



Available Online at EScience Press

Plant Health

ISSN: 2305-6835

<https://esciencepress.net/journals/planthealth>

***In-vitro* Effect of Auto-Vehicular Exhaust Emission on Seed Germination and Seedling Growth of Leguminous Plants from Different Localities of Karachi**

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ARTICLE INFO

ABSTRACT

Article History

Received: March 17, 2025

Revised: May 12, 2025

Accepted: May 29, 2025

Keywords

Pollution

Automobile exhaust emissions

Urban areas

Germination

Roadside trees

Automobile exhaust emissions are a dominant feature of an urban environment and are widely believed to have detrimental effects on plants. The impact of exhaust emission from automobile examined on four roadside species namely, *Caesalpinia pulcherrima* (L.) Sw., *Sesbania grandiflora* (L.) Poiret, *Senna alata* (L.) Roxb. and *Albizia lebbek* (L.) Bth., was assessed with respect to seed germination and seedling growth. Automobile emission showed significant effects on reducing seedling growth parameters of *C. pulcherrima*, *S. grandiflora*, *S. alata* and *A. lebbek* growing in the polluted site of different localities of Karachi. Drastic effect in decrease of seed germination was showed by the seeds collected from the Tariq road followed by Bahadurabad and Khalid Bin Waleed road. Whereas, Gulshan-e-Ali showed maximum growth, leaf area, fresh and dry weight of leaf and root as compared to Karachi University Campus (unpolluted area).

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INTRODUCTION

Air pollution due to vehicular discharge generally arises from cars, motorcycles, minibuses, rickshaws, buses, wagons and trucks emits abundance of harmful gases in the environment which directly affect to living-organisms and road side plants which exposed to it. Presently, alertness is made to focus on the global environmental problems (Hasanuzzaman *et al.*, 2010). Demand of rapid modes of transportation has increased during the last few decades has resulted in raise of gaseous and particulate pollutants concentrations (Joshi *et al.*, 2009). Petrol and diesel fuel emit from vehicles are responsible for the wide range of pollutants depending on vehicle technology and operating conditions (Colville *et al.*, 2001). Auto-vehicular pollution is much greater than that emission of dust and poisonous gases emits by different types of factories. Motor vehicles account for 60-70% of the pollution found in an urban location (Tripathi and Gautam, 2007; Dwivedi *et al.*, 2008).

Environmental conditions are becoming progressively worse in Pakistan and its major cities are plagued with environmental problems due to unchecked vehicular emission are continuously adding toxic gases and other substances to the environment. (Wahid and Marshall, 2006). Combustion of fuels contributes a considerable amount of pollutants to air (Sawidis *et al.*, 1995). These pollutants in combinations cause synergistic effects to plant life. Emissions discharge from automobiles and trucks operating on public roads represent a major portion of the air pollutants included in emission inventories (Skiba and Belitskaya, 2003). The major cities of Pakistan like Karachi, Faisalabad, Lahore and Hyderabad are suffering by environmental pollution problems. In the city like Karachi where the population is rapidly increasing gradually, automobile emission is a major source of atmospheric pollution (Qadir and Iqbal, 1991). The traffic in the Karachi city is not only noisy but also producing hazardous environmental effects on

plants. The plants growing adjacent to roadsides of the city exhibited considerable damage in response to automobile exhaust emission (Shafiq and Iqbal, 2003, 2005a). Roadside trees in the city are coming under pressure and are lost due to vehicular-traffic transportation (Jim, 1998). Shafiq and Iqbal (2005b) found that biomass production of some roadsides trees were more sensitive to air pollutant particularly at M.A. Jinnah road as compared to other less polluted areas of the city. Lots of work has been done to study the response of traffic load on road side plants (Angold, 1997). Air pollutants also have an effect on germination of seeds, pedicles length and number of flowers in inflorescence (Nithamathi and Indira, 2005). Pollutants interact with other environmental factors and may alter plant-environment relationships on a regional level (Winner, 1981). Present research was carried out to investigate the effects of automobile pollutants on seed germination and seedling growth of some important roadside plant species namely, (*Caesalpinia pulcherrima* (L.) Swartz, *Sesbania grandiflora* (L.) Poiret, *Senna alata* (L.) Roxb., and *Albizia lebbbeck* (L.) Benth), growing in different localities of Karachi.

