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OZONE BIOMONITORING IN PAKISTAN USING TOBACCO CULTIVAR BEL-W3

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Abstract

The present study depicts a comparison of ozone (O_3) concentrations over a decade time (1993-94 to 2006) using plant biomonitoring and continuous ozone monitors techniques in Lahore city of Pakistan. The variations in O_3 levels were assessed at city centre, suburbs and semi-rural/rural locations in and around the city of Lahore by using American O_3-sensitive tobacco biomonitor plant (*Nicotiana tabaccum* L. cv. Bel-W3) for the first time in Pakistan during 1993 and 1994 seasons through weekly assessment of visible damage to leaves. Results for both 1993 and 1994 seasons indicated significant differences between sites in the mean 6-h O_3 concentrations with a range of over 20 ppb and 15 ppb across the sites in 1993 and 1994, respectively. An inverse relationship between the levels of NO_2 and O_3 was found during investigation. The highest O_3 levels of 75-80 ppb were found at rural areas and the lowest at city centre sites. The extent of O_3 injury on the tobacco cv. Bel-W3 leaves reflected the trends seen in O_3 concentrations. The highest and lowest leaf injury indices of 18-27% and 5-7% occurred at the rural and city centre sites, respectively. Results for 2006 season indicated the highest seasonal mean O_3 concentration of 100 ppb in semi-rural areas compared with city centre sites (68 ppb). The highest 26% and 20% increase in O_3 levels was observed at rural/semi-rural and city centre sites, respectively when compared with 1993 O_3 survey. Application of O_3 biomonitoring technique proved very cost-effective and feasible for the estimation of atmospheric O_3 levels in South East Asian regions like Pakistan where shortage of electric supply, trained man power and poverty is already playing havoc.

Introduction

Ambient O_3 is produced by photochemical reactions of its precursors like hydrocarbons (HCs) and oxides of nitrogen (NOx). Industrialization and the growing number of motor vehicles are responsible for increased emissions of these precursors. Furthermore, O_3 producing photochemical reactions are favoured by high temperatures and high light intensities with relatively slow winds (Wahid *et al*., 2001; Nali *et al*., 2006). O_3 being a secondary air pollutant is generated photochemically by the action of light on NOx and reactive HCs and contribute strongly to the oxidizing ability of the atmosphere (Wahid, 2006a). Field studies have revealed that pollutants generated at urban-industrial locations may travel 50-1000 km from the point of origin covering vast tracts in the direction of the prevailing level of atmospheric turbulence and penetrate deep into rural areas with consequent risk to crops and wild vegetation (Emberson *et al*., 2009).

In Pakistan, Ghauri *et al*., (1994) reported maximum O_3 around noon at the inland sites of the Karachi city, which reached the levels of 40 and 50 ppb compared to 25 ppb at the upwind coastal site. Wahid *et al*., (1995 a, b) reported weekly mean O_3 levels (6 h/day) of 25-45 and 30-55 ppb during wheat and rice growth seasons, respectively in the city of Lahore, Pakistan. Wahid (2006a, b) reported 72 ppb seasonal mean of O_3 concentration (8-h daily mean) at a semi-urban site in Lahore, with a peak of 100 ppb in the month of April 2004.

Ambient O_3 is a serious phyto-toxicant (Bignal *et al*., 2007) and its toxicity symptoms are characterized by chlorotic flecking, mottling, bronzing, bleaching and darkening of leaves in a range of plant species (Taylor *et al*., 1987; Zhao *et al*., 2011). Bel-W3 tobacco cultivar, because of its extreme sensitivity to atmospheric O_3 is an ideal biological indicator plant and often used for O_3 biomonitoring in USA, UK, Germany, The Netherlands, Denmark, Sweden, Italy, Spain and various other cities in the Europe (Nali & Lorenzini 2007; De Souza *et al*., 2009). In the present study, extensive experimentations were carried out for the assessment and location-wise distribution of O_3 levels in and around the city of Lahore, Pakistan to generate a base line data for this important secondary pollutant using biological monitor along with ozone analyzers. The main objective of the investigation was to establish the low-cost biomonitoring technique in the South Asia region using tobacco plants (cv. Bel-W3) as O_3 bioindicator. This is the first ever ozone biomonitoring study using tobacco cv. Bel-W3 carried out in the Indian-subcontinent and presents profile of O_3 over a period of 13 years (1993-2006) in Lahore, which is the capital city of Punjab province of Pakistan.

Materials and Methods

Site selection: The distribution of O_3 was assessed three times during the study viz., April-June 1993 (survey-I); March-mid-June 1994 (survey-II); March-September 2006 (survey-III) in Lahore covering city center, suburban, semi-rural and rural areas. Site selection details in three surveys are summarized in Table (1).

Experimental set up: O_3 assessments were made at weekly intervals by ozone analyzer (Model 8810, Monitor Labs Inc., US-Environmental Protection Agency) over the period 1000-1600 hours and ozone biomonitoring was carried out using an ozone sensitive American tobacco cultivar Bel-W3 (Ashmore *et al*., 1980). In addition, measurement of NO_2 was also made at all sites using diffusion tubes (Atkins *et al*., 1978) to aid interpretation of the data obtained. The degree of injury on the tobacco Bel-W3 plants was also assessed at weekly intervals. In the first two surveys of 1993 and 1994, both techniques (ozone continuous monitor and ozone biomonitor) were used while in the final survey in 2006 only continuous O_3 analyzer was used.

