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Assessment of yield losses in tropical wheat using open top chambers

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Abstract

The present study deals with the evaluation of effects of ambient gaseous air pollution on wheat (*Triticum aestivum* L. var. HUW-234) growing in a suburban area situated in eastern Gangetic plain of India, using open top chambers. Eight hourly air monitoring was conducted for ambient concentrations of SO_2, NO_2 and O_3 in filtered chambers (FCs), non-filtered chambers (NFCs) and open plots (OPs). Various morphological, physiological and biochemical parameters were assessed during different developmental stages and finally yield parameters were quantified at the time of harvest.

Mean concentrations of SO_2, NO_2 and O_3 were 8.4, 39.9 and 40.1 ppb, respectively during the experiment in NFCs. Concentrations of SO_2, NO_2 and O_3 reduced by 74.6%, 84.7% and 90.4%, respectively in FCs as compared to NFCs. Plants grown in FCs showed higher photosynthetic rate, stomatal conductance, chlorophyll content and Fv/Fm ratio as compared to the plants in NFCs and OPs. Lipid peroxidation, proline, total phenol and ascorbic acid contents and peroxidase activity were higher in plants grown in NFCs. There were improvements in morphological parameters of plants growing in FCs as compared to those in NFCs and OPs. Yield of plants also increased significantly in FCs as compared to those ventilated with ambient air (NFCs) or grown in OPs. During the vegetative phase, NO_2 concentrations were higher than O_3, but O_3 became dominant pollutant during the time of grain setting and filling. The study concludes that O_3 and NO_2 are the main air pollutants in the sub-urban areas causing significant yield reductions in tropical wheat plants.

Keywords: Open top chambers; Ozone; Sulphur dioxide; Nitrogen dioxide; Effects; Wheat

1. Introduction

Ambient air pollution has been shown to reduce the growth and economic yield of a wide range of major crop species in the United States (Heck et al., 1988), Switzerland (Fuhrer et al., 1989), Sweden (Pleijel et al., 1991), Germany (Adaros et al., 1990), China (Wang et al., 2007), Pakistan (Wahid, 2006a, b), Japan (Kobayshi et al., 1995), Malaysia (Ishii et al., 2004) and Thailand (Ariyaphanphitak et al., 2005). Negative effect on crop yield under ambient air pollution was largely attributed to secondary air pollutant ozone (O_3), known to be highly phytotoxic. Ozone concentrations have increased considerably during the last century throughout the world. Ozone formation speeds up under conditions of bright sunlight, high temperatures and low wind speed. Other gaseous pollutants

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such as nitrogen dioxide (NO_2) and sulphur dioxide (SO_2) also pose negative influence on crop yield (Heck et al., 1988).

Impact of ambient air pollution particularly of O_3 on agricultural crops has been well documented in temperate countries through projects such as US National Crop Loss Assessment Network (NCLAN) (Heck et al., 1988) and European Crop Loss Assessment Network (EUCLAN) using open top chambers (OTCs). The magnitude of yield reductions varied from species to species and frequency of O_3 events that occurred during the growing season (Fuhrer et al., 1989). Both NCLAN and EUCLAN studies have clearly shown reductions of wheat yield due to elevated ambient O_3 concentrations over the growing period at different locations (Fuhrer et al., 1989; Pleijel et al., 1991).

Recent studies conducted in Asia showed high mean O_3 concentrations in Seoul (Jo et al., 2000), Hong-Kong (Lee et al., 2002), Tokyo (Wakamatsu et al., 1999), Pakistan (Wahid et al., 1995) and India (Agrawal et al., 2003). In contrast to the research outputs from developed nations on impact of ambient air pollution on crops, the numbers of case studies from developing countries are very few (Tiwari and Agrawal, 2006; Wahid, 2006a, b; Wahid et al., 1995, 2001). Air pollutant concentrations are increasing rapidly in many developing countries due to rapid economic transformation, industrialization and urbanization accompanied with increase in energy demands (Agrawal, 2005). Number of motor vehicles in India has increased from 21.3 million in 1991 to 67.0 million in 2003. Energy consumption in India is 12.8×10^{12} Btu. More traffic on roads and other transport sectors using dirtier fuels have changed the pattern of air pollutant concentrations in ambient air of urban areas. Atmospheric pollutants generated in urban–industrial areas spread to long distances from the place of origin to influence the suburban and rural areas supporting agriculture (Agrawal et al., 2003; Singh et al., 2005; Wahid, 2006a, b).

Agricultural productivity is needed to be enhanced to cope up the growing population of the country along with a steadily rising standard of living. Wheat (*Triticum aestivum* L.) is one of the major staple crops in India and grown over 26.4 million ha. Along with certain problems in agronomic practices and natural calamities, ambient air pollution could be one of the major factors for not achieving the target set for production of wheat crop in the country. During 2003–2004 the goal for yield was 78 Mt, however, only 72.06 Mt was achieved. Giles (2005) has predicted O_3 as a threat likely to cause major problems in countries that are rapidly industrializing, such as India and China. Keeping the above fact in view, the present study was undertaken to evaluate the pattern of changes in morphological, physiological, biochemical and yield characteristics of wheat (*T. aestivum* L. cultivar HUW-234) under current levels of ambient air pollutants using OTCs in a suburban area of a medium sized Indian city.

The hypothesis of the study was that O_3 and NO_2 are the major air pollutants at experimental site situated in suburban area, affecting the physiological, biochemical and growth characteristics of wheat plants negatively. The higher concentrations of O_3 particularly during anthesis period may cause more adverse impact on yield attributes of wheat as the sensitive stages of reproductive development are more severely affected due to their limited capacity to compensate for any losses of reproductive sites.