MATERIALS AND METHODS

Experiment was conducted to determine the effect of automobile pollution on the productivity (seed germination and seedling growth) of *C. pulcherrima*, *S. grandiflora*, *S. alata* and *A. lebbbeck* under the natural environmental conditions in the department of Botany, University of Karachi. Dry seeds were collected from the roadside of shrubs and trees like *C. pulcherrima*, *S. grandiflora*, *S. alata* and *A. lebbbeck* influenced by traffic activities. Enough seeds of each individual of a species were randomly collected from each area and all these seeds were soaked in 1% bleach solution (NaOCl) for 2 minutes and rinsed with distilled water to avoid any type of fungal contamination during germination and dried aseptically. 10 seeds of each species were kept in sterilized Petri dishes (90mm diameter) on Whatman filter paper No. 42 at room temperature (27-28°C) and replicate thrice. After 15 days of seed germination, growth parameters were recorded.

Statistical analysis

Analysis was carried out using the personal computer software packages SPSS version 13.0. The data collected for various parameters from different sites was subjected to statistical analysis that was calculated by

one-way analysis of variance techniques, ANOVA (Steel and Torrie, 1960) and Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Present research carried out to study the effects of auto vehicular exhaust emission impact on seed germination and seedling growth of *C. pulcherrima*, *S. grandiflora*, *S. alata* and *A. lebbbeck* which are important plant species growing along the main busy roads of the city of Karachi, Pakistan. These seeds collected from different polluted sites of the city behaved differently in terms of germination and growth. The effects of motor vehicle exhaust emission on seed germination varied from site to site. Significant ($p < 0.05$) reduction in seed germination was observed in the seeds of *C. pulcherrima*, *S. grandiflora*, *S. alata* and *A. lebbbeck* collected from Tariq Road followed by Bahadurabad, Khalid bin Waleed Road, Gulshan-e-Ali as compared to Karachi University campus which showed maximum ($p < 0.05$) length, leaf area, fresh and dry weight of leaf and root. Iqbal and Siddiqui in 1996 found reductions in seed germination of *Pongamia pinnata* (L.) Merr., and *Sesbania sesban* (L.) Merr., collected from the polluted areas. Krishnaya and Bedi in 1986 had observed the toxic effects of automobile exhaust on germination of *C. tora* L. and *C. occidentalis* L. The possible reason for reduction in seed germination of *C. pulcherrima*, *S. alata*, *S. grandiflora* and *A. lebbbeck* could be due to the development of unhealthy seeds produced by trees growing along the main road of the city. The deposition of lead and soot particles on the surface of pods from automobile are additional cause for low production of healthy seeds. The other reason of inhibitory factor in controlling the germination and seedling growth of *C. pulcherrima*, *S. grandiflora*, *S. alata* and *A. lebbbeck* can be attributed to the physical constraints induced by dust and diesel fuel particles available in the nearby soil and absorbed by the seed due to automobile activities. Diesel fuel would cause a film of oil which form around the seed and act as a physical barrier thus preventing or reducing both water and oxygen transfer to the seed. This physical impedance was shown to delay seed emergence and therefore could be an additional factor in the overall inhibitory effect of diesel fuel contamination on germination and growth of plants (Adam and Duncan, 2002). Seedling growth of *C. pulcherrima*, *S. grandiflora*, *S. alata* and *A. lebbbeck* seeds collected from different

