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EVALUATION OF AMBIENT AIR POLLUTION IMPACT ON CARROT PLANTS AT A SUB URBAN SITE USING OPEN TOP CHAMBERS

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Abstract. The present experiment was done to evaluate the impact of ambient air pollution on carrot (*Dacus carota* var. Pusa Kesar) plants using open top chambers (OTCs) ventilated with ambient (NFCs) or charcoal filtered air (FCs) at a suburban site of Varanasi, India. Various morphological, physiological and biochemical characteristics of the plants were studied at different growth stages. Air monitoring data clearly showed high concentrations of SO_2, NO_2 and O_3 in the ambient air of study site. SO_2 and NO_2 concentrations were higher during early growth stages of carrot, whereas O_3 concentration was highest during later growth stages. Filtration of air has caused significant reductions in all the three pollutant concentrations in FCs as compared to NFCs.

Plants growing in FCs showed significantly higher photosynthetic rate, stomatal conductance, water use efficiency and variable fluorescence as compared to plants growing in NFCs. Protein content also showed a similar pattern, however, lipid peroxidation, ascorbic acid content and peroxidase activity were higher in plants growing in NFCs as compared to FCs. Shoot length, number of leaves per plant, leaf area and root and shoot weight increased significantly upon filtration of ambient air. Total nitrogen decreased significantly in root, but increased significantly in shoot of plants grown in NFCs. Total P, Mg, Ca and K contents decreased significantly in plants grown in NFCs as compared to FCs. The individual pollutant concentrations were below threshold for plant injury, but the combined effect of all the three seems to act synergistically in causing greater adverse impact on dry weight and physiology of carrot plants. The study clearly indicates that air pollutants are high enough in the ambient air to cause significant unfavorable impact on carrot plants. The work further supports the usefulness of OTCs for assessing air pollution damage under field conditions in developing countries.

Keywords: ambient air, carrot plants, impact, open top chambers, suburban site

1. Introduction

Adverse effects of air pollution on biota and ecosystems have been demonstrated worldwide. Much experimental work has been conducted on the analysis of air pollutant effects on crops and vegetation at various levels ranging from biochemical to ecosystem levels (Saxe, 1991; Krupa *et al.*, 1995). Earlier, it was believed that any damage done to the vegetation would be confined to the isolated areas of high arial pollution like those in the vicinity of large industrial emissions. However, increased regional transportation have caused the pollutants to be more efficiently

dispersed leading to reductions in localized high concentrations and increase in rural areas (Hassan *et al.*, 1995; Agrawal *et al.*, 2003). Regional air pollution may cause significant yield losses on sensitive crops (Wahid *et al.*, 1995; Krupa *et al.*, 1995; Agrawal *et al.*, 2003). The instrumental and bio-monitoring of air pollution carried out at suburban sites have shown that often the major phytotoxic agents are present at levels above the threshold of plant damage (Wahid *et al.*, 1995a,b; Agrawal *et al.*, 2003). It has been observed that ozone concentrations are higher in suburban and rural areas as compared to the urban areas, whereas SO_2 and NO_2 concentrations are higher at urban sites (Hassan *et al.*, 1995; Wahid *et al.*, 1995a,b; Agrawal *et al.*, 2003).

The deleterious effects of the pollutants are caused by the production of reactive oxygen species (ROS) in plants, which cause peroxidative destruction of cellular constituents (Shimazaki *et al.*, 1980). Such effects of pollutants on plants include pigment destruction, depletion of cellular lipids and peroxidation of polyunsaturated fatty acid (Castillo *et al.*, 1984). Pollutants can cause leaf injury, stomatal damage, premature senescence, decrease photosynthetic activity, disturb membrane permeability and reduce growth and yield in sensitive plant species (Atkinson *et al.*, 1988; Darrall, 1989; Saxe, 1991).

Open top chambers are most widely used technology for evaluating the impacts of air pollutants on vegetation (Adams *et al.*, 1988; Pleijel *et al.*, 1991; Jager *et al.*, 1994). The merits of this technology include a better control over pollutant exposure to plants by minimally altering the microclimatic conditions. This technology has been extensively used world wide to examine the effects of pollutants on different plant species. It has been estimated that 10% yield reductions occur in corn wheat and kidney bean at seasonal 7 h mean concentrations of 75–132, 64–93 and 72–86 nl l^{-1} O_3, respectively (TERG, 1988). However, OTC experiments with low concentrations of SO_2 and NO_2 (10 and 12 nl l^{-1}, respectively) have shown a 13% increase in the yield of winter barley (Flower *et al.*, 1988). Experiments conducted on the outskirts of Lahore, Pakistan showed that ambient air pollution has caused large yield reductions in local varieties of wheat and rice using OTC's (Wahid *et al.*, 1995a,b).