2. Material and methods

2.1. Study area

The study was conducted at the Agricultural Farm of Banaras Hindu University, a suburban area of Varanasi located in the eastern Gangetic plains of the Indian subcontinent at 25°14′N latitude, 82°03′E longitude and 76.1 m above mean sea level between the months of December, 2004 and March, 2005. This period of the year is characterized by mean monthly minimum temperature varying from 10.4 to 17.9 °C and mean monthly maximum temperature from 22.1 to 32.7 °C. Total rainfall was 115.6 mm, maximum recorded during February. Maximum monthly relative humidity ranged from 72.8% to 86.8% and minimum monthly relative humidity from 33% to 55.8%. The monthly variations in climatological data are given in Table 1. Soil at the study site was sandy loam in texture (sand 45%, silt 28% and clay 27%) with pH of 7.2.

2.2. Open top chambers

Six OTCs of 1.5 m diameter and of 1.8 m height were established at the experimental site following the design of Bell and Ashmore (1986). The details of OTCs design are described in Tiwari and Agrawal (2006). Each of the chambers was attached

Table 1
Meteorological data of the experimental site during the study period

Month/year	Rainfall (mm)	Mean temperature (°C)		Relative humidity (%)		Sunshine (h)
		Max.	Min.	Max.	Min.	
December 2004	0.0	23.7	10.8	86.8	49.3	6.3
January 2005	30.1	22.1	10.4	87.6	54.0	5.9
February 2005	48.5	26.1	14.3	84.3	55.8	8.6
March 2005	37.0	32.7	17.9	72.8	33.0	9.5

to high speed blowers with air supplied at three changes min^{-1}. Three chambers were ventilated with ambient non-filtered air (non-filtered chambers: NFCs) and another three with air that passed through activated charcoal filters (filtered chambers: FCs). All chambers were provided with prefilters to remove the dust. Three open plots (OPs) were also kept for studying the chamber effects on crops growing in OTCs. The treatments were distributed randomly. Microclimatic measurements were taken within and outside the chambers at 0900, 1300 and 1700 h at canopy height. It was found that temperature and relative humidity were 0.1–0.2 °C and 2–4% more in the chambers. The light intensity in the chambers was 95% of the ambient level in the OPs.

2.3. Plant material

Wheat (*T. aestivium L.*) cultivar HUW 234 was chosen for experiment because it is a highly recommended and widely grown variety for North-eastern plain zone of India. This variety of wheat is double gene dwarf variety developed from pedigree method by involving HUW 12/Sparrow/HUW12. HUW 234 has drooping leaves and club shaped spikes, having a life span of 120 days and an average height of 85–105 cm. This variety has high resistance against rust, loose smut, karnal bunt, powdery mildew and moderate resistant to leaf blight. This variety of wheat has been shown to be particularly sensitive to O_3 using EDU as a tool to assess the O_3 impact on plants (Tiwari et al., 2005).

Seeds of wheat were hand sown in chambers and in OPs in December 2004. Recommended dose of fertilizers (120, 60 and 40 kg ha^{-1} N, P and K as urea, super phosphate and muriate of potash, respectively) were added during preparation of field. Half dose of N and full doses of P and K were given as basal dressing and another half dose of N was given as a top dressing. Plants were thinned to 1 plant every 15 cm. Manual weeding was performed three times over the course of the experiment. Field was irrigated from time to time to maintain the soil moisture uniformly.

2.4. Air monitoring

Eight hourly monitoring for O_3, NO_2 and SO_2 were conducted throughout the growth period of plant. Air samples were drawn through Teflon tube (0.25 m diameters) at canopy height from different chambers between 9:00 and 17:00 h. The sampling tube was moved up as the plants grew. SO_2 was estimated by a SO_2 photometer analyzer (Model 319, Kimoto, Japan). O_3 concentrations were monitored using UV absorption photometric ozone analyzer (Model 400A, API, Inc., USA). Recording of O_3 and SO_2 concentrations were done at 15 min interval. The calibrations of the instruments were conducted weekly by known concentrations of SO_2 and O_3. NO_2 was scrubbed in NaOH (0.1 N) and analyzed colorimetrically following the method described by Merrymann et al. (1973).

2.5. Morphological parameters

For growth and biomass determinations, two monoliths ($10 \times 10 \times 20\,cm^3$) containing intact roots were carefully dug at random from each chamber and open plot at 40, 60 and 80 days after germination (DAG). These were thoroughly washed by placing them on a sieve of 1 mm mesh size under running tap water to remove the soil particles. Growth parameters recorded were root and shoot length, leaf area, numbers of tillers and leaves. Leaf area was measured using a portable leaf area meter (Model LI-3100, LI-COR, Inc. USA). Component plant parts were separated and oven dried at 80 °C till constant weight was achieved for biomass determination.

2.6. Physiological and biochemical parameters

Photosynthesis rate (Ps), stomatal conductance (Cs), transpiration (Es) and water use efficiency (WUE) were analyzed using portable photosynthetic system (Model LI-6200, LI-COR, USA) between 60 and 64 DAG. The measurements were made on fully expanded flag leaf on cloud free days during 09:00 and 10:00 h local time on three randomly selected plants in each chamber. During the measurements, photosynthetically active radiation ranged between 1100 and 1200 $\mu mol\, m^{-2}\, s^{-1}$. The system was calibrated using a known CO_2 source of 509 ppm concentration.