Table 1. Site detail for ozone monitoring during survey-I (1993), survey-II (1994) and survey-III (2006).

Map ref. No.	Site name	Sites included in			Site class
		1993 (I)	1994 (II)	2006 (III)	
1	+Shahdara Town	No	No	Yes	Semi-rural
2	*Village Batth	Yes	No	No	Rural agricultural
3	*Rakh Dera Chahal	Yes	Yes	No	Rural agricultural
4	*Engg. University	Yes	Yes	Yes	Inner suburbs of walled city
5	*Rang Mahal	Yes	No	Yes	Old city center (Walled city)
6	*Balal Ganj	Yes	No	Yes	Inner suburbs of walled city
7	+Taj Bagh colony	No	No	Yes	Semi-rural
8	*Cantonment	Yes	Yes	Yes	Modern outer suburbs
9	+Samanabad	No	No	Yes	Modern outer suburbs
10	+Garrison Academy, Cantt.	No	No	Yes	Modern outer suburbs
11	*Punjab University	Yes	Yes	Yes	Modern outer suburbs
12	+Model Town	No	No	Yes	Modern outer suburbs
13	+WAPDA Town	No	No	Yes	Semi-rural
14	+Green Town	No	No	Yes	Semi-rural

* O_3 biomonitoring was carried out using tobacco plant cv. Bel-W3
\+ O_3 analyzers were used only

Biological monitoring: In search of a valuable indigenous bioindicator for O_3, certified seeds of 15 commercial cultivars of tobacco (*Nicotiana tabaccum* L.) were obtained from the office of Pakistan Tobacco Board, Lahore and sent to Imperial College, London for screening their sensitivities to O_3 and comparing them with the American tobacco cultivar Bel-W3 (sensitive) and Bel-B (resistant). From the results (data not shown), it was decided that the local tobacco cultivar KHG-11 should be used as a resistant one to compare its behavior with that of very sensitive cultivar Bel-W3 in the field experiments. Seeds of this cultivar were provided by Department of Pure & Applied Biology, Imperial College London, United Kingdom. Tobacco seedlings were raised in 18 cm diameter earthen pots containing soil, cow dung, humus and sand in a ratio of 50: 30: 15: 05, respectively. Tobacco biomonitors (*Nicotiana tabaccum* L.) cv. Bel-W3 were exposed at experimental sites enclosing them with $1.2m^3$ expanded metal cages which were covered with green coloured garden netting for protection from high light intensity, and pests etc. Biological monitoring of O_3 was carried out with tobacco plant *Nicotiana tabaccum* L. cv. Bel-W3 in two consecutive growth seasons of 1993 and 1994, respectively. Table (2) shows the schedule for various events of O_3 biomonitoring experiments in 1993 and 1994 seasons. Five plants of each cultivar were exposed at each site per batch in both surveys. Leaf injury assessments were made as percentage flecking occurring on each leaf of each of the plants on three occasions. When exposed to concentrations of O_3 above the threshold value for injury, characteristic O_3 flecking on the leaves of tobacco cultivar Bel-W3 occurred. The occurrence of this flecking was used to determine periods of high levels of O_3, through both the intensity and coloration of flecks produced.

Table 2. Schedule for O_3 biomonitoring with tobacco cultivar Bel-W3 in and around the city of Lahore during 1993 and 1994 seasons.

Events	1993			1994					
	Batches								
	A	B	C	A	B	C	D	E	F
Sowing of seeds	15.02.93	02.03.93	17.03.93	11.01.94	25.01.94	08.02.94	22.02.94	08.03.94	22.03.94
Germination of seeds	25.02.93	12.03.93	27.03.93	21.01.94	04.02.94	18.02.94	04.03.94	18.03.94	01.04.94
Transplantation of seedlings	11.03.93	27.03.93	11.04.93	05.02.94	19.02.94	05.03.94	19.03.94	02.04.94	16.04.94
Shifting in the field and first assessment	22.04.93	08.05.93	22.05.93	19.03.94	02.04.94	16.04.94	30.04.94	14.05.94	28.05.94
Second assessment	29.04.93	15.05.93	30.05.93	26.03.94	09.04.94	23.04.94	07.05.94	21.05.94	04.06.94
Third assessment	08.05.93	22.05.93	06.06.93	02.04.94	16.04.94	30.04.94	14.05.94	28.05.94	11.06.94

Symptomology: After penetrating the foliage via the stomatal pores, O_3 attacks some component of the palisade cells which subsequently collapse and dry out resulting in the formation of dead tissues. These usually become visible to the naked eyes as bleached necrotic lesions, forming a stipple pattern on the upper surface of the leaf. Within about 8 days from the start of the exposure to ambient air, the lesions assumed a whitish colour. With continued exposure, the flecks grew in size and number and covered a progressive area of the injured leaves, ultimately affecting about whole blade of the leaf. New leaves which grew meanwhile were also injured, and the extensively injured older leaves reached early senescence and dropped. The percentage of the surface area of each leaf covered by O_3-induced injury was assessed visually with the help of a Standard Spotting Density Card (card not shown) provided by Imperial College, London.