The objective of the present study was to assess the impacts of ambient air pollution on carrot plants grown at a suburban site in Varanasi, India using open top chambers. The effects of pollutants on morphological, biochemical and physiological characteristics of carrot plants were studied at different developmental stages. This report is the first known result on plant response assessment based on the use of open top chamber methodology of air filtration in India.

2. Materials and Methods

The study was conducted at Suswahi, a suburban area of Varanasi, located in the eastern gangetic plains of Indian subcontinent at 25°14′N latitude, 82°03′E

longitude and 76.19 m above mean sea level. The city has a population of 1.8 million and area of 112.3 sq. km. Suswahi is located south of the city, about 7.8 km. from the city centre.

The experiment was carried out between the months of December 2002 and March 2003. This period of the year is characterized by mean monthly maximum temperature ranging between 16.15–29.8 °C and mean monthly minimum temperature between 6.25–16 °C. Total mean rainfall was 26.2 mm of which 89.5% occurred in January. Maximum relative humidity varied from 74.8–93% and minimum ranged from 35.4–66%. Wind speed varied from 2.3–4.14 km hr^{-1}.

Six open top chambers were constructed according to the design of Bell and Ashmore (1986). These chambers have simple basic design and can be constructed easily and rapidly. Chambers are 1.8 m in height and 1.5 m in diameter, consisting of an aluminum framework with polythene walls, with air supplied at three changes per minute via high-speed blower. These OTCs have been extensively used in air-filtration studies in a number of locations in south–east England, and Pakistan over the last decade. Three of the six chambers were equipped with charcoal filters and were treated as filtered chambers (FCs) and the other three had empty filters and were treated as non-filtered chambers (NFCs). All the chambers were provided with prefilters to remove dust. During the experimental period, the OTCs were ventilated continuously by passing air through filters. Temperature and relative humidity were 0.1–0.2 °C and 2–4% more in chambers as compared to outside. The light intensity in the chambers was 95% of the ambient level in the open plots. The experimental design was a randomized block design with three OTCs each for filtered and non-filtered treatments.

The field was prepared using standard agronomic practices. Farmyard manure was added uniformly during field preparation. The soil of the experimental field was sandy loam in texture (sand 45%, silt 28% and clay 27%) having organic carbon 0.67%, pH 7.4, cation exchange capacity 17.8 meq%, nitrogen 0.12% and phosphorus 0.065%. Seeds of carrot (*Dacus carota* var. Pusa Kesar) were hand sown in rows on December 12, 2002. Germination occurred on December 20, 2002. Subsequent thinning was done manually such that the density of the plants was reduced to 10 plants per chamber. When the plants germinated, fumigation was started and continued till the plants obtained their maximum root length (90 day age). Similar irrigation was followed in each chamber to provide identical water regime.

For physiological characteristics, five plants per chamber were marked and parameters such as rate of photosynthesis (Ps), stomatal conductance (Cs), transpiration (Es), internal CO_2 and water use efficiency (WUE) were analysed using Portable Photosynthetic system (LI-6200, LI-COR, Inc., Lincoln, NE, USA). The system was calibrated using a known CO_2 source (509 ppm concentration). The measurements were made on cloud free dates between 9.00 and 10.00 h. During the measurements the PAR over the waveband 400–700 nm ranged between 1100 and 1200 μmol m^{-2} s^{-1}. Chlorophyll fluorescence was also determined in these plants

between 10.00 and 11.00 h using Portable Plant Efficiency Analyser (PEA, MK2 9414, Hansatech Instruments Ltd. England). Leaf clips for dark adaptation were placed on the adaxial side of the leaves 10 minutes before measurement at excitation irradiance set at 200 μmol m^{-2} s^{-1}. Minimum fluorescence (*Fo*) and maximum fluorescence (*Fm*) were measured from which variable fluorescence (*Fv*) and ratio of variable and maximum fluorescence (*Fv/Fm*) were calculated.

For plant biochemical analysis, one plant per chamber was taken and parameters such as contents of chlorophyll (Maclachlan and Zalik, 1963), carotenoid (Duxbury and Yentsch, 1956), soluble protein (Lowry *et al.*, 1951), ascorbic acid (Keller and Schwager, 1977) and phenol (Bray and Thorpe, 1954), lipid peroxidation (Heath and Packer, 1968) and peroxidase activity (Britton and Mehley, 1955) were analysed at 60 days after germination (DAG).

Morphological parameters like root and shoot length, number of leaves per plant leaf area and root and shoot dry weights were analysed at 60 and 90 DAG. For this purpose, two plants per chamber were taken. Leaf area was measured using a Leaf Area Meter (Model 3100, LI- COR, Inc., Lincoln, NE, USA). For biomass analysis, five plants per chamber were picked at 60 and 90 DAG. Monoliths ($10 \times 10 \times 20$ cm^3) containing single plant with intact roots were carefully dug at random. Each plant was washed thoroughly and their parts were then separated and oven dried at 80 °C till a constant weight was obtained. Dry weight of the plants was taken and expressed in g $plant^{-1}$. Relative growth rate (RGR) and root shoot ratio (RSR) of the plants were calculated from biomass data using the formulae modified by Hunt (1982).