sites of the city was found low as compared to seeds collected from the campus areas as shown in table 1a, b, c and d. Seeds taken from the polluted sites showed decline in almost all the growth parameters. It was observed that the plants growing close to the busy road of the city are highly affected by auto emission. Low percentages of seedling growth proved the failing viability of the seeds. This decrease in growth of the polluted plants could be attributed to the presence of motor vehicle pollutants in the environment of different types of polluted area from where the seeds of *C. pulcherrima*, *S. grandiflora*, *S. alata* and *A. lebbbeck* were collected. Similar results have been found on seed germination and root growth of roadside plants of *Brassica chinensis* and *Brassica parachinensis* (Wong *et al.*, 1984). In auto vehicular pollution, some hidden or physiological changes or metabolic disorders of plants take place which affect the reproductive capacity of plants and enforce plants to produce low seeds viability. Bhatti and Iqbal in 1988 noticed that the phenology and productivity of *Ficus bengalensis* L., and *Eucalyptus* sp., were highly affected due to automobile exhausts. They also concluded that automobile emission significantly reduced the productivity, leaf dry weight and leaf area of *Guaiacum officinale*, *F. bengalensis* and *Eucalyptus* sp., at the polluted sites of the city as compared to control. A significant ($p < 0.05$) decline in leaf area of a roadside plant, *Bougainvillea spectabilis* has been observed by Hussain *et al.*, 1997.

Lots of research has been done to study the response of traffic load on plants (Angold, 1997). The polluted air may have profoundly effect on the growth and development on vegetation because gases enter the leaves during the normal course of gaseous exchange and causes blocking of stomata, degradation of chlorophyll, reduced starch synthesis, damage leaves tissues, growth retarded and ultimately decreased in crop yield (Agarwal *et al.*, 2003). Preeti (2000) observed that leaves of *Cassia siamea* and *Thevetia nerifolia* in the polluted environment showed reduction in growth.

Earlier research has shown that at high concentrations, many of the pollutants present in exhaust gases can be damaging to plants (Wellburn, 1990; Ackerly and Bazzaz, 1995). The pollutants emitted from the auto exhaust adversely affect the germination of seeds (Guderian, 1977). Siddiqui and Iqbal, (1992) investigated the percentage reduction in dry biomass for

C. surattensis, *L. leucocephala*, *P. aculeata*, *S. sesban* was decreased significantly in seedlings raised from the polluted seeds as compared to control. The significant reduction was observed in shoot height, number of leaves and circumference of polluted seedlings. In growth experiment, *L. leucocephala* was found less affected by automobile pollution as compared to other species. Automobile activities has increased the level of metals in the environment of the major cities of the world and produced profound effects on plant growth (Aksoy *et al.*, 2000; Shafiq and Iqbal, 2007). Due to lead toxicity, plant growth stunted and germination was inhibited. (Wierzbicka and Obidzinska, 1998; Antosiewicz and Wierzbicka, 1999; Shaukat *et al.*, 1999; Iqbal and Shazia, 2004). Foliar application of Pb (lead) affected growth and yield of wheat (Rashid and Mukhirji, 1993). Lead produced highly significant effects on shoot and root lengths and seedling dry biomass of *Lythrum salicaria* (Joseph *et al.*, 2002). Influence of cadmium toxicity on germination and growth of several common trees were investigated by Iqbal and Mehmood in 1991. Vehicular traffic is main sources of heavy metal contamination, either in soils or roadside dusts in urban areas, and it has increased in the most recent years (Ovadnevaite *et al.*, 2006).

The relationship between plants growing in polluted and unpolluted areas affected by auto vehicular activities helps in understanding the impact of automobile pollution on the growth of plants. As a result, a significant amount of information can be gathered and loss of plant species may be related either directly or indirectly through matching of the findings of the research. The present study confirms that the seeds of *Caesalpinia pulcherrima*, *Sesbania grandiflora*, *Senna alata* and *Albizia lebbbeck* reduced seedling germination collected from the polluted area. It becomes essential to grow resistant plant species and low lead fuel to help in reducing the automobile pollution. Existing roadside plants must be saved otherwise would be lost due to constant increase in vehicular traffic density infrastructure. Problem due to automobile activities is not a new one but the importance of its impacts on plants growing along the busy road has recently become clear. It is essential to understand the relationships between plant and the environment in which they are growing, if we want to conduct safe and clean environment on a long-term basis.

Table 1a. Effect of automobile pollution on seedling growth of *Caesalpinia pulcherrima* (L.) Sw.

