conductance on industrial site whereas the trend was reversed in *C. procera* and *A. aspera* (Fig. 4a).

Significant reduction (58.14%, 36.11% and 30.55%, respectively) in stomatal conduction was observed in *Z. mays*, *S. bicolor* and *B. oleracea* in contrast to the *O. sativa* growing on roadside of Gujrat district where it was increased by 53.84%. Contrary,  $g_s$  was increased in all crop plants under industrial metal pollution except *Z. mays*. All four wild plants of the district showed reduction in  $g_s$  whereas the value showed upward trajectory in *C. procera* and *R. communis* growing under industrial metal pollution (Fig. 4b).

Stomatal conductance of three crop plants except *Z. mays* (decreased 30.43%) on roadside remained almost unchanged in Sialkot district. Under industrial metal pollution, *O. sativa* ( $0.12 \text{ mol m}^{-2} \text{ s}^{-1}$ ) and *Z. mays* ( $0.14 \text{ mol m}^{-2} \text{ s}^{-1}$ ) was improved (33.33% and 17.85%, respectively) their stomatal conductance compared to unchanged behavior of other two crop plants. Wild plants under both kinds of metal pollution did not show measurable change in  $g_s$  except 14.29% and 33.33% upward trend of *R. communis* at roadside and industrial sites, respectively (Fig. 4c). *O. sativa* and *B. oleracea* decreased (12.5% and 15.79%, respectively) their stomatal conductance on roadside site of Hafizabad district whereas the parameter was increased insignificantly in *S. bicolor* and *Z. mays*. Likewise, a decrease of 8.33% and 10.53% was found in *S. bicolor* and *B. oleracea* growing on industrial sites of this district. A slight reduction was observed in all four wild plants sampled from the roadside

whereas it remained almost unaffected in wild plants under industrial metal pollution (Fig. 4d).

Vehicular metal pollution of MBD district decreased  $g_s$  of all four crop plants whereas the characteristic was increased in crop plants growing on industrial site with maximum increase found in *O. sativa* (20.59%). Similarly, all wild plants on roadside of MBD district showed reduction pattern in  $g_s$  whereas it was increased (6.45% and 20%, respectively) in *P. hysterophorus* and *R. communis* under industrial metal pollution (Fig. 4e). Reduction trend in  $g_s$  continued in all crops and wild plants growing on roadside and industrial site of Narowal district compared to unchanged behavior in respect of this parameter by all plant species (Fig. 4f). Mean square values from ANOVA for  $g_s$  showed that all wild and crop plants as well all three sites varied each other significantly ( $p \leq 0.001$ ) (Table 2).

**5. Inter cellular  $\text{CO}_2$  Concentration ( $C_i$ ):** In Gujranwala district, all crop and wild plants growing on both kinds of metal pollution, except *B. oleracea* on roadside, showed an increase in  $C_i$  concentration whereas its value was declined in all eight species under study at all industrial sites (Fig. 5a).  $C_i$  value of all crop plants on roadside of Gujrat district increased considerably with maximum value in *B. oleracea* i.e.  $157.5 \mu\text{mol/mol}$ . The same parameter was reduced in *S. bicolor* (6.65%) and *O. sativa* (8.89%) under industrial metal pollution. Wild plants of the district also exhibited the similar pattern of increase under vehicular metal pollution except in *A. aspera* (decreased 1.92%) whereas it remained inconsistent under industrial metal pollution (Fig. 5b). All four crop plants on roadside in Sialkot district showed upward trend in  $C_i$  value (maximum  $167.5 \mu\text{mol/mol}$  in *B. oleracea*).  $C_i$  value was declined in *S. bicolor* and *O. sativa* (4.81% and 6.69%, respectively) under industrial metal pollution. Similarly, wild plants of the district exhibited increasing trend in  $C_i$  value at roadside with maximum value in *C. procera* i.e.  $149.25 \mu\text{mol/mol}$  whereas it remained almost unchanged under industrial metal pollution (Fig. 5c). Crop and wild plants growing in both kinds of metal pollution in Hafizabad district remained unaffected in terms of  $C_i$  value except slight reduction (5.54%) was found in *S. bicolor* under industrial metal pollution conditions and *C. procera* for both kinds of metal pollutions i.e. 8.86% at roadside and 10.10% at industrial site (Fig. 5d). In MBD district, a slight increase in  $C_i$  value was observed in all crop plants (maximum in *Z. mays* i.e. 14.39%) sampled from roadside whereas the value showed increase only in *Z. mays* (8.95%) and *B. oleracea* (10.35%) under industrial metal pollution.