Chlorophyll fluorescence was determined between 10.00 and 11.00 h using portable plant efficiency analyzer (Model, MK2 9414, Hansatech Instrument Ltd., UK) on the same leaf where Ps was measured. Leaf clips for dark adaptation were placed on the adaxial side of the leaves for 10 min before measurement at excitation irradiance of 200 $\mu mol\, m^{-2}\, s^{-1}$. Minimum fluorescence ($F_0$) and maximum fluorescence (F_m) were measured from which variable fluorescence (F_v) and ratio of variable and maximum fluorescence (F_v/F_m) were calculated.

Biochemical analyses were conducted only for the plants growing in FCs and NFCs at 40 and 60 DAG. Three plants were randomly harvested from each chamber and leaves were cut down and refrigerated for analyses of photosynthetic pigments, antioxidants and metabolites. Total chlorophyll and carotenoid contents were measured by using the methods of Maclachlan and Zalik (1963) and Duxbury and Yentsch (1956), respectively. Phenol content was determined in acetone extract by using the methodology of Bray and Thorpe (1954). Peroxidase activity and ascorbic acid content were estimated by the methods of Britton and Mehley (1955) and Keller and Schwager (1977), respectively. Proline content was measured by the method of Bates et al. (1973). The extent of lipid peroxidation was estimated as malondialdehyde (MDA) content following the protocol of Heath and Packer (1968).

2.7. Yield parameters

Plants were harvested at maturity to assess different yield parameters. Six plants were sampled from each replicate OPs, FCs and NFCs. Number and weight of ears plant^{-1}, number of grains ear^{-1}, number and weight of grains plant^{-1}, weight of above ground part and weight of 1000 grains (test weight) were recorded. Harvest index (HI) was calculated as the ratio of weight of grains plant^{-1} and total above ground biomass of the plant.

2.8. Statistical analysis

Growth and biomass data were subjected to two-way ANOVA test for assessing the significance of quantitative changes in different parameters due to treatments at different sampling intervals. Data of yield and physiological characteristics were analyzed for significance of changes due to treatments using one way ANOVA test. Duncan's multiple range test was performed as post hoc on parameters subjected to ANOVA tests. The significance of difference for pigments, chlorophyll fluorescence kinetics, metabolites and enzymes due to treatments was calculated by student's *t*-test. All the statistical tests were performed using SPSS software (SPSS Inc., version 10.0).

3. Results

3.1. Air quality monitoring

Air monitoring showed higher concentrations of SO_2, NO_2 and O_3 in NFCs and OPs as compared to FCs (Fig. 1). Air filtration reduced the concentrations of O_3, NO_2 and SO_2 by 90.4%, 84.7% and 74.6%, respectively in FCs as compared to NFCs. During the 4 months growing period of wheat, maximum variations were found with O_3 concentration, which increased from a mean concentration of 36.4 ppb in December 2004 to a mean concentration of 48 ppb in March 2005. Concentration of SO_2 was low and it decreased from mean concentration of 11.1 ppb in December to 4.8 ppb in March. Mean concentration of NO_2 was highest in the month of December (43.1 ppb), thereafter a decline was observed with minimum concentration recorded during March (38.2 ppb). There was no significant difference in the concentrations of SO_2, NO_2 and O_3 between NFCs and OPs.

3.2. Morphological parameters

Plants grown in FCs showed increments in growth parameters as compared to those grown in NFCs and OPs (Table 2). ANOVA test showed that variations in morphological parameters were

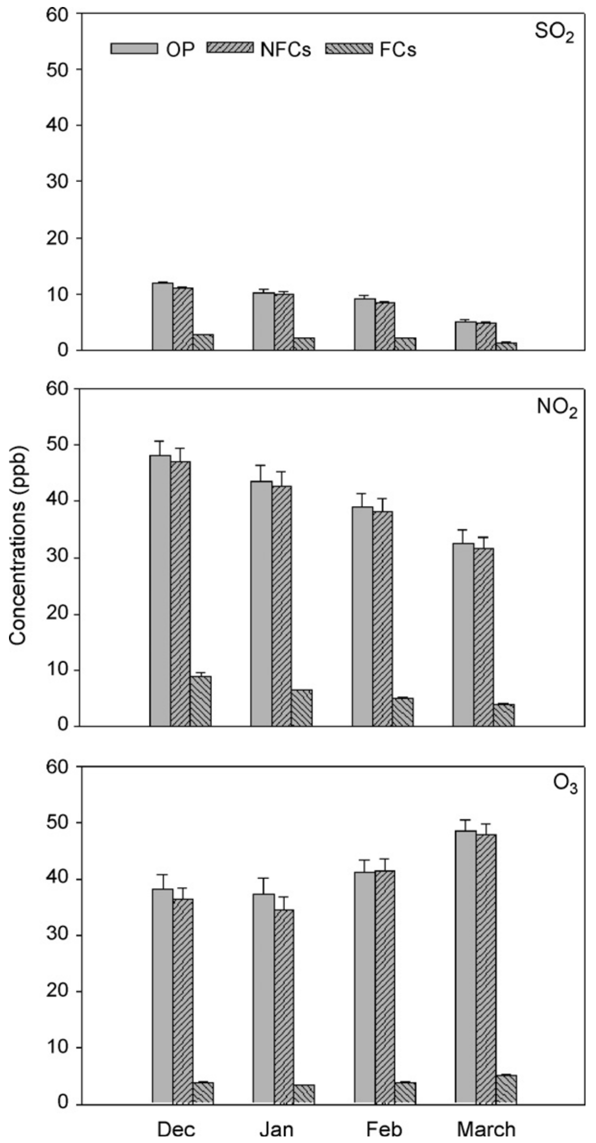

Fig. 1. Mean concentrations (ppb) of different pollutants at the experimental site (8-h daily mean). Values are mean ± 1 SE.