Oven dried plant samples were ground in a stainless steel grinder and passed through a 2 mm sieve. These powdered samples were used for determining nitrogen, sulphate-sulphur, total phosphorous, sodium, potassium, calcium and iron contents. Total nitrogen content was determined by Gerhardt Automatic N Analyzer (Germany). For determination of SO_4-S, total P, Ca^{++}, K^+, Mg^{++} and Fe^{++} contents, digestion of powdered root and shoot samples was done separately by the method given by Allen *et al.* (1974). Ca^{++}, K^+, Mg^{++} and Fe^{++} contents in digested material were determined with the help of Atomic Absorption Spectrophotometer (Model 2380, Perkin–Elmer, USA), whereas SO_4-S was determined by following turbidimetric method of Rossum and Villaruz (1961). Total P was estimated by method of Williams *et al.* (1970).

Eight hourly monitoring of air quality was done for SO_2 and NO_2 at the experimental site using Portable Gas Sampler following wet chemistry methodologies. Monitoring was done between 8.00 to 16.00 h daily. The instruments were placed successively in the filtered and non-filtered chambers in the centre. The height of the sampling was adjusted to crop height accordingly and varied from 33–45 cm at the end of the negative growth. SO_2 was estimated by the method of West and Gaeke (1956), NO_2 by Merrymann *et al.* (1973) and O_3 by Photometric Ozone Analyzer (Model 400 A, API, Inc., USA).

The significance of differences between treatments was calculated by student *t*-test.

3. Result

3.1. Concentration of gaseous pollutants

Air quality monitoring of non-filtered chambers showed that high concentrations of SO_2, NO_2 and O_3 were present at the experimental site. In the filtered treatment, daily mean concentrations of SO_2, NO_2 and O_3 were less as compared to the non-filtered treatment by 88, 83.8 and 89.5%, respectively (Table I). The air monitoring data collected during the entire experiment (December-March) suggested that SO_2 and NO_2 concentrations were higher during the earlier part of the experiment i.e. during the germination and vegetative growth of plants. O_3 concentration was, however, higher during the latter stage of the experiment (February-March), which was the time of root filling in the plants.

3.2. Physiological characteristics

Photosynthetic rate (Ps) showed significant increment of 12.06% in plants of FCs as compared to those of the NFCs (Figure 1). Stomatal conductance (Cs) and transpiration rate (Es) also increased significantly in plants growing in FCs (Figure 1). Chlorophyll fluorescence measurements showed (Table II) that F_0 decreased by 3.8% in FCs as compared to NFCs. Fm and Fv, however, showed increments of 23.17 and 27.5%, respectively in plants of FCs as compared to NFCs (Table II).

3.3. Biochemical characteristics

Total chlorophyll and carotenoid contents were higher in plants growing in FCs as compared to NFCs, but the increment was not significant (Figure 2). Ascorbic acid and phenol contents were significantly more in plants of NFCs as compared to those

TABLE I

Monthly Mean concentrations (ppb) of different pollutants at the experimental site in filtered (FCs) and non filtered (NFCs) chambers

	SO_2		NO_2		O_3	
Month	NFCs	FCs	NFCs	FCs	NFCs	FCs
December	40.33 ± 1.29	4.58 ± 0.48	39.58 ± 0.90	5.58 ± 0.55	35.33 ± 1.16	3.83 ± 0.36
January	39.32 ± 0.71	4.68 ± 0.23	41.48 ± 0.70	7.48 ± 0.39	35.48 ± 0.76	3.26 ± 0.18
February	36.57 ± 0.54	4.79 ± 0.21	38.14 ± 0.63	6.96 ± 0.41	38.96 ± 1.15	3.75 ± 0.22
March	33.61 ± 0.48	3.87 ± 0.20	35.71 ± 0.41	5.06 ± 0.21	43.74 ± 0.56	5.16 ± 0.24

Mean ± 1 S.E.

TABLE II

Changes in chlorophyll fluorescence (mV) of carrot plants grown in filtered and non filtered chambers (Mean ± 1 S.E.)

	Fo	Fm	Fv	Fv/Fm
Non filtered	519* ± 4.73	2741.66*** ± 44.77	2222.67*** ± 46.87	0.810*** ± 0.004
Filtered	500 ± 4.85	3568.66 ± 48.37	3068.00 ± 52.48	0.859 ± 0.003

Level of significance from FCs * = $p < 0.05$;** = $p < 0.01$;*** = $p < 0.001$; NS = Not significant; *Fo* = Initial Fluorescence; *Fm* = Maximum Fluorescence; *Fv* = Variable Fluorescence; *Fv/Fm* = Ratio of variable Fluorescence and Maximum Fluorescence.