Wild plants of the district did not respond noticeably with respect to change  $C_i$  value on both kinds of metal pollutions (Fig. 5e). *Z. mays* and *S. bicolor* on roadside of Narowal district showed a little increase (13.24% and 5.04%, respectively) whereas the remaining two crop plants and all wild plants remained consistent in  $C_i$  value when compared with the control. The behavior of all wild and crop plants was also unchanged for this parameter on industrial site of the district (Fig. 5f).

It was revealed from ANOVA for  $C_i$  value that all crop and wild plants differed significantly at ( $P \leq 0.05$ ) in Gujranwala and Narowal districts and at ( $p \leq 0.001$ ) in Gujrat, Sialkot, Hafizabad and MBD districts. The value of  $C_i$  at all three sites of Gujranwala, Gujrat, Sialkot and MBD varied significantly whereas this difference was non-significant in Hafizabad and Narowal (Table 2).

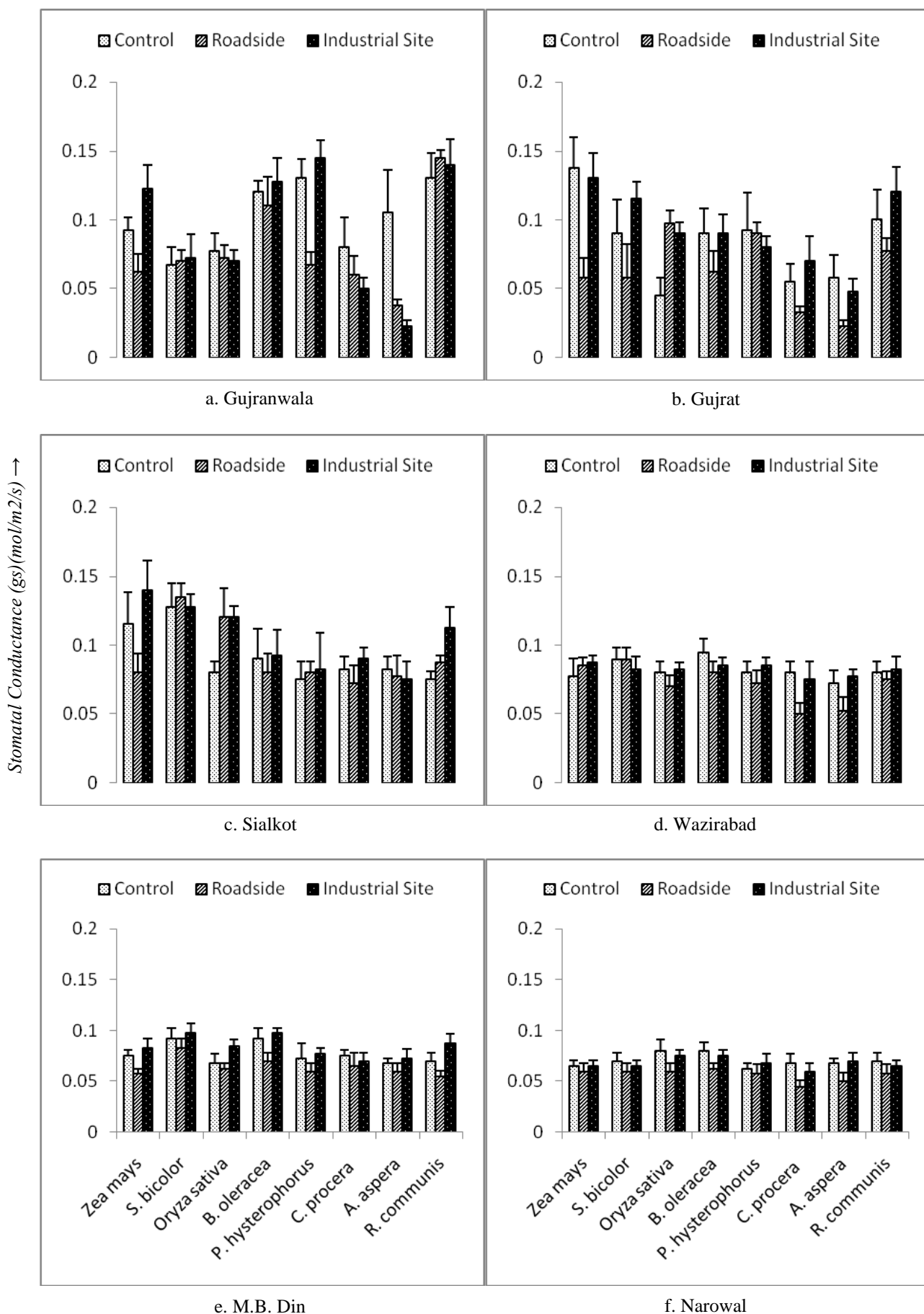


Fig. 4. Stomatal Conductance (Gs) of some wild and crop plant species growing under industrial and automobiles metal pollution in Gujranwala division.

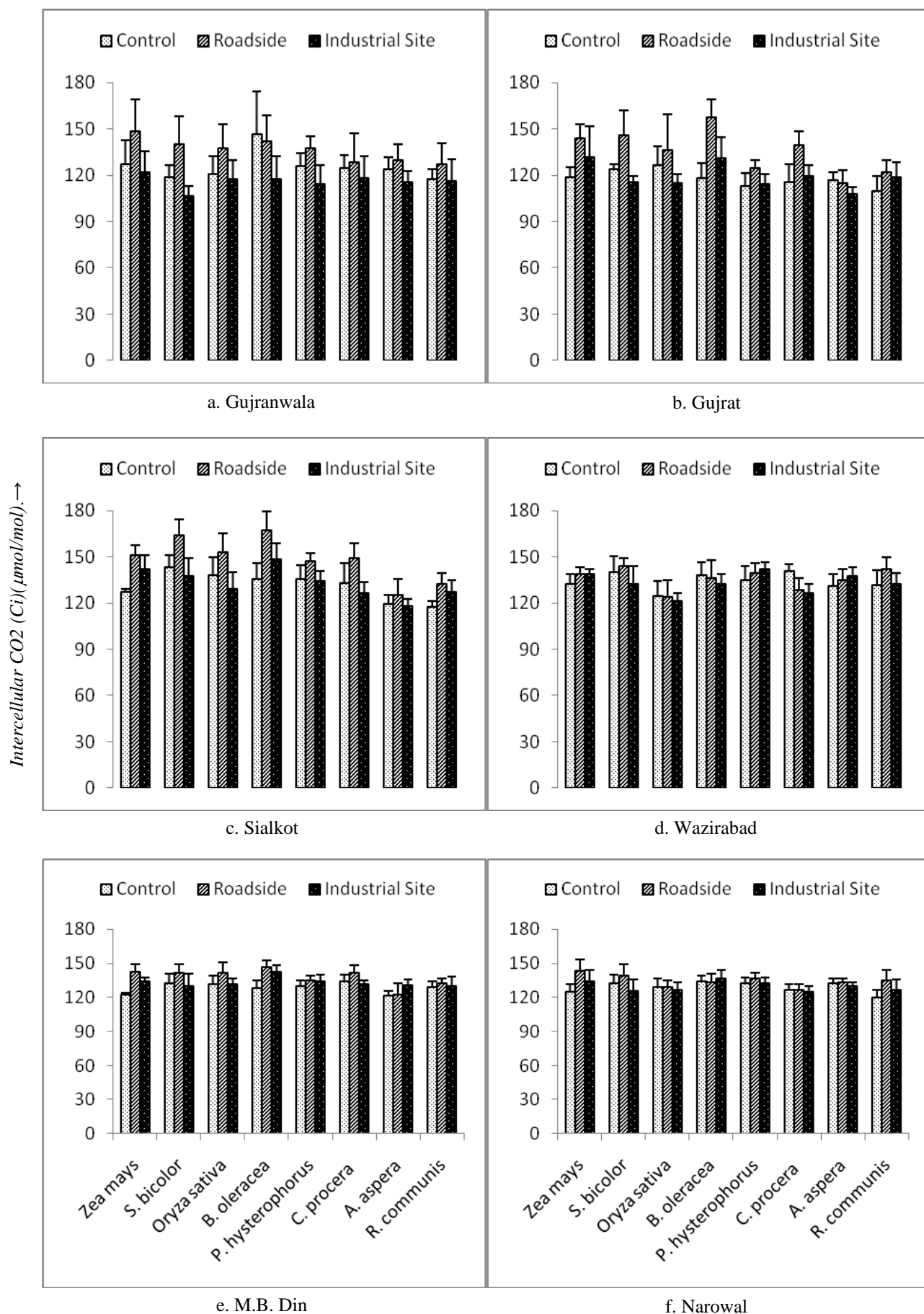


Fig. 5. Intercellular CO<sub>2</sub> concentration of some wild and crop plant species growing under industrial and automobiles metal pollution in Gujranwala division.

