Caspian J. Env. Sci. 2009, Vol. 7 No.1 pp. 9~16
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CJES
Caspian Journal of Environmental Sciences

[Research]

Total Flavonoids and Phenolics in *Catharanthus roseus* L. and *Ocimum sanctum* L. as Biomarkers of Urban Auto Pollution

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ABSTRACT
Present transplant study was carried out across Lucknow, the capital of Utter Pardesh using two medicinally important plants *viz.*, "*Catharanthus roseus* L." and "*Ocimum sanctum* L." to assess whether certain ecophysiological responses (change in total flavonoids and total phenolics) in these two plants may serve as the biomarkers of auto exhaust pollution. Samples were taken from five different sites which differed from each other in terms of the number and type of vehicles plying over and were loaded with different concentrations of air pollutants (such as, SO_2, NO_2, SPM, RSPM). During the study, it was observed that the concentration of the different air pollutants across the sites correlated with the number and type of vehicles. Total flavonoids and total phenolics in both plants increased by increasing pollution loads across the sites, hence, this validates their role as biomarkers of auto exhaust pollution.

Keywords: Total flavonoids, Total phenolics, Vehicular pollution.

INTRODUCTION
Air pollution, a global problem being faced by both the developed nations as well as the developing ones, has been aggravated by developments that typically occur as countries become industrialized: growing cities, increased traffic, rapid economic development and industrialization, and high levels of energy consumption. All these factors act as cause and effect for one another and act in a synergistic manner to befoul the sanctity of natural environment. According to study conducted during 1975-1995 to compare the rates of economic growth and the rates of growth of industrial pollution and the vehicular pollution projected that the Indian economy grew by 2.5 times, but the industrial pollution load grew by 3.47 times and the vehicular pollution load by 7.5 times (Kumar and Bhattacharya, 1999). Over the last three decades, motor vehicle numbers have been doubling every 10 or fewer years in many Asian countries as against a 2 - 5% annual growth rate in Canada, the United States, the United Kingdom and Japan (Faiz *et al*., 1992; Walsh, 1994). Useing automobiles is growing fast globally at large and with much greater pace in developing countries (Yunus *et al*., 1996). Currently, in India, air pollution is wide spread in urban areas where automobiles are the major contributors and a few other areas with a high concentration of industries and thermal power plants. Motor vehicles have been closely identified with increasing air pollution levels in urban centers of the world (Mage *et al*., 1996; Mayer, 1999) and are responsible for 60 to 70% of the pollution found in an urban environment (Singh *et al*., 1995). Depending upon the fuel type, the main exhaust emissions are oxides of nitrogen (NO_x), oxides of carbon, oxides of sulphur (SO_x), carbon particles, heavy metals, water vapor and hydrocarbons including aldehydes, single and poly aromatic hydrocarbons, alcohols, olefins, alkylnitriles

besides a number of secondary pollutants such as ozone, etc causing serious environmental and health impacts. (Pandey *et al*., 1999; Kammerbauer and Dick, 2000). It is estimated that vehicles account for 70% of CO, 50% of HC, 30-40% of NO_X, 30% of SPM and 10% of SO_2 of the total pollution load in the major metros of India, of which two thirds arecontributed by two wheelers alone (CSE,2001; CPCB, 2002). Besides the human and animal populations, this problem has drastic impacts on the local environment and cause extensive damage to vegetation including crops, fruit trees, medicinal plants and ornamentals. Plants being directly and constantly exposed (round the clock) to the pollutants (both gaseous and particulates) play a significant role as indicators and in mitigating the problem. They absorb, accumulate and integrate the pollutants impinging on their foliar surface, acting as the sinks for various pollutants and thus mitigating the problem. The plants don't render this service to the mankind without any serious implications; in turn they suffer from various deformities caused by the integrating pollutants and show diverse morphological, biochemical, anatomical and physiological responses. In this backdrop the present transplant study was planned using two medicinal plants namely "*Ocimum sanctum L." and "Catharanthus roseus L."* to asses whether certain ecophysiological responses (total flavonoids and phenolics) may be valid bioindicators of urban auto pollution.