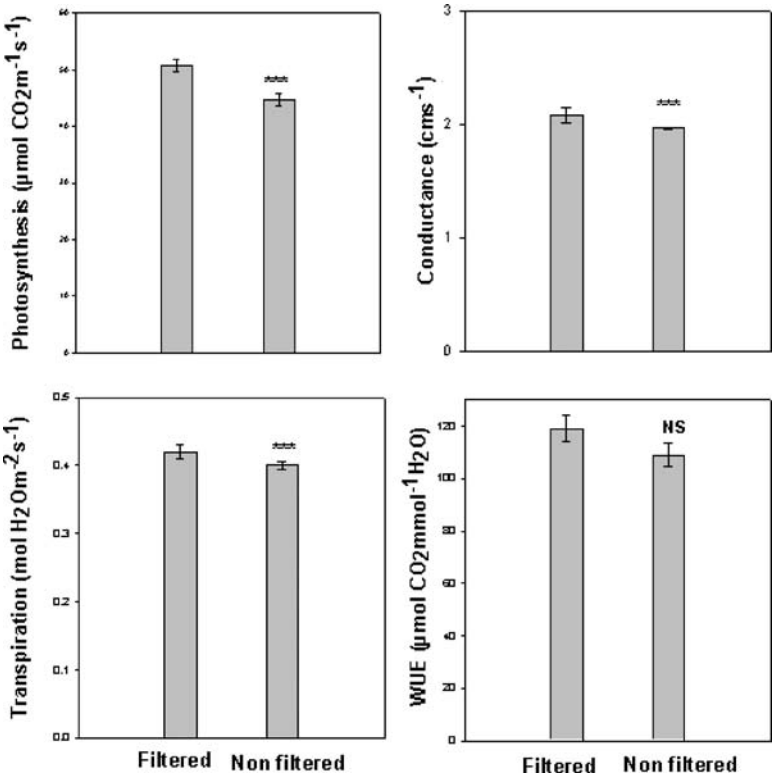

Figure 1. Selected physiological characteristics of carrot plants grown in filtered and non filtered chambers. Bars represent mean ± 1 SE Level of significant difference from FCs: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; NS = Not significant.

of FCs (Figure 3). Peroxidase activity also increased by 36.18% in plants growing in NFCs as compared to those growing in FCs (Figure 4). Lipid peroxidation was higher in plants of NFCs (Figure 4). Protein content, however decreased by 23.9% in plants of NFCs (Figure 3).

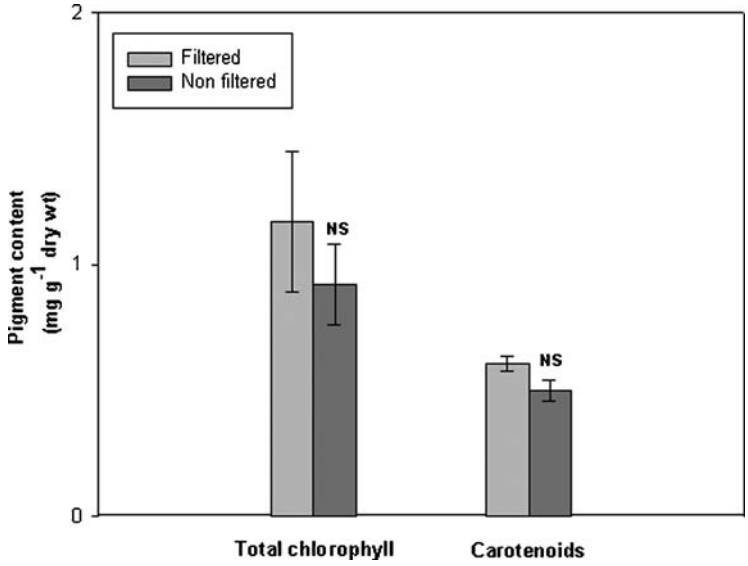

Figure 2. Total chlorophyll and carotenoid contents of carrot plants grown in filtered and non filtered chambers. Bars represent mean ± 1 SE Level of significant difference from FCs: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; NS = Not significant.

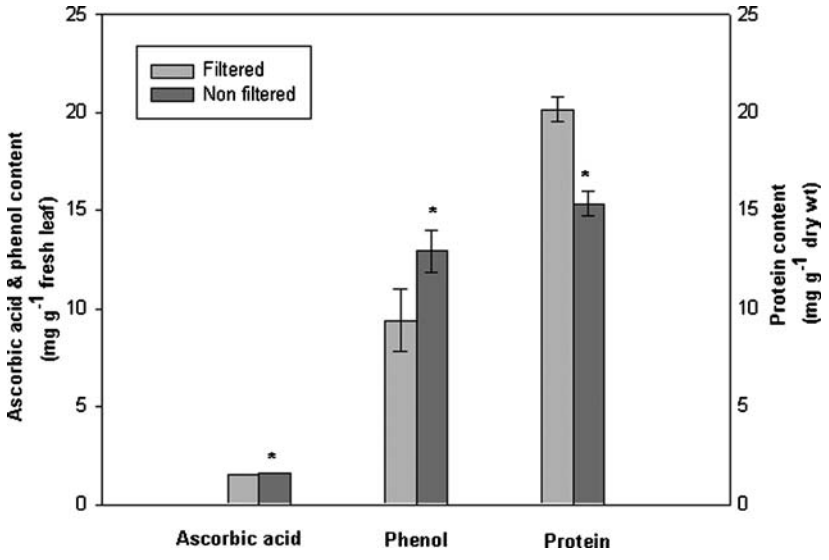

Figure 3. Ascorbic acid, phenol and protein contents of carrot plants grown in filtered and non filtered chambers. Bars represent mean ± 1 SE Level of significant difference from FCs: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; NS = Not significant.