significant due to treatment, age and treatment × age interaction except for root length (Table 3). There was an increment of 33.6% in number of tillers at 60 DAG in FCs as compared to NFCs. Number of leaves plant^{-1} also increased significantly at both the ages of observations. Leaf area increased significantly by 13%, 31.4% and 31.9% at 40, 60 and 80 DAG, respectively in FCs in comparison to NFCs. Root, shoot and leaf biomass were higher in FCs as compared to NFCs and OPs at 60 and 80 DAG (Fig. 2). Results of two way ANOVA showed that variations in root, shoot and leaf biomass were significant due to age, treatment and their interaction.

3.3. Physiological and biochemical parameters

Photosynthetic rate increased by 27.1% in plants of FCs as compared to those in NFCs (Table 4). Stomatal conductance and transpiration rate also increased significantly in plants grown in FCs (Table 4). No significant differences were observed in photosynthetic rate, stomatal conductance and transpiration rate in plants of NFCs and OPs. Chlorophyll fluorescence measurements showed reduction of 4% and 4.9% in F_0 at 40 and 60 DAG, respectively in FCs as compared to NFCs. Values of F_m and F_v showed significant increments in plants grown in FCs at all ages of observations. Similar trend was observed for F_v/F_m ratio, which was 3.5% and 5.4% higher in plants of FCs than NFCs at 40 and 60 DAG, respectively (Fig. 3).

At all ages of estimation, total chlorophyll and carotenoid contents were higher in plants of FCs as compared to those in NFCs (Fig. 3). Increments were, however, not significant for carotenoid content. Concentration of MDA, an indicator of lipid peroxidation was higher in plants of NFCs as compared to those in FCs (Fig. 3). These increments were 47.4% and 41.6% at 40 and 60 DAG, respectively. Total phenolics were significantly higher in plants of NFCs than FCs (Fig. 4). Observed increments were 23.5% and 38.5% at 40 and 60 DAG, respectively. Peroxidase activity and ascorbic acid content showed increments of 23.4–38.1% and 6.6–11.2%, respectively in plants of NFCs as compared to FCs. Similarly, proline content was 36% and 46.2% higher in NFCs as compared to FCs at 40 and 60 DAG (Fig. 4).

3.4. Yield responses

As compared to plants in NFCs and OPs, respectively, number of ears plant^{-1} increased by 22.2% and 29.6% and weight of ears plant^{-1} increased by 21.9% and 34.2% in FCs. Similarly, weight and number of grains plant^{-1} were significantly higher by 20.7% and 14.5%, respectively in FCs as compared to NFCs. There was no significant difference in weight and number of grains plant^{-1} between NFCs and OPs. Test weight showed an increase of 18.8% in FCs as compared to NFCs. Similarly, significant increase of 8.4% for HI was found in FCs (Table 5).

Table 2
Agewise changes in morphological parameters of wheat plants in filtered chambers (FCs), non-filtered chambers (NFCs) and open plots (OPs)

Parameters	40 DAG			60 DAG			80 DAG		
	FCs	NFCs	OPs	FCs	NFCs	OPs	FCs	NFCs	OPs
Root length (cm)	11.8±1.04[a]	9.8±0.46[b]	8.3±0.22[b]	13.9±0.34[a]	12±0.46[b]	10.3±0.41[c]	16.9±0.76[a]	16.1±1.36[a]	13.2±0.17[b]
Shoot length (cm)	59.8±1.9[a]	53.6±2.5[b]	45.6±0.7[c]	98.1±0.27[a]	95.7±0.88[a]	88.5±2.32[b]	106.2±2.02[a]	105±2.35[a]	97±1.15[b]
No. of tillers ($plant^{-1}$)	10.3±0.6[b]	7.3±0.42[b]	4.3±0.49[c]	13.5±1.75[a]	8.3±0.21[b]	7.5±0.43[b]	12±1.7[a]	11.7±1.2[a]	10.5±0.42[a]
No. of leaves ($plant^{-1}$)	32.8±2.2[a]	30.3±1.5[a]	19.8±0.6[b]	47±8.6[a]	27.5±2.4[b]	26.2±0.7[b]	29.8±1.16[a]	22.5±1.8[b]	18.8±0.7[b]
Leaf area (cm^2)	646.2±21.9[a]	562±2.3[b]	546.3±2.3[b]	933.7±230.7[a]	639.9±44.8[b]	621±3.1[b]	340.1±47.1[a]	231.6±20.4[b]	222.8±1.94[b]

Values are mean ± 1 SE.
Values followed by different letters are significantly different ($p<0.05$).

Table 3
F-ratio and level of significance for morphological parameters of wheat plants grown in filtered chambers (FCs), non-filtered chambers (NFCs) and open plots (OPs)

Parameters	Age	Treatment	Interaction (age × treatment)
Root length (cm)	42.3***	23.3***	0.4[N.S]
Shoot length (cm)	64.2***	13.0***	2.7*
No. of tillers ($plant^{-1}$)	7.5***	13.1***	3.5*
No. of leaves ($plant^{-1}$)	5.9**	15.4***	2.9*
Leaf area (cm^2)	99.6***	16.8***	2.6*
Root biomass (g)	128.8***	48.0***	7.3***
Shoot biomass (g)	759.3***	70.2***	21.0***
Leaf biomass (g)	209.7***	23.6***	3.7***

Level of significance: *$p<0.05$; **$p<0.01$; ***$p<0.001$; N.S: not significant.