MATERIALS AND METHODS

Lucknow (26o52/N latitude, 80o56/E longitude, 128 m above the sea level), the Capital of Uttar Pradesh, is spread over an area of 310 sq. km in the central plain of the Indian subcontinent, supporting a population of 36.48 lakh (Census, 2001). It has distinct tropical climate with a marked monsoonal effect. The year is divided into three distinct seasons; Summer (March to June), Rainy (July to October) and Winter (November to February). The temperature ranges from a minimum of 5^{o}C in winter to a maximum of 47^{o}C in summer. The mean average relative humidity is 60%, with a rainfall of 1006.8mm.

To conduct the aforesaid study, an extensive survey of Lucknow city was under taken to select the sites based on the number and type of vehicles plying over there. During the survey, five sites, which differ significantly in the number and type of the automobiles, were selected as:

Site I: Babasaheb Bhimrao Ambedkar University Campus (Vidya Vihar) Lucknow.
Sit II: Sardar Patel Institute of Dental and Medical Sciences, Lucknow (Lucknow - Raebarelly Road).
Sit III: Banthara Field Station of NBRI, Lucknow (Lucknow-Kanpur Road).
Sit IV: Central Institute of Unani Medicine (Lucknow -Kursi Road).
Sit V: Sikandar Bagh Crossing (NBRI Genetic Block), Ashok Marg, Lucknow (one of the busiest roads of Lucknow).

All the sites are quite different from each other as far as the number and type of vehicles plying through these sites are concerned. The Babasaheb Bhimrao Ambed -kar University Campus, Vidya Vihar (Site: I), an area of 250 acers, which is not open for thorough passes and has not developed the connecting roads, as it is a newly established campus of the university, has been marked as the 'control site' (least polluted) as the expected pollution levels were negligible, much below the threshold values (almost an healthy environment).

Air Quality Monitoring

At all the three sites, traffic density was recorded (between 9.00 AM to11.00 AM) between the peak traffic hours. Air monitoring was also carried out at all these sites and the parameters studied were SO_2, NO_2, RSPM and SPM. Monitoring of RSPM and SPM was carried out with the help of a high volume air sampler (Envirotech make-APM 460) and an attachment device (APM 411) with the high volume air sampler was used for the monitoring of gaseous pollutants. Air was allowed to bubble at a flow rate of 1.2 l/min in glass impingers with 30 ml of absorbing solutions i.e. Potassium tetrachloromercurate for SO_2 and basic Sodium arsenate for NO_2. The sampler was run for six hours (between 9.00 AM to3.00 PM) and the samples were brought to the laboratory for analysis as per the standard methods of West and Gaeke, (1956) and Jacobson and Hochheiser, (1958) respectively for SO_2 and NO_2.

Plant Transplantation

Seeds of both plants were procured from Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow and were sown in October, 2006 in 12' earthen pots (thirty each) filled with equal quantities of uniformly mixed farmyard manure and garden soil. These pots were kept for four weeks in the green house at the horticulture field station of Babasaheb Bhimrao Ambedkar University and then transferred to the study sites. One set of these plants (i.e.10 each) was kept at the Babasaheb Bhimrao Ambedkar University, main campus (LP), which served as the control (least polluted site).Same procedure was followed during the other two seasons (i.e., summer and rainy). During the study period, full care of the pots was taken and were uniformly watered. No synthetic fertilizer was applied during the study periods.

Sample Collection and Analysis

At the end of each season, the plants were harvested. The roots were cleaned off the adhering soil with mild water flush and were separated from the aerial parts. The leaves were also separated from the stem and used for the analysis. The leaves were wrapped in bloating paper and kept in an oven for drying at a temperature below 40^{O}C to avoid any degradation of the active principles. After drying, the material was ground to fine powder and analyzed. Total flavonoid content was determined as per the method described by Chang *et al.* (2002). Extracts of both plants, "*C. roseus* L." and "*O. sanctum* L." (0.5 ml of 1:10 mg ml^{-1}) in methanol from the respective sites, were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The mixture was kept at room temperature for 30 minutes and the absorbance was recorded at 415 nm with the help of SHIMADZU, UV-Visible spectrophotometer. The calibration curve was prepared using Routin as the standard and results were expressed as µg g^{-1}dw.

Total phenolic content was determined as per the method described by McDonald *et al.* (2001). Extracts of both plants (*C. roseus* L. and *O. sanctum* L.) (0.5 ml of 1:10 mg ml^{-1}) in methanol from the respective sites were mixed with Folin Ciocalteu (5 ml, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (4 ml, 1M). The mixtures were allowed to remain for 15 minutes and then absorbance was recorded at 765 nm with the help of SHIMADZU, UV-Visible spectrophotometer. Standard curve was prepared using Gallic acid as the reference. Total phenol values were expressed in terms of Gallic acid equivalent (µg g^{-1}dw).