All the seven damage categories were observed (Fig. 1) on leaves of sensitive tobacco cv. Bel-W3 during this investigation on the pattern similar to that of Standard Spotting Density Card. From respective categories, the percent surface area injured of each leaf was estimated subsequently and changes in the percent leaf injury for each time period were then used to calculate leaf injury index (LII) similar to that used by Ashmore *et al*., (1980).

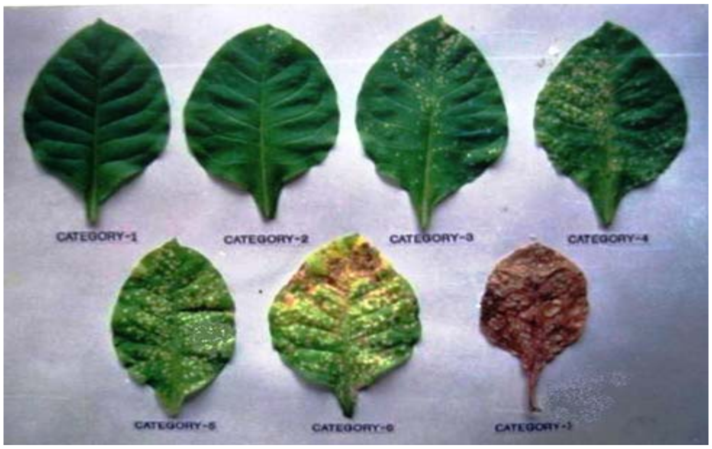

Fig. 1. Leaves of tobacco cultivar Bel-W3 showing ozone damage categories after exposure of plants to the field conditions of Lahore, Pakistan.

Results

O_3 monitoring with analyzers: The temporal variation in O_3 concentrations during surveys of 1993, 1994 and 2006 are summarized in figures (2a, 2b & 3), respectively. In each year considerable differences were found among site classes in the mean 6-hour O_3 concentrations. In 1993 season, the lowest 6-hour mean O_3 concentration in the range 49-57 ppb was found at city centre area compared to 55-67 ppb and 62-79 ppb at suburban and rural areas respectively, throughout the season (Fig. 2a). Similar trends were observed in 1994 season with lowest 6-hour mean O_3 concentrations range of 12-58 ppb at city centre area compared to 14-69 and 20-80 ppb at suburban and rural areas, respectively (Fig. 2b). Reduction in O_3 concentration at initial stages of 1994 season may be attributed to lower mid-day temperature recorded on those days.

Results of 1993 and 1994 surveys showed a considerable impact of sites on O_3 levels in and around the city. Therefore in 2006, further classification of sites was carried out as city centre, inner suburbs, outer suburbs and semi-rural areas to confirm this trend and for better understanding of recent results. Figure 3 showed the results of weekly O_3 and NO_2 monitoring at various site classes during 2006. O_3 survey of 2006 confirmed the trends observed in 1993 and 1994 surveys. The lowest 6-hour mean O_3 concentration of 55-76 ppb was found at city centre areas compared to 61-83, 72-91 and 83-107 ppb at inner suburbs, outer suburbs and semi-rural areas, respectively. A consistent relationship between levels of O_3 and levels of NO_2 monitored at each site was apparent, with an inverse relationship existing between the two gases. This can be explained, in part, by the scavenging reaction between O_3 and NO: $NO+O_3 \Leftrightarrow NO_2+O_2$.

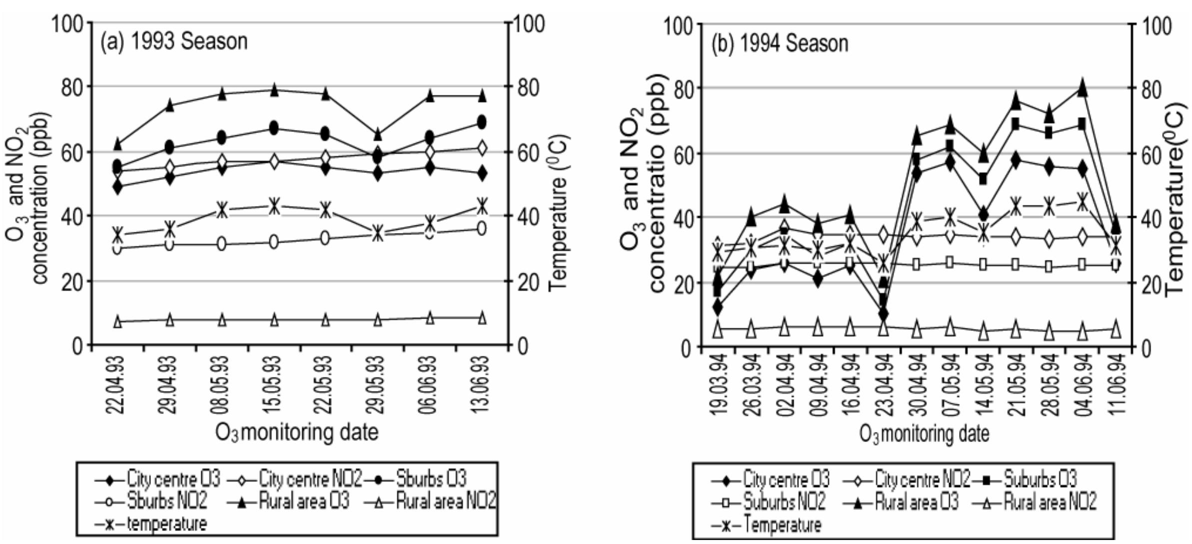

Fig. 2. Class-wise chemical monitoring of O_3 and NO_2 levels in and around the city of Lahore during survey-I (a) and survey-II (b).