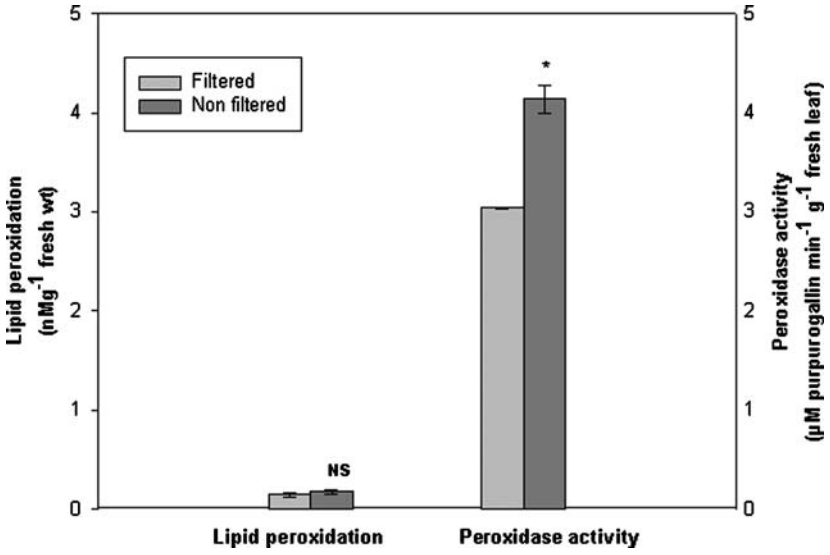

Figure 4. Lipid peroxidation and peroxidase activity of carrot plants grown in filtered and non filtered chambers. Bars represent mean ± 1 SE Level of significant difference from FCs: $^{*} = p < 0.05$; $^{**} = p < 0.01$; $^{***} = p < 0.001$; NS = Not significannt.

3.4. GROWTH AND BIOMASS

A comparison of treatment means of morphological parameters between the plants growing in FCs and NFCs chambers is shown in Table III. At 60 DAG, the plants growing in FCs showed increase in the root and shoot lengths. However, this increase was not significant (Table III). At 90 DAG, however, shoot length of the plants of FCs significantly increased by 14.10% (Table III). Number of leaves per plant showed increments in the plants growing in FCs as compared to those of NFCs. But this increase was significant only at 90 DAG (Table III). Leaf area of the plants

TABLE III

Agewise changes in morphological characteristics of carrot plants grown in filtered (FCs) and non filtered (NFCs) chambers (Mean ± 1 S.E.)

	60 DAG		90 DAG	
Parameters	NFCs	FCs	NFCs	FCs
Root length (cm)	$4.13^{NS} \pm 0.37$	5.20 ± 0.25	$18.27^{NS} \pm 0.73$	19.10 ± 0.55
Shoot length (cm)	$30.28^{NS} \pm 0.73$	38.18 ± 1.61	$142.13^{**} \pm 6.43$	165.46 ± 3.96
Number of leaves plant^{-1}	$6.33^{NS} \pm 0.66$	7.00 ± 0.57	$11.50^{***} \pm 0.71$	18.16 ± 0.77
Leaf area (cm^2)	$300.31^{*} \pm 47.93$	343.58 ± 26.60	–	–

Level of significance from FCs $^{*} = p < 0.05$;$^{**} = p < 0.01$;$^{***} = p < 0.001$; ^{NS} = Not significant.

TABLE IV
Agewise changes in biomass accumulation of carrot plants grown in filtered (FCs) and non filtered (NFCs) chambers (Mean ± 1 S.E.)

	60 DAG		90 DAG	
Parameters	NFCs	FCs	NFCs	FCs
Root weight (g)	$0.25^{NS} \pm 0.05$	0.40 ± 0.14	$1.52^{***} \pm 0.09$	2.78 ± 0.29
Shoot weight (g)	$3.13^{NS} \pm 0.36$	4.21 ± 0.93	$32.00^{**} \pm 3.47$	52.06 ± 5.45
Root shoot ratio (g g^{-1})	$0.064^{NS} \pm 0.012$	0.162 ± 0.070	$0.051^{NS} \pm 0.004$	0.061 ± 0.007
Relative growth rate	–	–	$0.07^{***} \pm 0.001$	0.08 ± 0.001

Level of significance from FCs $^{*} = p < 0.05$; $^{**} = p < 0.01$; $^{***} = p < 0.001$; ^{NS} = Not significant.

growing in FCs increased significantly by 12.59% as compared to the plants of NFCs (Table III).