Statistical Analysis

Data were statistically analyzed by two-way ANOVA to check the authenticity of the results using STATISTICA- 7.1 (Stat Soft) USA.

RESULTS AND DISCUSSION

Air Analysis

Marked variations were recorded in the traffic density across the five sites during the different seasons with site I recording the minimum number and site V the maximum (Fig. 1). Seasonal variations in the concentrations of SO_2, NO_2, RSPM and SPM were observed at all the five sites (Table 1). The results showed that concentrations of gaseous compounds were highly dynamic with significant seasonal variations characterized by high winter and low monsoon levels. The concentration of all the pollutants was in general more during winter followed by summer and least during the rainy season. During the winter months, increased atomspheric stability and less atmospheric circulation makes the air mass more stagnant. As a result, minimum atmospheric dispersion throughout the planetary boundary layer is observed. Moreover, the lack of precipitation during winter months reduces the potential for wet deposition and associated cleansing mechanisms. Conversely, during monsoons, low pollutant concentrations can be ascribed to precipitation driven washout (especially for SO_2 and NO_2). Monsoon rains have the most dramatic effect in lowering the gaseous pollutant levels in the atmosphere. Despite low solubility of oxides of nitrogen in water, rains in the monsoon season effectively reduce their concentrations in the air. Gupta *et al*. (2007), have observed similar situation under Kolkata conditions. During all the three seasons site V recorded maximum concentration of SO_2, NO_2, RSPM and SPM,

decreasing significantly down the sites with site I registering minimum values, which may be attributed to varying vehicular frequency. Singh *et al*., 1995; Jafary and Faridi, 2006; Verma and Singh, 2006; Gupta *et al*., 2007 and Joshi and Swami (2007) too have reported similar relation between pollution level and vehicular frequency.

In addition to the different meteorological conditions and traffic volumes at the different sites, the concentration of the gaseous and particulate pollutants are associated with some other conditions as a high rate of abrasion in vehicular engines and greater emission of particulates including dust by slow running vehicles over the fast ones, addition pollution produced by vehicles halting at traffic signals and less favorable conditions of ventilation and dispersion of pollutants in areas surrounded by high buildings. Mandavilli *et al*., 2007 and Verma and Singh, 2006 have also attributed the increase in pollution levels to these factors.

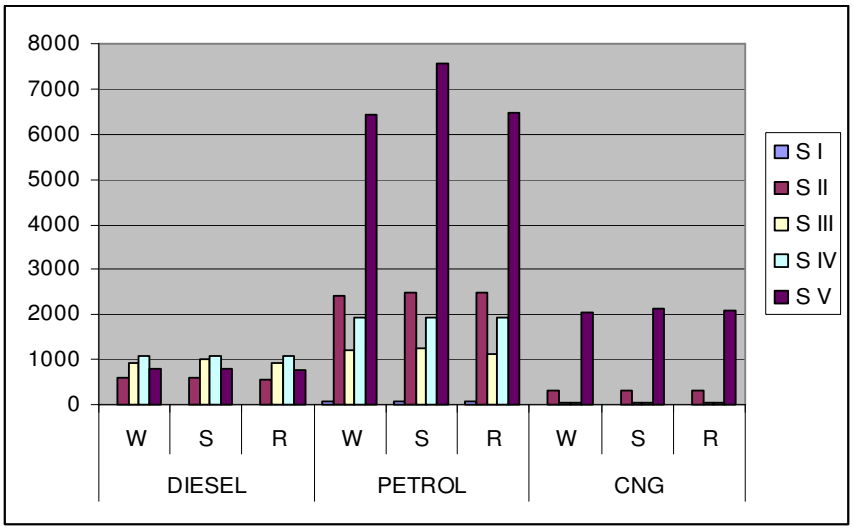

Fig 1. Season wise traffic density at the study sites during peak traffic hours (between 9.00 AM to 11.00 AM).

Table 1. Concentrations (μg m^{-3}) of different air pollutants at five sites during different seasons.