Thus in the city centre, where NO emissions are high, substantial amount of the O_3 reacts with NO giving a corresponding increase in NO_2 levels. This relationship between O_3 and NO_2 was found in all the three surveys of 1993, 1994 and 2006. There is a substantial variation from week to week in O_3 levels in three years, much of which can be explained by the corresponding variations in temperatures (Figs. 2a, 2b & 3). In each year the lowest

O_3 concentration coincides with the lowest mid-day temperatures. This is consistent with the current understanding that O_3 concentration will be highest on days of high temperatures. The formation of O_3, however, is also dependent upon light intensity. This fact is more apparent from results in 1994 and 2006, which cover a longer period extending over March and April through mid-June and September, respectively.

Results from 1993 survey clearly show less variation in O_3 levels at urban sites when compared to rural sites; excluding the first week of observations, the range was 14 ppb at rural sites, 9 ppb at suburban sites but only 5 ppb at city centre sites (Fig. 2a). The range of values seen in 1994 was considerably greater than those seen in 1993 (Fig. 3b) due to wider range of temperatures recorded during the period of study, but the trend was still the same. The range for rural sites was 58 ppb, whilst those for suburban and city centre sites were 52 and 46 ppb, respectively. In the recent survey of 2006, the range was 18-22 ppb at city centre and suburbs except semi-rural sites where it was 26 ppb (Fig. 3). Overall, less variation in the O_3 levels was found at all site classes located in and around the city of Lahore, throughout the monitoring duration 2006. This trend confirmed the substantial increase in O_3 levels throughout the city of Lahore compared to previous two surveys carried out during 1993 and 1994 seasons.

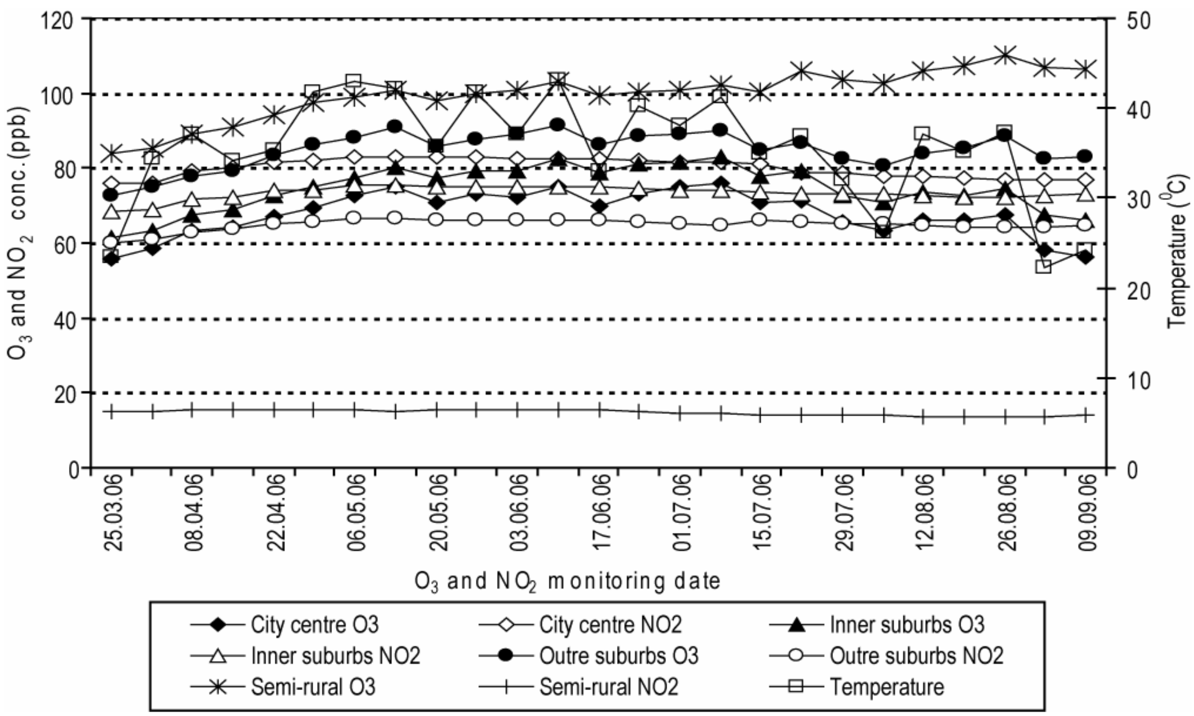

Fig. 3. Class-wise chemical monitoring of O_3 and NO_2 levels in and around the city of Lahore during 2006 (survey-III.).