4. Discussion

The field experiment conducted in OTCs clearly showed significant adverse effects of ambient air pollution on wheat plants growing in the suburban areas of Varanasi. The microclimatic conditions only varied to a small extent between the chambers and OPs. The increases in temperature (0.1–0.2 °C) within chambers as compared to OPs during the present study were much less than those reported by Wahid (2006a), 1.4 °C and Vandermeiren et al. (1992), 0.8–1 °C. However, the increments in relative humidity (2–4%) inside the chamber observed during the present study were similar to that reported by Wahid (2006a). A 5% reduction in light intensity was observed during the present study, while Ashmore et al. (1988) and Wahid (2006b) reported 7% and 2–6% reductions in light levels, respectively. The lower magnitude of increase in temperature inside the chambers reflected a better air circulation as compared to other studies (Vandermeiren et al., 1992; Wahid, 2006a). Charcoal filters have very efficiently removed all the pollutants, with maximum filtration of O_3. Wahid et al. (1995) and Wahid (2006b) also found filtration efficiencies of 87–92% for O_3. In the present study NO_2 filtration (85%) efficiency was higher than the reported efficiencies of 67% by Wahid (2006b) and 47–58% by Wahid et al. (1995). The air monitoring data clearly showed that SO_2 being low in concentration may not have a major impact on plants in the area. However, the concentration of SO_2 was much above the natural background level and also higher than some of the critical levels used to protect vegetation in Europe (Ashmore and Wilson, 1994). NO_2 and O_3 were the major air pollutants in the area, which may be ascribed to the high vehicular emission in the urban areas of Varanasi.

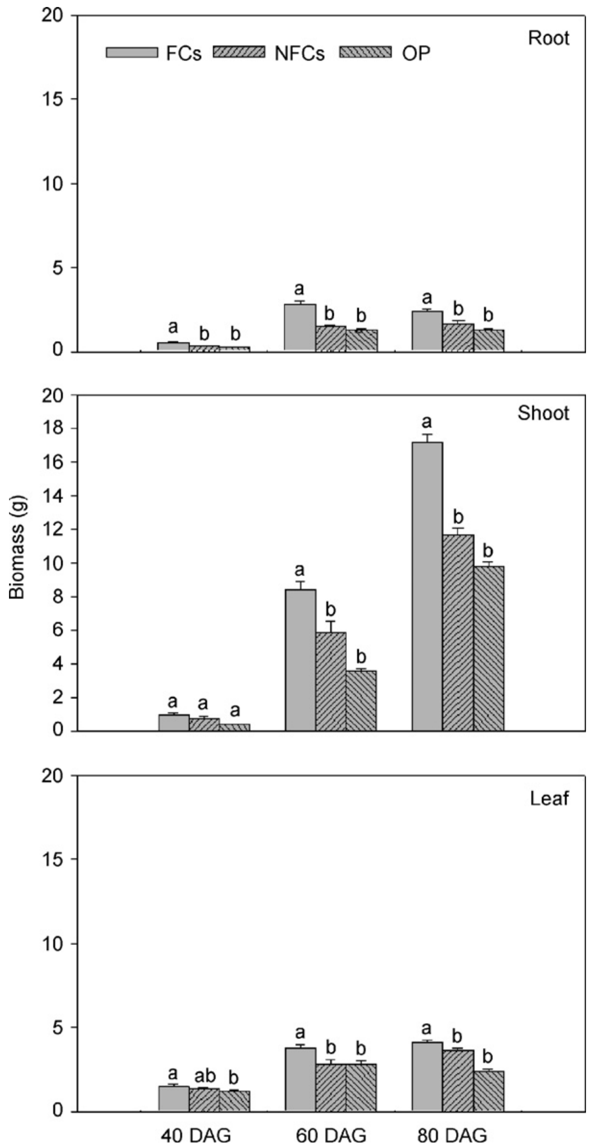

Fig. 2. Root, shoot and leaf biomass of wheat plants grown in FCs, NFCs and OPs at 40, 60 and 80 DAG. Values followed by different letters are significantly different ($p<0.05$).

During the vegetative stage of wheat growth (December–January), temperature and sunlight intensity were low and NO_2 concentrations were higher than O_3. But, during reproductive period (February–March) temperature and light intensity gradually increased which favoured more O_3 formation thus causing a lower mean concentration of NO_2, which is a primary precursor for O_3 production. Agrawal et al. (2003) have also reported higher mean concentration of O_3 during summer than winter at suburban and rural sites around Varanasi. The seasonal mean concentrations of all the three pollutants recorded during the present study were lower than the concentrations reported by Wahid (2006a, b) for the same period of time. The pollutant concentrations in NFCs and OPs were similar.