Treatments	Germination (%)	Root Length (cm)	Shoot Length (cm)	Seedling length (cm)	Leaf Area (cm ²)	Root Fresh Weight (g)	Stem Fresh Weight (g)	Leaf Fresh Weight (g)	Root Dry Weight (g)	Stem Dry Weight (g)	Leaf Dry Weight (g)	Total Seedling Fresh Weight (g)	Total Seedling Dry Weight (g)	Root/ Shoot ratio Fresh Weight	Root/ Shoot ratio Dry Weight
Site A	100	4.53b ± 1.58	5.36ab ± 1.58	9.90b ± 1.72	1.15a ± 0.33	0.12b ± 0.01	0.21a ± 0.02	0.23b ± 0.04	0.02b ± 0.005	0.07b ± 0.008	0.04a ± 0.006	0.56b ± 0.01	0.13c ± 0.01	0.59a ± 0.01	0.31a ± 0.08
Site B	63	1.83a ± 0.14	4.73a ± 0.47	6.56a ± 0.49	0.04a ± 0.006	0.16a ± 0.02	0.14ab ± 0.02	0.01a ± 0.00	0.02a ± 0.006	0.03a ± 0.003	0.34a ± 0.03	0.05a ± 0.008	0.24a ± 0.04	0.78a ± 0.22	0.64a ± 0.04
Site C	70	2.50ab ± 0.51	4.53a ± 0.17	7.03a ± 0.33	0.06ab ± 0.01	0.16a ± 0.04	0.12a ± 0.01	0.01ab ± 0.003	0.04ab ± 0.01	0.03a ± 0.003	0.35a ± 0.03	0.08ab ± 0.01	0.40a ± 0.10	0.44a ± 0.14	0.67a ± 0.01
Site D	70	2.76ab ± 0.16	4.63a ± 0.20	7.40ab ± 0.36	0.08ab ± 0.02	0.16a ± 0.03	0.13a ± 0.02	0.02b ± 0.003	0.04ab ± 0.008	0.03a ± 0.003	0.37a ± 0.04	0.09abc ± 0.003	0.61a ± 0.34	0.80a ± 0.35	0.75a ± 0.01
Site E	87	2.63ab ± 0.26	6.33b ± 0.12	8.96ab ± 0.18	0.11ab ± 0.04	0.19a ± 0.05	0.22b ± 0.02	0.02ab ± 0.003	0.06b ± 0.008	0.03a ± 0.006	0.53b ± 0.05	0.11bc ± 0.01	0.82a ± 0.53	0.26a ± 0.03	0.79a ± 0.08
LSD.05=	0.437	0.158	0.087	0.066	0.074	0.430	0.750	0.101	0.172	0.053	0.572	0.126	0.219	0.112	

Table 1b. Effect of automobile pollution on seedling growth of *Sesbania grandiflora* (L.) Poiret.