Increase in O_3 levels from 1993 to 2006: The highest 26% increase in O_3 levels was observed at rural/semi-rural sites compared to city centre and outer suburbs where 20% increase was found in O_3 levels. Inner suburbs lie in between these two extremes with 22% increase in O_3 levels (Table 3). This higher increase in O_3 levels compared to outer suburbs (20 ppb) may be attributed to higher NO_2 levels (73 ppb), HCs and temperature occurrence in the immediate vicinity of city centre. An opposite trend of gradual decline in NO_2 levels from city centre outwards was also observed during both surveys compared to O_3 levels trend which showed gradual increase on the same transects from city centre to rural sites.

Table 3. Class-wise seasonal means and increase (%) in O_3 and NO_2 levels during 2006 survey compared to 1993 survey carried out in and around the city of Lahore.

Site class	1993 Season (survey-I)		2006 Season (Survey-III)		Percent increase during 2006 season relative to 1993 season	
	*O_3 (ppb)	**NO_2 (ppb)	*O_3 (ppb)	**NO_2 (ppb)	*O_3 (ppb)	**NO_2 (ppb)
City center	54.00	57.30	67.76	80.10	20.30	28.50
Inner suburbs	57.80	37.26	74.50	73.32	22.42	49.20
Outer suburbs	68.10	27.10	84.77	64.84	19.70	58.20
Rural/Semi-rural	73.94	7.73	99.76	14.70	25.88	47.40

* Weekly means of 6hrs/day (1000hrs-1600hrs)

** Weekly means during experimental season

Biological monitoring: Site-wise seasonal LII values (%) for the periods of study in the respective seasons of 1993 and 1994 are shown in figures 4a and 4b, respectively. Results of biomonitoring in 1993 and 1994 followed similar trends to those of the O_3 monitoring with analyzers. In both the years, the lowest leaf injury was found at city centre sites whilst the highest was found at sites in rural areas. In 1993, Leaf Injury Index values at rural sites of Rakh Dera Chahl and Village Batth were 26% and 20%, respectively compared to sub-urban and city centre values of 19% and 5-7%, respectively (Fig. 4a). In 1994, results of the limited four site survey confirmed the previous year's observations. The highest leaf injury index, 18.1% was found at rural location of Rakh Dera Chahl whilst the lowest, 2.8%, occurred at the University of Engineering and Technology situated close to the city centre. The LII value at the sub-urban areas of Cantonment and Punjab University Botany Garden was in between the two, at about 6-7% and 16.0%, respectively (Fig. 3b).

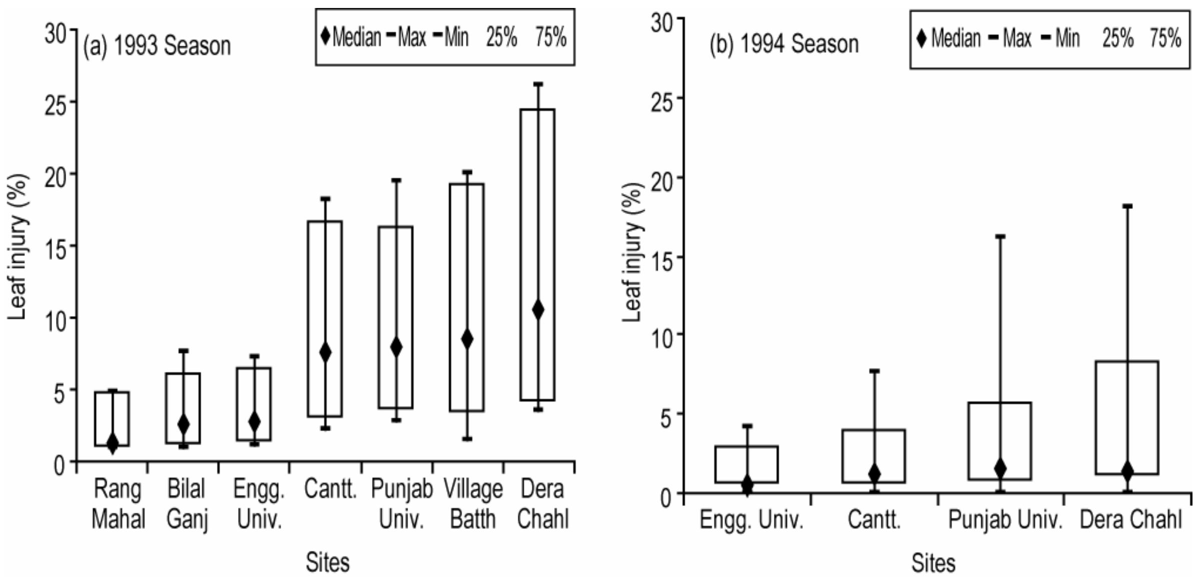

Fig. 4. Evaluation of foliar necrosis on tobacco (*Nicotiana tabaccum* L.) cv. Bel-W3 leaves exposed at various sites in and around the city of Lahore, Pakistan.

Discussion

The highest O_3 concentrations were observed at rural and semi-rural locations compared to suburbs and city centre sites (Figs. 2a, 2b and 3). Seasonal mean O_3 levels at the same sites are shown in Table 3.