At 60 DAG, the increments in root and shoot weights of FCs were however not significant as compared to those in NFCs (Table IV). At 90 DAG, however, root and shoot weights showed significant increases of 45.32 and 38.63%, respectively in plants growing in FCs as compared to those in NFCs (Table IV). RGR and RSR were 12.5 and 16.3% lower in plants growing in NFCs as compared to those in FCs at 90 DAG (Table IV).

3.5. Nutrients concentrations

Total nitrogen content of roots in NFCs was significantly lower (14.73%) as compared to those in FCs (Table V). However, in shoots significant increase of 15% in N content was observed in the plants growing in NFCs as compared to those of FCs (Table V). Total phosphorus content increased significantly by 57.14 and 73.6% in roots and shoots, respectively of plants in FCs as compared NFCs (Table VII). Sulphate sulphur content was significantly higher in plants growing in NFCs as

TABLE V
Nutrient concentrations (mg g^{-1} dry weight) in carrot plants growing in filtered (FCs) and non filtered (NFCs) chambers (Mean ± 1 S.E.)

	Root		Shoot	
Characteristics	NFCs	FCs	NFCs	FCs
Total Nitrogen	$2.54^{*} \pm 0.08$	2.99 ± 0.01	$1.09^{*} \pm 0.03$	0.95 ± 0.01
Total Phosphorus	$0.09^{**} \pm 0.005$	0.21 ± 0.01	$0.10^{**} \pm 0.008$	0.38 ± 0.02
Sulphate sulphur	$0.73^{*} \pm 0.04$	0.17 ± 0.01	$1.35^{*} \pm 0.20$	0.38 ± 0.12

Level of significance from FCs$^{*} = p < 0.05$; $^{**} = p < 0.01$; $^{***} = p < 0.001$; ^{NS} = Not significant.

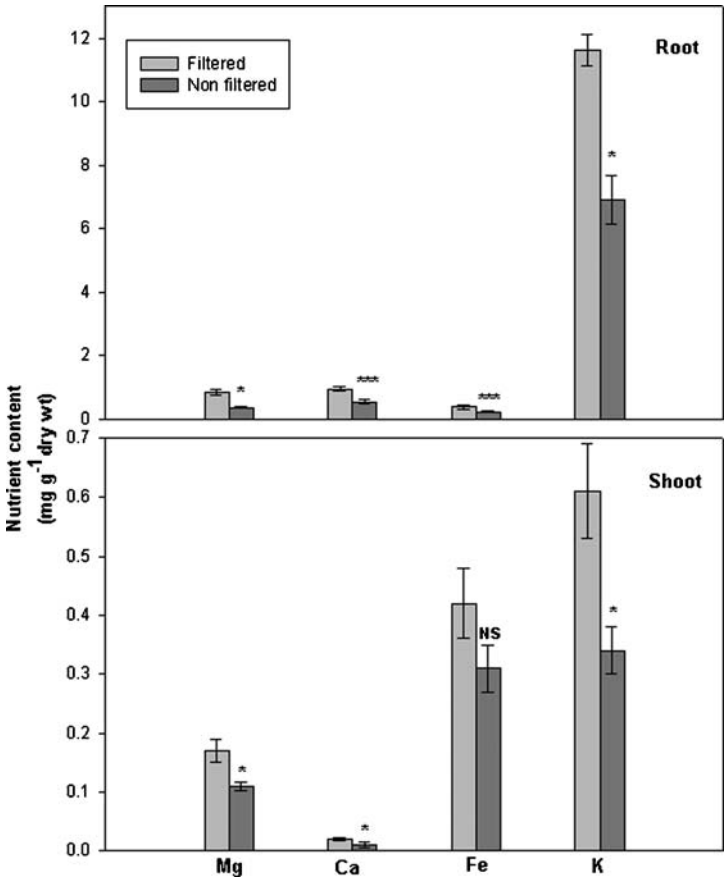

Figure 5. Changes in nutrient contents (mg g^{-1} dry wt) in roots and shoots of carrot plants grown in filtered and non filtered chambers. Bars represent mean ± 1 SE Level of significant difference from FCs: $^{*} = p < 0.05$; $^{**} = p < 0.01$; $^{***} = p < 0.001$; NS = Not significant.

compared to those in FCs. The increase was 329.41 and 255.02% in roots and shoots, respectively of the plants of NFCs.

Mg, Ca, Fe, and K contents showed higher values in plants of FCs compared to NFCs. Mg, Ca, Fe and K contents respectively decreased by 57, 42.5,40.5 and 40.58% in the roots (Figure 5) and by 35.2, 48, 50 and 44.2% in shoots of the plants of NFCs as compared to those growing in FCs (Figure 5).