Treatments	Germination (%)	Root Length (cm)	Shoot Length (cm)	Seedling length (cm)	Leaf Area (cm ²)	Root Fresh Weight (g)	Stem Fresh Weight (g)	Leaf Fresh Weight (g)	Root Dry Weight (g)	Stem Dry Weight (g)	Leaf Dry Weight (g)	Total Seedling Fresh Weight (g)	Total Seedling Dry Weight (g)	Root/ Shoot ratio Fresh Weight	Root/ Shoot ratio Dry Weight
Site A	93	15.73c ± 1.01	6.20b ± 0.05	21.93c ± 1.07	0.67b ± 0.02	0.03b ± 0.00	0.05a ± 0.008	0.10a ± 0.01	0.02b ± 0.003	0.01a ± 0.003	0.03b ± 0.005	0.19b ± 0.02	0.07b ± 0.005	0.56a ± 0.09	1.50b ± 0.28
Site B	47	3.66a ± 0.52	2.06a ± 0.16	5.73a ± 0.53	0.02ab ± 0.003	0.03a ± 0.01	0.05a ± 0.01	0.01a ± 0.00	0.01a ± 0.00	0.01a ± 0.003	0.11a ± 0.01	0.03a ± 0.003	0.83a ± 0.34	1.00ab ± 0.00	0.41a ± 0.06
Site C	63	8.50ab ± 2.86	4.06ab ± 1.07	12.56ab ± 3.94	0.02a ± 0.00	0.04a ± 0.008	0.06a ± 0.005	0.01a ± 0.00	0.01a ± 0.00	0.02ab ± 0.00	0.12ab ± 0.01	0.04a ± 0.00	0.49a ± 0.09	1.00ab ± 0.00	0.46ab ± 0.07
Site D	70	7.43ab ± 2.97	5.40b ± 0.80	12.83ab ± 2.19	0.01a ± 0.003	0.04a ± 0.003	0.07a ± 0.02	0.01a ± 0.00	0.01a ± 0.003	0.01a ± 0.003	0.13ab ± 0.02	0.04a ± 0.005	0.36a ± 0.08	0.66a ± 0.16	0.53ab ± 0.06
Site E	73	11.20bc ± 1.99	4.43ab ± 1.06	15.63bc ± 3.06	0.01a ± 0.003	0.06a ± 0.008	0.08a ± 0.008	0.01a ± 0.00	0.01a ± 0.003	0.01a ± 0.003	0.16ab ± 0.01	0.04a ± 0.006	0.27a ± 0.06	0.66a ± 0.16	0.62ab ± 0.10
LSD.05=	0.154	0.064	0.083	0.088	0.119	0.078	0.053	1.00	0.124	0.256	0.113	0.212	0.065	0.216	

Site A= University of Karachi (Control, that is non-polluted area), Site B= Tariq Road (Extremely/Heavily polluted area), Site C= Bahadurabad (maximum polluted area), Site D= Khalid Bin Waleed Road (mostly Polluted area), Site E= Gulshan-e-Ali (Less polluted area). Numbers followed by the same letter in the same column are not significantly different according to Duncan Multiple Range Test at p<0.05 level. ± Standard Error.

Table 1c. Effect of automobile pollution on seedling growth of *Senna alata* (L.) Roxb.

Treatments	Germination (%)	Root Length (cm)	Shoot Length (cm)	Seedling length (cm)	Leaf Area (cm ²)	Root Fresh Weight (g)	Stem Fresh Weight (g)	Leaf Fresh Weight (g)	Root Dry Weight (g)	Stem Dry Weight (g)	Leaf Dry Weight (g)	Total Seedling Fresh Weight (g)	Total Seedling Dry Weight (g)	Root/ Shoot ratio Fresh Weight	Root/ Shoot ratio Dry Weight
Site A	93	4.76a ± 0.06	4.40a ± 0.36	7.03a ± 0.33	1.50b ± 0.19	0.16a ± 0.06	0.21a ± 0.02	0.24b ± 0.03	0.08b ± 0.03	0.15b ± 0.02	0.15b ± 0.04	0.61b ± 0.11	0.39c ± 0.06	0.70a ± 0.26	0.52b ± 0.16
Site B	63	4.46a ± 0.92	4.80a ± 0.17	6.30a ± 1.57	0.03a ± 0.006	0.14a ± 0.01	0.14a ± 0.02	0.14a ± 0.02	0.06a ± 0.008	0.07a ± 0.01	0.32a ± 0.03	0.14a ± 0.01	0.25a ± 0.03	0.16a ± 0.02	0.57a ± 0.08
Site C	77	2.93a ± 0.72	4.43a ± 0.44	6.56a ± 0.88	0.08a ± 0.005	0.18a ± 0.05	0.15a ± 0.01	0.15a ± 0.01	0.11ab ± 0.03	0.07a ± 0.008	0.41ab ± 0.06	0.20ab ± 0.03	0.49a ± 0.11	0.15a ± 0.06	0.62a ± 0.04
Site D	80	4.36a ± 1.53	4.80a ± 0.51	6.56a ± 0.49	0.10a ± 0.04	0.19a ± 0.03	0.17ab ± 0.02	0.17ab ± 0.02	0.08a ± 0.005	0.08ab ± 0.003	0.47ab ± 0.08	0.18ab ± 0.01	0.53a ± 0.13	0.20a ± 0.03	0.66a ± 0.12
Site E	83	2.73a ± 0.49	4.83a ± 0.12	6.86a ± 1.05	0.11a ± 0.05	0.21a ± 0.02	0.20ab ± 0.008	0.20ab ± 0.008	0.12ab ± 0.01	0.12ab ± 0.01	0.53ab ± 0.07	0.27b ± 0.01	0.50a ± 0.19	0.20a ± 0.07	0.67a ± 0.005
LSD.05=		3.23	1.286	2.619	0.399	0.142	0.108	0.083	0.052	0.064	0.073	0.253	0.124	0.540	0.273