At city center sites, weekly mean O_3 levels of 60-65 ppb during third week of May 1993, 1994 and 65 ppb during 2006 (Fig. 3) are comparable to weekly mean O_3 levels of 65 ppb reported by Nali & Lorenzini (2007) in Italy. Seasonal mean urban centre O_3 levels of 54 ppb found in our investigation of 1993 season are in agreement with findings of Nali *et al*., (2004). They reported O_3 concentration of 49 ppb at urban centre in Italy. Manes *et al*., (2003) and Adeeb & Shooter (2004) recorded summer mean O_3 levels of 35 ppb in Rome (Italy) and Auckland (New Zealand), respectively. These levels are 50% lower than our findings of 68 ppb at urban centre during the survey of 2006 (Table 3). Rural area seasonal mean O_3 concentrations of 75-100 ppb found in Pakistan during this investigation, are 3 fold to that reported by Girgzdiene & Girgzdys, 2001 (Lithuania); Rodriguez & Guerra, 2001 (Spain); Jonnalagadda *et al.*, 2001 (Zimbabwe); Tonneijck, 1989 (The Netherlands) and Adeeb & Shooter, 2004 (New Zealand). They recorded seasonal mean O_3 levels of 30-45 ppb at rural areas of their respective countries. Ashmore, 1987 (UK), Shenone & Lorenzini, 1992 (Italy) and Agrawal *et al.*, 2003 (India) recorded 50-60 ppb seasonal mean O_3 levels in their respective countries. These levels are 50% lower than our findings of 100 ppb at rural areas of Pakistan (Table 3).

The biomonitoring techniques provide information on actual effects on living organisms that compliment the data obtained by physico-chemical measurements and modeling of emissions and ambient pollutant concentrations (Klumpp *et al*., 2004). By the exposure of sensitive cultivar, a clear spatial pattern of O_3 pollution and O_3 effects on plants with increasing levels from city centre to rural areas was demonstrated. The highest O_3 concentrations (Fig. 3) and the strongest O_3 damages (Fig. 4) to leaves were observed at rural and suburban sites; which are characterized by low levels of primary air pollutants, especially NO_2. These results are consistent with findings of Ashmore & Dalpra (1978) and Agrawal *et al*., (2003). Seasonal mean O_3 injury results for 1993 and 1994 seasons are shown in figure (4) while weekly O_3 and NO_2 monitoring results for the same seasons during 2006 are represented in Fig. 4. Maximum seasonal LII values of 26 and 20% observed at rural locations of Dera Chahl and Village Batth, respectively in 1993 season and highest LII of 18% observed at rural location of Dera Chahl during 1994 season are comparable to 20%, 23% and 17% leaf damages reported by Goren & Donagi (1980). The lowest LII values of 5-7% found at city centre sites are in agreement with 5%, 4-10%, 5%, 5.73% and 6-12% leaf damages found by Varbiro *et al*., (2004). Minimum seasonal mean leaf damage of 1-3% found in this investigation (Fig. 4) is consistent with a number of studies carried out in various countries of the world, for instance, Italy (Nali *et al*., 2001, 2004). The sensitive tobacco cultivar Bel-W3 has been used for even larger projects, such as to survey O_3 in the British Isles (Ashmore *et al*., 1978, 1980). Fig. (5) represents not much variation in LII values between city center sites of Rang Mahal and inner suburbs of Bilal Ganj and Engineering University in 1993. Outer suburbs of Cantonment area and Punjab University sites showed almost similar LII values but higher than city centre sites. Rural sites of Village Batth and Dera Chahl indicated highest LII values during 1993 and 1994 seasons. These results are attributable to higher O_3 concentrations and temperature measured during exposure periods of 1993 and 1994 (Fig. 3). O_3 concentration exceeded during exposure periods mostly beyond the threshold value of 40ppb. Ball (1981) and Flagler (1998) reported positive correlation between weekly LII and number of hours per week when O_3 concentration exceeded 40 ppb. Smith *et al.*, (2003) and Zbierska and Karolewicz-Borowaik (2004) found more damage with sever symptoms on tobacco cultivar Bel-W3 leaves compared to low level areas.

Reduction in LII values during initial stages of 1994 season (Fig. 5b) is due to lower O_3 levels and temperature during this period (Fig. 3b). Not much variation in LII values was observed during 1994 season although the sites have different geographical and urban characteristics. Several external meteorological factors like temperature, water pressure deficit and global radiation as well as internal plant factors like the water balance are known to exert strong influence on the stomata regulation and thus on O_3 uptake (Klumpp *et al*., 2004).

O_3 enters the leaf through stomata and reacts in the apoplast to produce reactive oxygen species (ROS), such as super oxide anion (O^{-2}), hydrogen peroxide (H_2O_2), hydrogen radical (OH^{-}) and singlet oxygen (Mudd, 1997). This triggers the plants to produce an oxidant burst by as yet unknown mechanism (Schraudner *et al*., 1996). Short high-peak concentrations induce cell death lesions, reminiscent of lesions activate during plant-pathogen interaction (Rao *et al*., 2000). The classical O_3 symptoms in tobacco cultivar Bel-W3 plants occur as sharply defined dot-like lesions on the adaxial side of the leaf resulting from death of group of palisade cells (Loreto *et al*., 2001).