4. Discussion

The experiment performed in the open top chambers clearly suggests the adverse effects of ambient air pollution on plants growing in the suburban areas of Varanasi. The air quality data indicate that SO_2, NO_2 and O_3 were present in concentrations

high enough to cause growth reductions in carrot plants. Gaseous pollutants showed considerable monthly variations during different stages of the plants. High levels of SO_2 and NO_2 observed at the experimental site may be associated to its close proximity to the national highway from Delhi to Howrah experiencing heavy vehicular transportation. During the earlier stages of experiment, when the temperature and light intensity were comparatively lower, NO_2 showed higher concentration than O_3. However, during the latter stages of the experiment, increase in temperature and high light intensity favoured the formation of O_3, thus O_3 concentrations were higher. Agrawal *et al.* (2003) observed similar concentrations of SO_2 (30 ppb $\pm$1.14) and NO_2 (33.8 ppb $\pm$ 3.20) at a site closer to the present experimental site. However, O_3 concentration (11.3 ppb $\pm$ 1.34) was lower as compared to those in the present study. Hassan *et al.* (1995) have also observed higher levels of ambient oxidant in rural areas of Egypt.

Plants growing in NFCs showed significant reductions in Ps, Es and Cs as compared to those growing in FCs. Studies conducted by Paludan-Muller *et al.* (1999) showed that seedlings of *Fagus sylvatica* L., when exposed to ambient air (NF) and charcoal filtered air (CF) showed a decrease of 2, 4.58 and 4.1% in Ps, Es and Cs in NF plants as compared to CF plants. Chronic exposure to air pollutants can inhibit, stimulate or have no effects on Ps, Es and Cs depending on the exposure dose and species involved (Darrall, 1989). Low concentrations of SO_2 for prolonged duration have been found inhibitory to photosynthetic rate even after initial stimulation of Ps at very low concentration (Atkinson *et al.*, 1988). Black *et al.* (1982) showed that 40 ppb of SO_2 and 50 ppb of O_3 together caused greater reduction in Ps than O_3 alone. Bull and Mansfield (1974) reported an additive effect of SO_2 and NO_2 combination in reducing Ps in *Pisum sativum* L. seedlings. In the present study, decrease in Cs may be responsible for reduction in Ps. Decrease in Cs of plants exposed to pollutants indicates an avoidance mechanism of plants under air pollution stress. The lower value of WUE of plants in NFCs than FCs may be ascribed to the decline of Ps of these plants.

A decrease in *Fv* was observed in plants growing in NFCs, which reflects the decrease in light quenching capacity of the chlorophyll in these plants. *Fv/Fm* ratio also showed a significant decline in plants of NFCs. Decrease in *Fv/Fm* ratio can be directly correlated to decrease in Ps in plants growing in NFCs. The relationship between decrease in *Fv/Fm* and photoinhibition of Ps is reported in the leaves of *Swietenia* where Fv/Fm ratio decreased to the same extent as O_2 evolution (Krause *et al.*, 1995).

Reductions in total chlorophyll contents of plants under ambient air pollution have been reported by Agrawal *et al.* (2003) in various crops grown in suburban and rural areas of Varanasi. In this experiment, however, chlorophyll reductions were not significant. Ascorbic acid is known to provide stability to the plant cell membranes during pollution stress and scavenges cytotoxic free radicals, which can otherwise cause lipid peroxidation and destruction of membranes (Dindsa *et al.*, 1982). In the present study, the plants growing in NFCs showed an increase

in ascorbic acid content. This increase might be a consequence of substantial oxidative stress. Lee *et al.* (1984) reported accumulation of ascorbic acid content in plant cells after pollutant exposure. A similar result was obtained by Varshney and Varshney (1984), where resistant species showed increase in ascorbic acid content, whereas sensitive species showed decline. Carrot plants seem to increase defense level by enhancing ascorbic acid content in response to air pollution stress. 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). Protein concentration decreased significantly in the leaves of the plants grown in non filtered chambers. Deepak and Agrawal (2001) have shown reductions in protein content of two cultivars of soybean at SO_2 concentration of 60 ppb for 8 h daily for 70 days. Agrawal and Agrawal (1990) observed a reduction of 39% and 6.8%, respectively in *Vicia faba* and *Cicer arietinum*, 30 days after exposure of 99 ppb O_3 for 2 h daily.

Level of lipid peroxidation was higher in plants grown in NFCs as compared to those in FCs, which confirms an oxidative stress in plants caused due to SO_2, NO_2 and O_3. Lipid peroxidation measured as MDA concentration has been correlated with the degree of injury to membrane under O_3 exposure (Ranieri *et al.*, 1996). Calatayud *et al.* (2002) reported significant increase in lipid peroxidation of *Lactuca sativa* growing in NFCs as compared to those growing in O_3 free air. Increase in peroxidase activity as observed in the present study was also reported by Peters *et al.* (1989). Castillo *et al.* (1984) showed that the activity of extracellular peroxidase, especially the basic form whose activity was supported by ascorbate oxidation increased markedly and rapidly after fumigation with pollutants.