## Discussion

Photosynthesis is the one of the most important features of plants used to assess their tolerance to environmental stresses. Heavy metals exercise their toxic actions mostly by causing injury to chloroplasts and disturbing photosynthesis. Metal pollution toxicity caused reduction in photosynthetic rate in all plant species but extent of reduction was varied in all species. Photosynthetic rate of *B. oleracea* was least affected by the metal pollution stress compared to other crop plants. It is correlated with comparatively more decrease in chlorophyll contents of other crop plants as these pigments are the most important structures in the photosynthetic mechanism. Heavy metal ions interfere with photosynthetic enzymes and chloroplast membranes which lead to photosynthesis inhibition (Aggarwal *et al.*, 2012; Hafeez *et al.*, 2023). Leave stomata functions are influenced by heavy metal accumulation in leaves causing indirect reduction in photosynthesis and transpiration in higher plants. Chlorophyll content reduction due to heavy metals indirectly impacts photosynthesis; hence measurement of photosynthetic pigments is used to determine metal stress (Aggarwal *et al.*, 2012; Hafeez *et al.*, 2023).

Rate of transpiration in all crop and wild plants was decreased under vehicular metal pollution but was slightly enhanced under industrial metal pollution with insignificant effect on *B. oleracea* and *C. procera*. Whereas it remained unchanged or less affected in comparatively less polluted sites of Hafizabad, M.B.D and Narowal districts. In soil-water medium, mixed heavy metals compete with each other which probably led to inconsistent segments of the gas exchange data (Chandra & Kang, 2016). The stomatal conductance was reduced in all crop and wild plant species under roadside metal stress. It is viewed that metals dust deposition from road traffic on the leaf surfaces choked the stomatal pores led to limited uptake of oxygen. Generally, leaf surfaces are also affected by these contaminants and thus interject light absorption process (Dhal *et al.*, 2024).

In both crop and wild plant species of metal polluted districts, the congested stomatal pores also decreased the rate of transpiration. Results were in line with earlier findings of Khalid *et al.*, 2017 and Shakeel *et al.*, 2023. Different noxious metal pollutants present in the automobiles exhausts critically affected the physiological attributes of plants (Khalid, 2017). They also damage membranous structures, induce stomatal adjustments and harm electron transport chain (Kulshrestha & Saxena, 2016; Jorjani & Karakaş, 2024).

Water use efficiency (WUE) affected differently to different plant species. It was increased in *Z. mays* under vehicular pollution in district Gujranwala, Gujrat and Sialkot whereas decreased in Districts Hafizabad, M.B.D and Narowal. WUE was increased in all wild plants under industrial metal pollution in all districts. This reflects that plants attempt to preserve maximum water in response to ecological stresses. Metal and other ecological stresses hinder the uptake of water by roots so it is a water conservation strategy by plants (Feng *et al.*, 2023). Higher value of WUE exhibited by wild plants seems to be a main contributor in making them comparatively more metal pollution tolerant.

A significantly declining tendency ( $p < 0.05$ ) in intercellular carbon dioxide concentration was shown by all plants in highest industrial metal polluted district i.e. Gujranwala and vice versa in least polluted district i.e. Narowal. The highest decrease in  $C_i$  was found in *S. bicolor* at Gujranwala compared to control plants. The  $C_i$  remained less affected or unchanged in all plants at Narowal, the least polluted district. Similar findings were reported by Souri *et al.*, (2019) while exploring effects of heavy metals on photosynthetic activities. Owing to the blocking of stomata under severe metals stress plants utilize the stored water for metabolic activities. Stomatal blocking also obstructs uptake of  $CO_2$  from the atmosphere leading to decrease in photosynthesis, thus affecting plant growth (Shomali *et al.*, 2023). *B. oleracea* among crop plants and *C. procera* among wild plants showed maximum tolerance against metal stress by maintaining gas exchange attributes.

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