BACKGROUND INFORMATION
RESPONSES OF PLANTS TO AIR POLLUTANTS

A: Plant growth and morphological traits

Effect of sulphur dioxide (SO₂)

Leaves, the highly exposed organs of plants, are affected the most by air pollution. By virtue of their unique position and specialized functions (gaseous exchange), the leaves experience the maximum brunt of exposure and accordingly have to undergo changes in form, structure and function which often serve as markers of environmental pollution (Saxe, 1996). Leaf injuries like chlorosis, necrosis, browning and burning adversely affect physiological functions especially the photosynthetic activity of plants. Hindawi (1970) holds that leaf structure plays the most important role in building carbohydrates and other vital plant products and foods. Therefore, injury to the leaf will affect vitality of the whole plant.

Dry weight of the foliage is indicative of plant growth (Dubey, 1994). Dry weight of plants exposed to SO₂ may decrease severely even in the absence of visible injury (Kumawat and Dubey, 1989; Rao and Dubey, 1989). On the contrary, a continuous exposure to low concentrations of SO₂ can result in growth stimulation; 20 nl l⁻¹ SO₂ enhanced the growth of nine crop species by about 5-10% (Roberts, 1984). Coleman et al. (1989) have demonstrated that a radish plant exposed to 4000 nl l⁻¹ SO₂ for 15 days had a reduced root/shoot ratio, respiration and the rate of nitrogen absorption by roots, compared to the non-fumigated plants, due to a decrease in assimilate partitioning to root growth.
The coexisting environmental conditions, such as drought and salinity may decrease SO$_2$-uptake rate by increasing the stomatal resistance (Huang and Murray, 1993), thereby reducing the internal accumulation of SO$_2$ and its toxicity in plants. Many factors such as salt stress that can disturb metabolic processes like photosynthesis, respiration and protein metabolism (Levitt, 1980), may finally decrease or destroy the homeostatic safety margin in terms of energy and enzyme capacity required to counter the detrimental effects of SO$_2$ (Qifu and Murray, 1994). Both salinity and SO$_2$ can decrease the leaf area, dry weights of roots and shoots and the fresh weight of root nodules. SO$_2$ may increase the shoot:root ratio. Low salinity pretreatment protected plant growth from SO$_2$ injury, probably by decreasing SO$_2$ uptake via increase in stomatal resistance. However, high salinity treated plants, despite stomatal closure, were severely injured by the subsequent SO$_2$ exposure (Qifu and Murray, 1994).

Effect of nitrogen dioxide (NO$_2$)

Air borne nitrogen compounds are rapidly assimilated. Thus, the influx of nitrogen oxides from air may be considered as a small additional nitrogen supply to growth, which does not cause detectable damage (Slovik et al., 1996). Although most studies have indicated phytotoxic effects of NO$_2$, some have suggested growth-stimulatory effects of exposure to low concentration of NO$_2$ (Okano, et al., 1985; Edelbauer and Maier, 1988; Sandhu and Gupta, 1989).

Black and Black (1979) and Neighbour et al. (1988) have reported damaged epidermal cells on the surfaces of polluted leaves. Reduction in turgor pressure of epidermal cells adjacent to guard cells would likely prevent stomata from closing.
properly. Similarly, Lendzian and Kersteins (1988) have suggested that exposure to NO\textsubscript{2} can increase water permeability of cuticles. Cuticular damage and increased permeability in response to wet acid deposition are quite common (Barker and Ashenden, 1992).

**Effect of pollutant combinations**

SO\textsubscript{2} and NO\textsubscript{2} are apparently detoxified in tissue layers outside the vascular cylinder as the direct effects of fumigation are observed only in mesophyll cells (Rantanen et al., 1994). Direct leaf injury due to NH\textsubscript{3}\textsuperscript{+} or NH\textsubscript{4}\textsuperscript{+} occurs only at a relatively high concentration (Vander Eerden, 1982). These ions interfere with several physiological processes even without causing tissue injury. At high concentrations or during long-term exposures adverse effects become dominant.

Plants growing under field conditions near thermal power plants are exposed to an array of air pollutants which interact and affect plant morphology and metabolism. These effects may be synergistic, additive or antagonistic depending upon environmental factors and the species involved (Tingey and Reinert, 1975). Plant growth may increase or decrease under SO\textsubscript{2} or NO\textsubscript{2} effect depending upon the exposure conditions (Murray et al., 1994). Dry deposition of SO\textsubscript{2}, NO\textsubscript{x} and O\textsubscript{3} may occur through absorption by canopy surfaces (Slovik et al., 1996) as well as through stomatal uptake into the needle mesophyll. SO\textsubscript{2} and NO\textsubscript{2} can prove to be potential nutrients to the leaf as these can be oxidized to sulfate and nitrate, reduced and then metabolized to amino acids and proteins (Garsed, 1984; Welburn, 1990). The effects of long-term plant exposure to mixtures of SO\textsubscript{2} and NO\textsubscript{2} may be either synergistic or antagonistic, but in the case of grasses these are commonly synergistic (Whitmore, 1985).
Studies on grasses have shown that combination of SO\textsubscript{2} and NO\textsubscript{2} may be toxic to plant growth (Ashenden, 1979; Ashenden and Williams, 1980; Whitmore and Freer-Smith 1982). *Phleum pratense* L. was more sensitive to SO\textsubscript{2} when growth was slow and it was suggested that exposures during winter may be particularly phytotoxic. SO\textsubscript{2} and NO\textsubscript{2} not only contribute to acidity in rain which may seriously affect plant growth, but also assert substantial effect by their direct action as gaseous pollutants.