Parameter	Season	Site				
		I	II	III	IV	V
NO_2	W	9.50 ± 6.51^{d}	23.34 ± 2.76^{c}	32.73 ± 2.69^{b}	36.59 ± 9.56^{b}	55.86 ± 2.76^{a}
	S	7.09 ± 4.55^{c}	13.71 ± 2.76^{bc}	18.53 ± 6.51b^{c}	22.14 ± 3.13^{b}	39.18 ± 6.10^{a}
	R	0.76 ± 1.31^{b}	8.29 ± 4.17^{ba}	12.51 ± 3.76^{a}	14.91 ± 4.78^{a}	19.13 ± 2.09^{a}
SO_2	W	8.57 ± 3.22^{c}	20.14 ± 4.28^{b}	22.35 ± 4.35^{b}	26.24 ± 3.59^{b}	39.28 ± 3.51^{a}
	S	6.36 ± 3.16^{c}	16.67 ± 1.74^{b}	17.62 ± 1.49^{b}	19.93 ± 4.78^{b}	30.34 ± 3.18^{a}
	R	4.47 ± 2.10^{d}	10.67 ± 3.46^{bcd}	13.41 ± 1.38^{ac}	13.72 ± 6.98^{ab}	22.24 ± 3.01^{a}
RSPM	W	89.96 ± 10.34^{c}	213.08 ± 27.99^{b}	291.35 ± 71.86^{b}	292.85 ± 13.77^{b}	401.22 ± 76.84^{a}
	S	86.87 ± 7.36^{c}	222.27 ± 43.53^{b}	246.38 ± 32.26^{b}	253.05 ± 42.67^{b}	369.49 ± 40.04^{a}
	R	77.73 ± 11.61^{b}	251.48 ± 52.47^{a}	254.94 ± 44.11^{a}	247.35 ±63.50^{a}	332.80 ±39.06^{a}
SPM	W	153.45 ± 11.83^{d}	439.23 ± 38.90^{c}	498.80 ± 35.81^{c}	572.91 ± 30.48^{b}	744.98 ± 49.09^{a}
	S	115.26 ± 17.85^{c}	313.59 ± 28.10^{b}	319.26 ± 23.60^{b}	342.86 ± 29.64^{b}	564.98 ± 19.43^{a}
	R	135.53 ± 22.95^{c}	370.56 ± 34.78^{b}	345.91 ± 72.23^{b}	340.35 ± 34.14^{b}	454.22 ± 52.41^{a}

Values in rows followed by same superscript are not significantly different at $p<0.05$ according to Newman Kuels test.

Total Flavonoids

Vehicular pollution resulted in significant increase in the total flavonoid content of both plants during different seasons (Table 2). In both plants, site I recorded the lowest values of total flavonoids during all seasons, which increased significantly along the sites attaining maximum values at site V. In case of "*C. roseus*" an increase of 38.8, 29.0 and 10.0% was recorded at site V in comparison to site I during winter, summer and rainy seasons, respectively.

However, the effect was maximum on "*O. sanctum*" registering an increase of 42.6, 30.7 and 11.0%, respectively, during winter, summer and rainy seasons at site V in comparison to site I. This increase in the total flavonoid content may be attributed to vehicular pollution. These findings are in agreement with the findings of Nikolova and Ivancheva (2005), who have reported increase in total flavonoids in "*Artemisia vulgaris* L." and "*Veronica chamaedrys* L." in relation to air pollution stress. Enhancement of phenolic compounds and flavonoids as a result of pollution impact has been observed in "*Betula pubescens*" and white birch leaves (Loponen, *et al*., 1997; 1998).

Table 2. Total Flavonoid content (µg g^{-1} dw) of *Catharanthus roseus* L. and *Ocimum sanctum* L. at five sites during different seasons.