Increasing levels of tropospheric O_3, currently dominates the air pollution problems, particularly in the Asian regions where climate is also supportive in the generation of this pollutant which damages plants, human health and pristine environment. In the present study, both techniques viz., O_3 monitoring with continuous analyzers and biomonitoring (Ashmore *et al*., 1980) using tobacco cv. Bel-W3 were employed and it was found that high O_3 concentrations existed in the atmosphere of Lahore region. It was also noted that comparatively much higher O_3 levels were recorded throughout the research duration in the rural areas than that of other experimental sites confirming that higher O_3 levels were often associated with rural background than in the city centres (Bell 1986; Ashmore 2005) where it is known to have a substantial effect on the agricultural production in North American, European and Asian countries including Pakistan (Emberson *et al*., 2001, 2003) because O_3 impairs plant metabolism that in turn influence the yield of crops (Wahid 2006a, b; Emberson *et al*., 2009; Zhao *et al*., 2011). Present study has successfully demonstrated the feasibility of using biomonitor plants (viz., Tobacco cv. Bel-W3) in Pakistan for the monitoring of ambient ozone. This is cost-effective and reliable monitoring technique in the region like Pakistan where shortage of electricity supply and other socio-economic problems are already aggravated.

Conclusions

- High O_3 concentrations existed in the atmosphere of Lahore region and higher O_3 levels were often associated with rural background than in the city centers.
- Data in the present study have revealed AOT 40 ppb O_3 levels at many sites in Lahore which is matter of serious concern; as at or above this level O_3 is considered to be injurious to a range of plant species including human beings.
- The O_3 data based on more than one decade (1993-2006) in this study, indicated the continuous deteriorating atmospheric quality of Lahore region and is comparatively worst than other developing nations which reflect an alarming situation with respect to environmental degradation & protection.
- The exposure of O_3-sensitive tobacco cultivar Bel-W3 proved very suitable low-cost technique for the effect-related monitoring of phytotoxic ambient O_3 concentrations in developing countries like Pakistan.

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References

Adeeb, F. and D. Shooter. 2004. Variation of surface ozone in the ambient air of Auckland, New Zealand. *Environmental Monitoring and Assessment*, 95: 201-220.

Agrawal, M., B. Singh, M. Rajput, F. Marshall and J.N.B. Bell. 2003. Effect of air pollution on peri-urban agriculture: a case study. *Environmental Pollution*, 126: 323-329.

Ashmore, M. and C. Dalpra. 1978. Effects of London' air on plant growth. Environmental Bulletin, London. pp. 3-5.

Ashmore, M.R. 2005. Assessing the future global impacts of ozone on vegetation. *Plant Cell and Environment*, 28: 969-964.

Ashmore, M.R., J.N.B. Bell and C. Dalpra. 1980. Visible injury to crop species by ozone in the United Kingdom. *Environmental Pollution*, Series *A*, 21: 209-215.

Atkins, D.H.F., C. Healy and J.B. Tarrant. 1978. The use of simple diffusion tubes for the measurement of nitrogen dioxide levels in homes using gas and electricity for cooking. *Report R-9184*, AERE, Harwell, Didcot. Oxon, U.K.

Ball, D.J. 1981. A study of plants as indicators of photochemical pollution in the London area. *The London Naturalist*, 60: 27-42.

Bell, J.N.B. 1986. Air pollution injury to vegetation. In: *Proceedings of 93rd Environmental Health Congress*, Scarborough, 22-25 Sept. 1986. Institute of Environmental Health Officers, London.

Bignal, K. L., M. R. Ashmore, A. D. Headley, K. Stewart, and K. Weigert. 2007. Ecological impacts of air pollution from road transport on local vegetation. *Applied Geochemistry*, 22: 1265-1271.

De Souza, S.R., S.M.R. Sant'Anna, M.C.S. Rinaldi and M. Domingos. 2009. Short term leaf responses of *Nicotiana tabaccum* 'Bel-W3' to ozone under the environmental conditions of Sao Paulo, SE–Brazil. *Brazilian Archives of Biology and Technology*, 52(1): 251-258.

Emberson, L., M. Ashmore and F. Murray. 2003. Air pollution Impacts on Crops and Forests- A Global Assessment. *Air Pollution Reviews*, 4, Imperial College Press. U.K.

Emberson, L.D., M.R. Ashmore, F. Murray, J.C.I. Kuylenstierna, K.E. Percy, T. Izuta, Y. Zheng, H. Shimizu, B.H. Sheu, C.P. Liu, M. Agrawal, A. Wahid, N.M. Abdel-Latif, M. Van Tienhoven, L.I. de Bauer and M. Domingos. 2001. Impacts of air pollutants on vegetation in developing countries. *Water, Air and Soil Pollution*, 130: 107-118.

Flagler, R.B. 1998. Recognition of Air Pollution injury to vegetation: A pictorial Atlas. Second ed. *Air and Waste Management Association*, Pittsburgh, USA.

Ghauri, B., M. Salam and M.I. Mirza. 1994. An assessment of air quality in Karachi, Pakistan. *Environmental Monitoring and Assessment*, 32: 37-45.

Girgzdience, R. and A. Girgzdys. 2001. Spatial and Temporal variability in ozone concentration level at two Lithuanian Stations. *Water, Air and Soil Pollution*, 130: 1547-1552.

Goren, A. I. and A.E. Donagi. 1980. Assessment of atmospheric ozone levels in Israel through foliar injury to Bel-W3 tobacco plants. *Oecologia*, 44: 418-421.