Air pollutants are known to induce oxidative stress in plants after absorption. Presence of O_3 triggers an oxidative burst, leading to production of reactive oxygen species (ROS) (Wohlgemuth et al., 2002) and subsequent lipid peroxidation. In the present study, lipid peroxidation was indicated to be higher in plants of NFCs as compared to those grown in FCs. Lipid peroxidation has been correlated with the damage to membrane in O_3 exposed plants (Calatayud and Barreno, 2004). Cell wall ascorbate provides a first line defence against O_3 and SO_2 fumigations (Castillo and Greppin, 1988). In the present experiment, foliar ascorbic acid content was higher in plants grown in NFCs, which might be a consequence of substantial oxidative stress. Recent studies have shown higher levels of ascorbic acid in the leaf apoplast of O_3 tolerant snap bean (*Phaseolus vulgaris* L.) genotypes compared with sensitive lines (Burkey et al., 2003). The present test cultivar (HUW-234) of wheat might have increased the defense against oxidative stress by enhancing ascorbic acid content in response to air

Table 4
Variations in physiological characteristics of wheat plants at 60 DAG grown in filtered chambers (FCs) and non-filtered chambers (NFCs)

Parameters	Treatments		
	FCs	NFCs	OPs
Photosynthesis rate ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	16.98 ± 0.03^a	12.37 ± 0.06^b	12.24 ± 0.02^b
Stomatal conductance ($cm\ s^{-1}$)	2.91 ± 0.01^a	2.33 ± 0.18^b	2.26 ± 0.03^b
Transpiration rate ($mmol\ m^{-2}\ s^{-1}$)	19.43 ± 0.08^a	15.86 ± 0.09^b	15.73 ± 0.02^b
Water use efficiency ($\mu mol\ CO_2\ m^{-2} s^{-1}\ mmol\ m^{-2}\ s^{1}$)	0.87 ± 0.004^a	0.78 ± 0.07^b	0.77 ± 0.001^b

Values are mean $\pm$ 1 SE.
Values followed by different letters are significantly different ($p<0.05$).

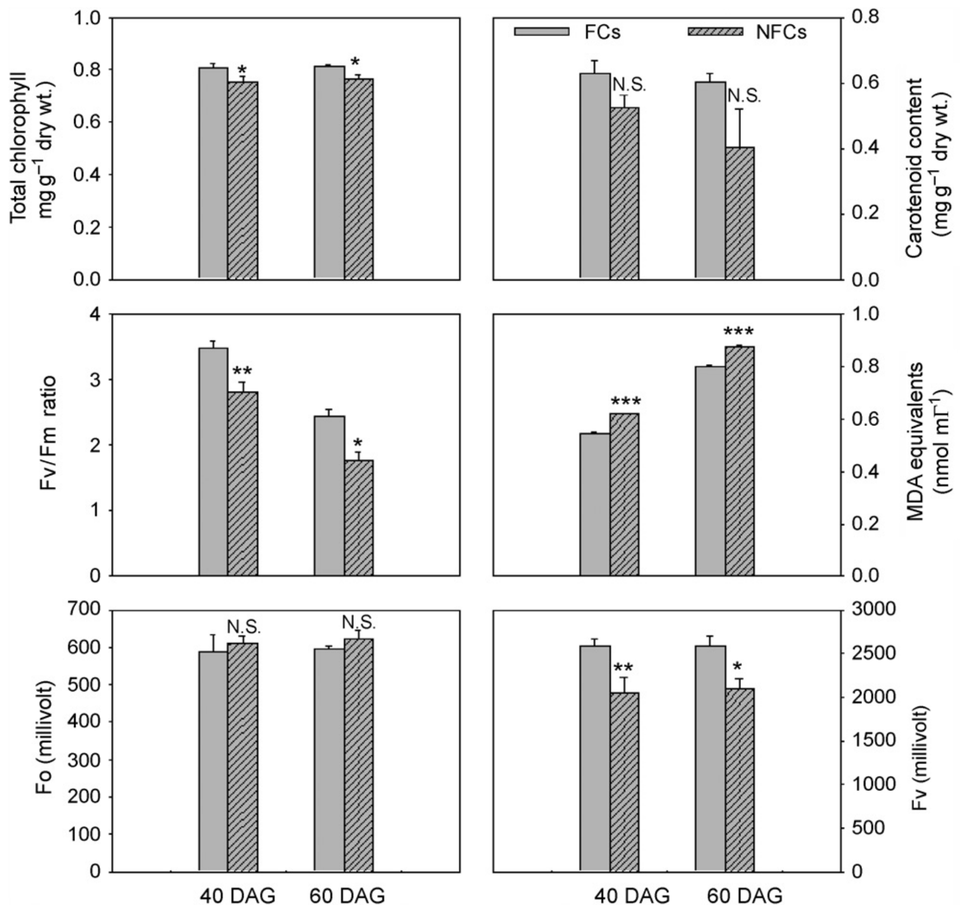

Fig. 3. Total chlorophyll and carotenoid contents, F_0, F_v and F_v/F_m ratio and lipid peroxidation in wheat plants grown in filtered (FCs) and non-filtered chambers (NFCs) at 40 and 60 DAG. Level of significance: $*p<0.05$, $**p<0.01$, $***p<0.001$ and NS = not significant.

pollution stress. Increments in peroxidase activity have also been reported as defensive response under O_3 (Tingey et al., 1975) and NO_2 (Horsman and Wellburn, 1975) stresses. In the present study, ascorbic acid content and peroxidase activity increased with age in wheat plants in NFCs as compared to FCs.

Proline accumulates in plants under environmental stresses to scavenge ROS (Matysik et al., 2002). In the present study, proline content increased in plants grown in NFCs. Pollutants after dissolving in the intercellular fluid of leaves give rise to a series of reactions, which alter the levels of major anions and lead to accumulation of proline (Mansfield and Freer-Smith, 1981). Total phenol content also increased in plants of NFCs. Langerbartels et al. (1990) showed an increase in the phenolic compounds in presence of O_3. Phenols are responsible for plant resistance against many stresses. Air pollutants including NO_2 and O_3 produce ROS causing widespread damage to membranes and associated molecules, including chlorophyll pigments. In the present investigation total chlorophyll content was lower in plants growing in NFCs as compared to those in FCs. Pleijel et al. (2006) showed significant reduction in chlorophyll content of wheat cv. Dragon and Lantvete under NFCs with additional ozone (+40 ppb) as compared to those in FCs. Agrawal et al. (2003) have also reported reductions in total chlorophyll content of pot grown wheat plants grown under ambient air mainly O_3 and NO_2 in the suburban areas of Varanasi.