Plant	Season	Site I	Site II	Site III	Site IV	Site V
C. roseus	W	123.26 ± 1.13^{e}	142.85 ± 1.19^{d} (-16.0)	154.18 ± 1.13^{c} (-26.0)	162.77 ±1.22^{b} (-32.1)	171.03 ± 1.15^{a} (-38.8)
	S	115.01 ± 1.32^{e}	130.72 ± 1.34^{d} (-13.7)	140.18 ± 1.11^{c} (-22.0)	145.68 ±1.14^{b} (-26.7)	148.31 ± 1.31^{a} (-29.0)
	R	117.18 ± 1.42^{d}	120.84 ± 1.55^{c} (-3.1)	123.01 ± 1.59^{b} (-5.0)	127.34 ±1.18^{a} (-8.7)	128.83 ± 1.41^{a} (-10.0)
O. sanctum	W	230.15 ± 1.23^{e}	263.53 ± 1.19^{d} (-14.5)	282.36 ± 1.10^{c} (-22.7)	308.39 ±1.06^{b} (-34.0)	328.27 ± 1.19^{a} (-42.6)
	S	214.47 ± 1.01^{c}	234.00 ± 0.80^{d} (-9.1)	255.29 ± 0.91^{c} (-19.0)	264.08 ±0.90^{b} (-23.1)	280.25 ± 1.05^{a} (-30.7)
	R	212.05 ± 1.54^{d}	222.87 ± 1.27^{c} (-5.1)	226.67 ± 1.19^{b} (-7.0)	234.70 ±1.11^{a} (-10.7)	235.15 ± 1.11^{a} (-11.0)

Values in rows followed by same superscript are not significantly different at p<0.05 according to Newman Kuels test. Values in parenthesis represent percentile variation.

Table 3. Total Phenolic content (µg g^{-1} dw) of *Catharanthus roseus* L. and *Ocimum sanctum* L. at five sites during different seasons.

Plant	Season	Site I	Site II	Site III	Site IV	Site V
C. roseus	W	97.09 ± 0.94^{e}	101.02 ± 1.06^{d} (-8.8)	106.77 ± 0.99^{c} (-13.8)	112.99 ± 1.26^{b} (-18.4)	123.83 ± 1.29^{a} (-21.6)
	S	90.94 ± 1.11^{d}	98.02 ± 1.09^{c} (-7.3)	100.70 ± 1.23^{b} (-9.0)	102.44 ± 1.09^{b} (-11.3)	110.50 ± 1.05^{a} (-17.7)
	R	94.93 ± 1.12^{c}	96.92 ± 1.07^{b} (-2.6)	98.94 ± 1.15^{ab} (-2.4)	98.65 ± 1.13^{b} (-4.4)	101.33 ± 1.10^{a} (-6.3)
O. sanctum	W	105.97 ± 1.44^{e}	142.55 ± 1.30^{d} (-13.6)	171.68 ± 1.38^{c} (-27.1)	203.34 ± 1.31^{b} (-39.5)	235.46 ± 1.50^{a} (-55.0)
	S	122.47 ± 0.78^{e}	137.71 ± 0.67^{d} (-10.3)	168.12 ± 0.84^{c} (-19.7)	187.64 ± 0.76^{b} (-34.2)	209.26 ± 0.77^{a} (-41.5)
	R	184.13 ± 1.49^{c}	180.20 ± 0.86^{d} (3.1)	184.78 ±1.26^{c} (-7.0)	192.64 ± 1070^{b} (-9.3)	198.74 ± 1.55^{a} (-7.4)

Values in rows followed by same superscript are not significantly different at p<0.05 according to Newman Kuels test. Values in parenthesis represent percentile variation.

Total Phenolics

Significant increase in the total phenolic content of both plants was experienced in response to the varying pollution load during different seasons (Table 3). In both the plant species, site I recorded lowest values of total phenolics during all the seasons, which increased significantly along the sites attaining maximum values at site V. In case of "*C. roseus*" an increase of 21.6, 17.7 and 6.3% was recorded at site V in comparison to site I during winter, summer and rainy seasons, respectively. However, the effect was maximum on "*O. sanctum*" registering an increase of 55.0, 41.5 and 7.4%, respectively during winter, summer and rainy seasons at site V in comparison to site I. This increase in the total phenolic content may be attributed to vehicular pollution. Air pollution can induce qualitative and quantitative changes in secondary metabolite composition (Zobel, 1996; Kanoun *et al.*, 2001; Lopanen *et al.*, 2001). An increase of the phenolic compound level has been observed after the exposure of plants to several toxic pollutants (Giertych *et al.*, 1999). Kanoun *et al.* (2001) have reported a significant change of foliar phenolic accumulation in chronically stressed individuals of "*Phaseolus vulgaris* cv." Nerina due to ozone fumigation. Pasqualini *et al* (2003) have similarly shown the change in foliar phenol concentration in Aleppo pine (*Pinus halepensis* Mill.) needles exposed to various atmospheric pollutants, which increased with exposure to SO_2 but reduced with exposure to Nitrogen oxide pollution. This negative correlation between nitrogen oxides and total phenolics may be explained by the positive impact of these pollutants on the activity of nitrate reductase (Krywult *et al.*, 1996). This enzyme promotes nitrogen assimilation and several studies have shown negative correlations between nitrogen and phenolic compound concentrations in needles or leaves of various "*Pinus*" species (Giertych *et al.*, 1999). Agrawal and Deepak (2003) reported significant increase in total phenolics of two wheat cultivars (*Triticum aestivum* L. cv. Malviya 234 and HP1209) under CO_2 and CO_2 + SO_2 exposures. Stimulation of total phenolics due to pollutant exposure has also been reported earlier (Howell, 1974). Accumulation of phenolics in leaves may reduce carbon fixation and ATP synthesis, and may stimulate the respiration and disintegration of chloroplasts (Howell, 1974). Rai *et al.* (2007) have also reported significant increase in the total phenolic contents in wheat (*Triticum aestivum* L.) under air pollution stress.