Jonnalagadda, S.B., J. Bwila and W. Kosmus 2001. Surface ozone concentration in Eastern Highlands of Zimbabwe. *Atmospheric Environment*, 35: 4341-3446.

Klumpp, A., G. Klumpp and W. Ansel. 2004. Urban air quality in Europe-results of three years of standardized Biomonitoring studies. In: *Urban Air Pollution, Bioindication and Environmental Awareness* (Eds.): A. Klumpp, W. Ansel and G. Klumpp. *Euro-Bionet 2002 conference*, November 5-6, 2002. University of Hohenheim, Germany.

Loreto, F., M. Mannozzi, C. Maris, P. Nascetti, F. Ferranti and S. Pasqualini. 2001. Ozone quenching properties of isoprene and its antioxidant role in leaves. *Plant Physiology*, 126: 993-1000.

Manes, F., F. De Santis, M.A. Giannini, C. Vazzana, F. Capogna and I. Allegrini. 2003. Integrated ambient ozone evaluation by passive samples and clover Biomonitoring mini-stations. *The Science of the Total Environment*, 308: 133-141.

Nali, C. and G. Lorenzini. 2007. Air quality survey carried out by school children: An innovative tool for urban planning. *Environmental Monitoring and Assessment*, 131: 201-210.

Nali, C., A. Francini and G. Lorenzini. 2006. Biological monitoring of ozone: the twenty-year Italian experience. *Journal of Environmental Monitoring*, 8: 25-32.

Nali, C., L. Crocicchi and G. Lorenzini. 2004. Plants as indicators of urban air pollution (ozone and trace elements) in Pisa, Italy. *Journal of Environmental Monitoring*, 6:636-645.

Nali, C., M. Ferretti, M. Pellegrini and G. Lorenzini. 2001. Monitoring and Biomonitoring of surface ozone in Florence, Italy. *Environmental Monitoring and Assessment*, 69: 159-174.

Rao, M. V., J.R. Koch and K.R. Davis. 2000. Ozone: a tool for probing programmed cell death in plants. *Plant Molecular Biology*, 44: 345-358.

Rodriguez, S. and J.C. Guerra. 2001. Monitoring of ozone in a marine environment in Tenerife (Canary Islands). *Atmospheric Environment*, 135: 1829-1841.

Schenone, G. and G. Lorenzini. 1992. Effects of regional air pollution on crops in Italy. Agriculture, *Ecosystem and Environment*, 38: 51-59.

Schraudner, M., C. Langebartels and H. Sandermann. 1996. Plant defence systems and ozone. *Biochem. Soc. Trans.*, 24: 456-461.

Smith, C., J. Coulston, E. Jepsen and T. Prichard. 2003. A national ozone Biomonitoring program results from field surveys of ozone sensitive plants in North Eastern forest. *Environmental Monitoring and Assessment*, 87: 271-291.

Taylor, H.J. M.R. Ashmore and J.N.B. Bell. 1987. Air pollution Injury to vegetation: *a guidance manual commissioned by Her Majesty's Air Pollution Inspectorate of the Health and Safety Executive*. Institution of Environmental Health Officers, London.

Tonneijck, A.E.G. 1989. Evaluation of ozone effects on vegetation in the Netherlands. In: *Atmospheric ozone Research and its Policy Implications*. (Eds.): T. Schneider, G.S.D. Lee, G.J.R. Wolter and L.D. Grant. pp. 251-260. Elsevier, Amsterdam, The Netherlands.

Varbiro, G., G. Borics and C. Balogh. 2004. The East Hungrian Biomonitoring Network (EHBN). In: *Urban Air Pollution, Bioindication and Environmental Awareness.* (Eds.): A. Klumpp, W. Ansel and G. Klumpp. *Euro-Bionet 2002 Conference.* 5-6 November, 2002. University of Hohenheim, Germany. pp 59-65.

Wahid, A. 2006a. Productivity losses in barley attributable to ambient atmospheric pollutants in Pakistan. *Atmospheric Environment*, 40(28): 5342-5354.

Wahid, A. 2006b. Influence of atmospheric pollutants on agriculture in developing countries: a case study with three new varieties in Pakistan. *The Science of the Total Environment*, 371(1-3):304-313.

Wahid, A., E. Milne, S.R.A. Shamsi, M.R. Ashmore and F.M. Marshall. 2001. Effects of oxidants on soybean growth and yield in the Pakistan, Punjab. *Environmental Pollution*, 113: 271-280.

Zbierska, J. and K. Karolewicz-Borowiak. 2004. Initial recognition of the reaction of tobacco plants (*Nicotiana tabaccum* L.) to tropospheric ozone in the urban area (Poznan, Poland). In: *Urban Air Pollution, Bioindication and Environmental Awareness.* (Eds.): A. Klumpp, W. Ansel and G. Klumpp. *Euro-Bionet 2002 Conference.* 5-6 November, 2002. University of Hohenheim, Germany. pp. 329-336.

Zhao, Y., J.N.B. Bell, A. Wahid and S.A. Power. 2011. Inter and intra-specific differences in the response of Chinese leafy vegetables to ozone. *Water, Air and Soil Pollution*, 216: 451-462.ss

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