Table 1d. Effect of automobile pollution on seedling growth of *Albizia lebbeck* (L.) Bth.

Treatments	Germination (%)	Root Length (cm)	Shoot Length (cm)	Seedling length (cm)	Leaf Area (cm ²)	Root Fresh Weight (g)	Stem Fresh Weight (g)	Leaf Fresh Weight (g)	Root Dry Weight (g)	Stem Dry Weight (g)	Leaf Dry Weight (g)	Total Seedling Fresh Weight (g)	Total Seedling Dry Weight (g)	Root/ Shoot ratio Fresh Weight	Root/ Shoot ratio Dry Weight
Site A	93	9.60d ± 0.77	8.50b ± 0.81	25.00b ± 6.39	1.91b ± 0.18	0.19a ± 0.03	0.18b ± 0.01	0.15c ± 0.005	0.12b ± 0.03	0.11b ± 0.01	0.08b ± 0.003	0.52d ± 0.04	0.32b ± 0.04	1.00a ± 0.15	1.04b ± 0.14
Site B	60	4.93a ± 0.52	4.43a ± 0.34	9.36a ± 0.78	0.05b ± 0.01	0.08a ± 0.02	0.05a ± 0.02	0.01a ± 0.003	0.05a ± 0.005	0.01a ± 0.003	0.19a ± 0.03	0.08a ± 0.003	0.79a ± 0.41	0.35a ± 0.10	0.84a ± 0.34
Site C	63	5.66ab ± 0.38	4.53a ± 0.31	10.20a ± 0.40	0.06b ± 0.01	0.10a ± 0.02	0.09ab ± 0.008	0.02a ± 0.005	0.05a ± 0.003	0.02a ± 0.005	0.25ab ± 0.04	0.09a ± 0.003	0.60a ± 0.07	0.36a ± 0.08	1.30ab ± 0.29
Site D	70	6.90bc ± 0.20	5.30a ± 0.37	12.20a ± 0.25	0.12ab ± 0.01	0.13ab ± 0.01	0.11bc ± 0.008	0.02a ± 0.003	0.06a ± 0.00	0.02a ± 0.003	0.36bc ± 0.02	0.10a ± 0.003	0.93a ± 0.15	0.38a ± 0.05	1.54ab ± 0.22
Site E	77	8.00c ± 0.40	5.80a ± 0.17	13.80a ± 0.51	0.15a ± 0.02	0.15ab ± 0.03	0.11bc ± 0.01	0.03a ± 0.003	0.06a ± 0.008	0.02a ± 0.00	0.41cd ± 0.02	0.11a ± 0.008	1.10a ± 0.32	0.54a ± 0.08	1.60ab ± 0.21
LSD.05=		1.26	1.421	10.013	0.775	0.085	0.077	0.046	0.050	0.023	0.013	0.122	0.064	0.888	0.361

Site A= University of Karachi (Control, that is non-polluted area), Site B= Tariq Road (Extremely/Heavily polluted area), Site C= Bahadurabad (maximum polluted area), Site D= Khalid Bin Waleed Road (mostly Polluted area), Site E= Gulshan-e-Ali (Less polluted area). Numbers followed by the same letter in the same column are not significantly different according to Duncan Multiple Range Test at p<0.05 level. ± Standard Error

CONFLICT OF INTEREST

The authors affirm that the research was conducted without any commercial or financial affiliations that could be perceived as potential conflicts of interest.

ACKNOWLEDGMENTS

The authors extend their appreciation to the University of Karachi, Karachi, for all types of support.

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