CONCLUSION

Changes in concentrations of total flavonoids and phenolics in "*Catharanthus roseus* L." and "*Ocimum sanctum* L." may serve as biomarkers of urban auto pollution as both the parameters showed a positive relationship with the vehicular pollution load across the different sites.

REFERENCES

Agrawal, M. and Deepak, S.S. (2003) Physiological and biochemical response of two cultivars of wheat to elevated levels of CO_2 and SO_2 singly and in combination. *Environ. Pollut.* **121**, 189-197.

Census, (2001) Dissemination of Census - 2001 results by Directorate of Census Operation, (U.P.), Lucknow. One day workshop at BBAU Lucknow on 4th Jan. 2008.

Chang, C., Yang, M., Wen, H. and Chern, J. (2002) Estimation of total flavonoid content in propolis by two complementry colorimetric methods. *J. Food Drug Analysis.* **10**, 178-182.

Centre for Science and Environment (2001) 'A Special Presentation by CSE.' To the Committee on Auto Fuel Policy (Mashelkar Committee), New Delhi.

Central Pollution Control Board (2002) 'Benzene in Air and its Effects on Human Health. Parivesh Newsletter. CPCB Ministry of Environment and Forests, New Delhi, Government of India.

Faiz, A., Weaver, C., Sinha, K., Walsh, M. and Carbajo, J. (1992) Air pollution from motor vehicles: issues and options for developing countries. The World Bank, Washington, DC, pp. 280.

Giertych, M.J., Karolewski, P. and De Temmerman, L.O. (1999) Foliage age and pollution alter content of phenolic compounds and chemical elements in *Pinus nigra* needles. *Water, Air and Soil Pollution*. **110**, 363-377.

Gupta, A.K., Karar, K., Ayoob, S. and John, K. (2007) Spatio-temporal characteristics of gaseous and particulate pollutants in an urban region of Kolkata, India. *Atmos.Res.*

doi: 10.1016/*j.atmosres*. **2007**, 07-008.

Howell, R.K. (1974) Phenols, ozone and their involvement in the physiology of plant injury. In: M. Dugger (Ed.), Air Pollution Related to Plant Growth, A.C.S. Symposium Series 3. Washington D C, pp. 94-105.

Jacobs, M.B. and Hochheiser, S. (1958) Continuous sampling and ultra micro determination of nitrogen dioxide in air. *Anal. Chem.* **30**, 426-428.

Jafary, Z.A. and Faridi, I.A. (2006) Air pollution by roadside dust and automobile exhaust at busy road-crossings of Lahore. *Pak J. Physiol*. **2**, 31-34.

Joshi, P.C. and Swami, A. (2007) Physiological response of some tree species under roadside automobile pollution stress around city of Haridwar, India. *Environmentalist.* 27, 365-374.

Kammerbauer, J. and Dick, T. (2000) Monitoring of urban traffic emission using some physiological indicators in *Ricinus comm.-unis* L. Plants. *Environ. Cont. and Toxicol*. **39**, 161-166.

Kanoun, M., Goulas, M.J.P.and Biolley, J.P. (2001) Effect of chronic and moderate ozone pollution on the phenolic pattern of bean leaves (*Phaseolus vulgaris* L. cv. Nerina): relations with visible injury and biomass production. *Biochem. Syst. Ecol*. **29**, 443-457.