Plants grown in NFCs and OPs showed lower rates of Ps, Es and Cs as compared to those in FCs. Temple et al. (1988) found significant reductions in Ps of cotton (*Gossypium hirsutum* L. cv. SJ-2) grown in NFCs and NFCs receiving additional O_3 as compared to NFCs. Decrease in WUE of plants in NFCs as compared to FCs suggest that CO_2 assimilation is more severely reduced than

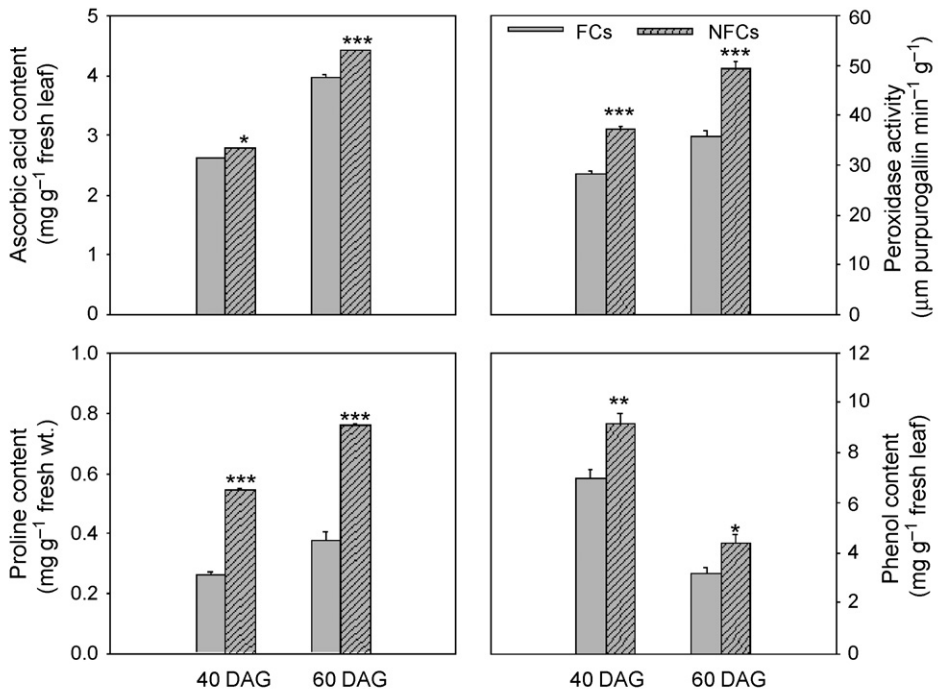

Fig. 4. Ascorbic acid, proline, total phenol content and peroxidase activity in wheat grown in filtered (FCs) and non-filtered chambers (NFCs) at 40 and 60 DAG. Level of significance: $*p<0.05$, $**p<0.01$, $***p<0.001$ and NS = not significant.

Table 5
Variations in yield characteristics of wheat plants grown in filtered chambers (FCs) non-filtered chambers (NFCs) and open plots (OPs)

Parameters	Treatments		
	FCs	NFCs	OPs
Number of ears (plant^{-1})	14.5 ± 0.42^{a}	11.6 ± 0.66^{b}	10.2 ± 0.47^{b}
Weight of ears (g plant^{-1})	29.6 ± 0.88^{a}	23.2 ± 1.01^{b}	19.5 ± 0.76^{c}
Number of grains (plant^{-1})	568 ± 14.3^{a}	485.8 ± 18.89^{b}	475.1 ± 12.9^{b}
Weight of grains (g plant^{-1})	27.2 ± 1.02^{a}	21.6 ± 0.94^{b}	20.3 ± 0.13^{b}
Test weight (g)	45.3 ± 2.30^{a}	36.8 ± 1.06^{b}	32.4 ± 1.08^{b}
Harvest index (g g^{-1})	0.39 ± 0.01^{a}	0.37 ± 0.04^{a}	0.33 ± 0.008^{a}

Values are mean ± 1 SE.
Values followed by different letters are significantly different ($p<0.05$).

transpirational water loss, which indicated more pollutant effect at the mesophyll level. Wahid (2006a) reported significant reductions in Ps and Cs of three cultivars of wheat grown in OTCs with mean seasonal concentrations of 71 ppb O_3, 30 ppb NO_2 and 16 ppb SO_2. The decrease in Ps could also be attributable to decrease in Cs in plants of NFCs as compared to FCs during the present study. O_3 is found to induce stomatal closure of varying degree leading to reduction in stomatal conductance (Guidi et al., 1997). The reduction in Ps due to O_3 exposure has often been correlated with lower carboxylation efficiency due to O_3 effects on enzymes of Calvin cycle (Zheng et al., 2002). Rate of photosynthesis of flag leaves declined by 40% in wheat cv. Satu due to exposure of 45 ppb O_3, 8 h daily for 4 weeks during anthesis (Ojanpera et al., 1998). In the present study also mean O_3 concentration was more than 40 ppb during the anthesis period of wheat. This period is the most important for O_3 effects on grain yield in cereals (Pleijel et al., 1998).