Height and biomass accumulation of plants growing in NFCs decreased as compared to those growing in FCs. Pandey and Agrawal (1994) showed significant reductions in height of *Delonix regia*, *Cassia fistula* and *Carissa carandas* when grown at a site experiencing SO_2, NO_2 and O_3 concentrations of 17.86, 24.38 and 12.24 ppb, respectively at Varanasi. Agrawal *et al.* (2003) also recorded reductions of 21.02, 2.69, 51.7 and 43.75%, respectively in wheat, mustard, mung and palak, growing at a site having mean pollutant concentrations of 17.16 ppb SO_2, 28.05 ppb NO_2 and 10.33 ppb O_3. Reductions in leaf area and leaf number may be due to decreased leaf production rate and enhanced senescence (Pandey and Agrawal, 1994). The effect of reduced leaf area resulted in reduced absorbed radiations and subsequently in reduced Ps rate.

In the present study, plants grown in NFCs showed significant reductions in root and shoot biomass, at 90 DAG (final harvest). Yield reductions in beans from ambient air have been previously demonstrated through air filtration studies in USA (Heggestad *et al.*, 1980) and Europe (Bonte *et al.*, 1988). The root fraction was more severely damaged than the top fraction as evidenced by decrease in root: shoot ratio between the plants grown in NFCs as compared to those grown in FCs. Reduced root growth may be caused by reductions in available C from photosynthesis (Saxe,

1991), an increased C demand for above ground repair or replacement mechanisms (Kelly *et al.*, 1993) and/or by impaired phloem functioning (Spence *et al.*, 1990). Reduction in root: shoot ratio was also reported in *Plantago major* L. upon O_3 exposure in OTC's (Reiling and Davison, 1992). RGR of the plants of NFCs was lower as compared to plants growing in FCs. This decrease can be attributed to the reduced rate of photosynthesis of the plants due to reduced leaf number and leaf area. Reiling and Davison (1992) also reported reduction in RGR of *Plantago major* L. when fumigated with 70 nl l^{-1} of O_3 for 7 h d^{-1} for 2 weeks.

Nitrogen content increased significantly in shoots, but decreased significantly in the roots of carrot plants growing in NFCs as compared to those growing in FCs. This increase in N content may be ascribed to the foliar absorption of NO_2 present in NFC because soil nitrogen content is uniform in filtered and non-filtered chambers. NO_2 has been shown to be absorbed in and assimilated into various organic nitrogen compounds leading to elevated foliar nitrogen content (Sandhu and Gupta, 1989). O_3 and SO_2 have, however, been shown to reduce nitrogen content in plants (Wellburn *et al.*, 1981; Agrawal and Agrawal, 1990). Rajput and Agrawal (2004) showed significant reductions in N content of seed of *Pisum sativum* grown in ambient air of Varanasi. Interestingly, in roots, a contrasting response was observed, which suggests that the absorbed N from NO_2 was retained in shoot only and did not translocate to roots in plants growing in NFCs.

Nutrients such as Mg, Na, Ca, Fe and K in both root and shoot fractions of plants showed significant decrease in plants growing in NFCs as compared to those in FCs. This response may have been caused by the combination of factors such as changes in root development pattern due to air pollution stress and reduction in soil nutrient absorption due to disturbed plant growth. Reductions in K and Ca contents of SO_2 exposed plants have been reported for *Vicia faba*, *Cicer arietinum* and *Glycine max* (Keller and Jager, 1980).

SO_4-S concentrations increased significantly in root and shoot portions of the plants grown under NFCs as compared to those under FCs. Sulphur accumulation in plants growing in SO_2 enriched environment is a well-known phenomenon (Dekok, 1990). SO_2 enters mainly through stomata, dissolves on cellular moist surfaces of mesophyll cells and is hydrated into sulphurous acid. The rapid dissociation of this acid leads to SO_3^{2-} and HSO_2^- ions which are subsequently oxidized to less toxic sulphate in course of detoxification (Huve *et al.*, 1995). Pandey and Agrawal (1994) also found sulphate accumulation in leaves of three woody transplants grown in the urban environment of Varanasi.

The present experiment clearly suggests that the gaseous pollutants were present in concentration high enough to cause unfavourable changes in physiological and biochemical characteristics leading to significant reductions in growth and biomass of carrot plants. Nutrient quality of the plants was also inferior at concentrations of air pollutants in ambient air. The combined effects of all the three pollutants may have acted synergistically in causing unfavourable impact on carrot plants rather than the individual pollutants. The study also confirms the usefulness of OTCs

in assessing crop loss under field conditions. Use of OTCs is particularly recommended for developing countries, where air pollutant concentrations are likely to increase severalfold in future.

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