Krywult, M., Karolak, A. and Bytnerowicz, A. (1996) Nitrate reductase activity as an indicator of ponderosa pine response to atmospheric nitrogen deposition in the San Bernardino Mountains. *Environ. Pollut*. **93**, 141-146.

Kumar, P. and Bhathacharya, S. (1999) *Down to Earth*. **7**, 32-40.

Loponen, J., Lempa, K., Ossipov, V., Kozlov, M.V., Girs, A., Hangasmaa, K., Haukioja, E., Pihlaja, K. (2001) Patterns in content of phenolic compounds in leaves of mountains birches along a strong pollution gradient. *Chemosphere*. **45**, 291-301.

Loponen, J., Ossipov, V., Koricheva, J., Haukioja, E. and Pilhlaja, K. (1997) Low molecular mass phenolics in foliage of *Betula pubescens* Ehrh. in relation to aerial pollution. *Chemosphere*. **34**, 687-697.

Loponen, J., Ossipo, V., Lempa, K., Haukioja, E. and Pilhlaja, K. (1998) Concentrations and among-compound correlation of individual phenols in white birch leaves under air pollution stress. *Chemosphere*. **37**, 1445-1456.

Mage, D., Ozolins, G., Peterson, Y., Webster, A., Orthofer, R., Vandeweerd, R. and Gwyane, R. (1996) Urban Air Pollution in Mega City of the World. *Atmospheric Environment*. **30**, 681-686.

Mandavilli, S., Rys, M.J. and Russell, E.R. (2007) Environmental impact of modern roundabouts. *Int. J. Ind. Ergonomics* doi: 10/*j. ergon*. **2006**, 11-003.

McDonald, S., Prenzler, P.D., Autolovich, M. and Robards, K. (2001) Phenolic content and antioxident activity of olive extracts. *Food Chemistry*. **73**, 73-78.

Mayer, H. (1999) 'Air Pollution in Cities.' *Atmospheric Environment.* **33**, 4029-4037.

Nikolova, M.T. and Ivancheva, S.V. (2005) Quantitative flavonoid variations of *Artemisia vulgaris* L. and *Veronica chamaedrys* L. in relation to altitude and polluted environment. *Acta. Biol. Szeged*. **49**(3-4), 29-32.

Pandey, V., Kumar, A., Pal, A., Singh, N. and Yunus, M. (1999) Status of ambient air quality in Lucknow city. *Ind. J. Env. Prot.* **19**, 181-184.

Pasqualini, V., Robles, C., Garzino, S. Greff, S., Bousquet-Melou, A. and Bonin, G. (2003) Phenolic compounds content in *Pinus halepensis* Mill. needles: a bioindicator of air pollution. *Chemosphere.* **52**, 239-248.

Rai, R., Agrawal, M. and Agrawal, S.B. (2007) Assessment of yield losses in tropical wheat using open top chambers. *Atmospheric Research*. **41**, 9543-9554.

Singh, N., Yunus, M. Srivastava, K., Singh, S. N., Pandey, V., Misra, J. and Ahmad, K. J. (1995) Monitiring of auto exhaust pollution by road side plants. *Environmental Monitoring and Assessment.* **34**, 13-25.

Verma, A. and Singh, S.N. (2006) Biochemical and ultra structural changes in plant foliage exposed to automobile pollution. *Environmental Monitoring and Assessment.* **120**, 585-602.

Walsh, M.P. (1994) Trends in automotive transport: implications for environmental protection, energy efficiency and sustainnable development. Paper presented at the International Conference Toward Clean Transport: Fuel Efficient and Clean Motor

Vehicles, Mexico City, March, pp. 28-30.

West, P.W. and Gaeke, G.C. 1956. Fixation of sulfur dioxide as sulfitomercurate (II) and subsequent colorimetric estimation. *Anal. Chem.* **28**, 1816-1819.

Yunus, M., Singh, N. and Iqbal, M. (1996) Global status of air pollution: An overview. In Plant Response to Air Pollution. Yunus, M. and Iqbal, M. (Eds.), John Wiley and Sons. pp. 1-34.

Zobel, A.M. (1996) Phenolic compounds in defense against air pollution. In: Yunus, M., Iqbal, M. (Eds.), Plant Response to Air Pollution. John Wiley, Chichester, pp. 241-266.

(Received: Sep. 10-2008, Accepted: May. 17-2009)