The fluorescence induction kinetics measurement done during the present study showed higher constant yield (F_0), whereas variable fluorescence

(F_v) reduced for plants grown in NFCs. Increase in F_0 suggests structural alteration at PSII (Krause and Weis, 1984), whereas lowering of F_v (F_m-F_0) indicates thylakoid damage. Significant reduction in F_v/F_m ratio in plants of NFCs as compared to FCs indicated that polluted ambient air modified the photochemistry of PSII. Wahid (2006a) also reported significant reductions in F_v/F_m ratio of three varieties of wheat grown in unfiltered air and ambient air as compared to those in filtered air.

The negative effects of NO_2 and O_3 present in ambient air of NFCs on physiological and biochemical characteristics of plants led to significant reductions in growth and biomass accumulation in plants as compared to those in FCs. Reduction in leaf area may reduce absorption of radiation and subsequently reduction in net assimilation per unit ground area. Heagle et al. (2000) have reported 7% decline in leaf area in NFCs and 69% in NFCs with additional O_3 for a wheat variety C9904. Wahid (2006a) recorded significant decline in flag leaf area of three varieties of wheat grown in NFCs and OPs as compared to FCs. Number of tillers declined in NFCs and OPs as compared to FCs. Wahid et al. (1995) reported 25.9% and 31.8% reductions in number of tillers in wheat varieties Pak-81 and Chakwal-86, respectively grown in NFCs as compared to those in FCs.

No significant effects of filtration were recorded on biomass of root, shoot and leaves at 40 DAG. However, at 60 and 80 DAG reductions were significant in NFCs. During early vegetative growth of wheat NO_2 concentrations were higher than O_3, but at anthesis period O_3 became predominant pollutant. NO_2 is less phytotoxic than O_3 at comparable concentrations causing significant yield losses (Capron, 1994). Reductions in component wise biomass of plants in NFCs are directly correlated to the pollutant-induced changes in morphological, physiological and biochemical characteristics. Results of two-way ANOVA test showed that there is a consistent significant effect of treatment on growth parameters and biomass of wheat plants along with its increasing age.

In the present study, yield decreased by 20.7% at seasonal mean concentrations of 41.8, 39.9 and 8.5 ppb O_3, NO_2 and SO_2, respectively. Yield reductions recorded in the present study are greater than other studies conducted in Europe (Fuhrer et al., 1989; Pleijel et al., 1991) using OTCs, but lower than observations made by Wahid et al. (1995). Wahid et al. (1995) recorded yield reductions of 35% and 47% in wheat cultivars Pak-81 and Chakwal-86, respectively grown in chambers ventilated with ambient air compared with charcoal filtered air. Wahid (2006a) reported reductions of 43%, 39% and 18% in seed weight plant^{-1} of Pasban 90, Punjab 96, and Inqilab 91 varieties of wheat, respectively at seasonal mean concentrations of 70, 28 and 15 ppb O_3, NO_2 and SO_2, respectively in Lahore, Pakistan. Pleijel et al. (1991) reported yield reductions of 13% in wheat at 7-h mean concentrations of 42 and 44 ppb O_3 during two seasons in Sweden. In Switzerland, Fuhrer et al. (1989) also reported 6% reduction in 1000 grain weight in NFCs as compared to FCs. In European studies cited above yield reductions were found at mean O_3 concentrations of 40–50 ppb throughout the growth period of wheat, while in the present investigation such concentrations were achieved during later stages of plant growth.

HI is a parameter, which indicates the partitioning of dry matter between grain and above ground biomass. Reduction in HI of plants in NFCs and OPs as compared to FCs indicated that relatively less dry matter was partitioned into grain under pollutant stress of ambient air. Fuhrer et al. (1989) and Pleijel et al. (1991) have also found reductions in HI of wheat at higher mean O_3 concentrations causing significant yield losses. Sexual reproductive development is a crucial stage in the life cycle of seed crop plants and any impairment of the process has significant implications for productivity of these plants. Huxiang et al. (2005) reported that winter wheat grown in Yangtze Delta in China was adversely affected by O_3 because it is typically sown in October or November and harvested in May or June. The high springtime O_3 concentrations are of particular concern as they coincide with the timing of the environmentally sensitive stages in the winter wheat i.e. anthesis and grain filling (Slaughter et al., 1989). A similar seasonal variation in O_3 concentration was observed during the anthesis and grain filling stages of wheat during the present study. Black et al. (2000) have shown that current ambient levels of O_3 in many industrialized countries reduced the grain and fruit yields and adversely affected the yield quality.

5. Conclusion

The present study showed that NO_2 and O_3 were the main pollutants of concern in ambient air of suburban areas of Varanasi. Higher concentrations of NO_2 along with the low mean O_3 concentration during vegetative phase did not cause significant effects on growth and biomass accumulation in plants in NFCs and OPs. But during anthesis and grain filling stages at higher mean O_3 concentrations, significant reductions in various parameters were observed in plants grown in NFCs. This indicates that higher O_3 concentrations particularly at the reproductive stage of wheat led to significant yield reductions. Comparison of wheat responses grown in FCs with NFCs and OPs clearly showed improvements in different physiological, biochemical and morphological parameters of wheat plants under condition of pollutant exclusion in FCs. Yield attributes have also showed improvements when grown in FCs as compared to NFCs and OPs. This suggests that ambient air quality in suburban areas of Varanasi has potential negative impact on wheat growth and yield. Comparison of yield losses reported for temperate wheat cultivars at more or less similar O_3 concentrations as measured during the present study, showed that the test tropical cultivar is more sensitive than temperate cultivars.

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