Exposure of field crops to SO\textsubscript{2} + NO\textsubscript{2} may reduce growth, yield and quality of crops (Roberts, 1985; Whitmore, 1985). Toxicity of these gases depend on many factors including the rate of uptake and the capacity of the plant to detoxify, metabolize, transport and use or store their derivatives within the plant (Murray and Qifu, 1994).

Growth of deciduous species may be severely decreased by exposure to SO\textsubscript{2} + NO\textsubscript{2} mixtures during the growing season. Sensitivity differs between species (Rantanen et al., 1994), and the order of relative sensitivity coincides well with the degree of chronic injury at sites near the pollution source (Whitmore and Freer-Smith, 1982). The autumn-sown smooth-stalked meadow grass (*Poa-pratensis* L.) exposed to SO\textsubscript{2} or SO\textsubscript{2} + NO\textsubscript{2} underwent marked growth reductions during the winter and spring; the plants recovered during the summer but flowering was inhibited. Broad-leaved tree species have also shown substantial decreases in growth when exposed to SO\textsubscript{2} plus NO\textsubscript{2} (Whitmore and Freer-Smith, 1982).

The growth of leaf, petiole, flower and pod is normally inhibited in heavily-polluted areas. Length and frequency of trichomes on leaf surface may increase, while the stomatal frequency may decrease in the polluted habitats. Subsidiary-cell complex
are normally similar in all plant populations of the polluted or relatively unpolluted habitats (Sharma et al., 1980).

Coal-smoke pollution also causes chlorosis and browning of leaves, suppressed plant vigour, inhibition in fruit-setting and low yield, as observed in trees like *Dalbergia sissoo*, *Mangifera indica* and *Psidium guajava* (Ghouse and Amani, 1978; Ghouse and Khan, 1978; Lone et al., 1995) and *Zizyphus mauritiana* L. (Trag et al., 2001) weeds like *Commelina benghalensis*, *Croton bonplandianum*, *Calotropis procera* and *Euphorbia hirta* (Zaidi et al., 1979; Mishra, 1982; Gupta and Ghouse, 1987; Lone et al., 1995), grasses such as *Cyodon dactylon*, *Dactylis glomerata* and *Lolium perenne* (Asheiden, 1987; Khan et al., 1988), cereals such as wheat and barley (Lorenzini et al., 1990; Atkinson et al., 1991) and vegetables like tomato, egg plant, okra and cucurbits (Gupta and Ghouse, 1987; Khan et al., 1991; Khan and Khan, 1991, 1994a,b).

Decrease in the total number of leaves developed per plant, together with a higher degree of defoliation, indicates that coal-smoke pollutants inhibit the production of leaves (Gupta and Ghouse, 1987) and stimulated the formation of abscission layers leading to a premature leaf fall.

**Effect of carbon dioxide (CO₂)**

Rising carbon dioxide level generally stimulates plant growth but some species seem to remain relatively unaffected (Eamus and Jarvis, 1989; Idso, 1989; Krupa and Kickert, 1989). CO₂ enrichment which is widely used in growth chambers, green houses and *in vitro* cultures to increase the growth rate and quality of plants (Wittwer, 1983; Lakso et al., 1986), should decrease O₂-induced inhibition of photosynthesis.
and increase net photosynthesis (Ehleringer, 1979; McHale et al., 1987; Kimball, 1983). CO$_2$ enrichment has been shown to increase plant height (Imai and Murata, 1979b), dry weight (Imai and Murata, 1979a), tillering (Imai and Murata, 1976) and yield (Yoshida, 1973; Cock and Yoshida, 1973) of rice. The number of panicles per plant was the yield component primarily responsible for high grain yields with higher CO$_2$ concentration in rice (Baker et al., 1988). The extra C in plant leaves induced by high CO$_2$ concentrations may enhance leaf size, number of branches (or tillers) and number of node along the branches which support leaves, thus leading to a greater leaf area (Roger et al., 1984; Bhattacharya et al., 1985; Allen et al., 1988). Trees and tree crops have generally shown positive growth responses to CO$_2$ enrichment (Tolley and Strain, 1984; Luxmore et al., 1986; Norby et al., 1986; Downton et al., 1987; Brakke, 1989).

Effect of ozone (O$_3$)

Ozone stress can reduce total plant dry weight, leaf area, leaf area duration; relative growth rate of leaves, stems and roots and the net assimilation rate (Endress and Grunwald, 1985). Ozone may react at the leaf surface and within the apoplastic space outside the plasmalemma. The surface of the leaf includes the cuticle and in many species, trichomes, glands and wax deposition. Large amounts of ozone can be absorbed by the leaf surface, adding to the apparent leaf diffuse conductance.

Ozone can cause foliar injury, crop loss, reduced growth and increased susceptibility to plant pathogens and insect herbivory in many geographic regions (Krupa and Manning, 1988). The mechanisms by which O$_3$ induces these responses may be varied but some effects may be associated with accelerated foliar senescence which so often occurs in the exposed plants (Pell and Dann, 1991).
The symptoms of visible injury take many forms and usually include changes in pigmentation. The visible ozone injury symptoms vary with species and may include chlorotic or necrotic spotting, flecking or blotching and browning or reddening. Chlorosis may appear as an upper leaf surface stipple, and necrosis may be bifacial. Yield decreases with increased tropospheric ozone have been demonstrated for many crop plants (Heck et al., 1988; Krupa and Kickert, 1989).

The strong oxidizing properties of ozone affect various biological components and metabolites present in or on plant cells. It oxidizes the sulfhydryl and fatty acid double bonds as well as other cellular scavenging compounds and greatly increases the membrane permeability (Mudd, 1982; Guderian et al., 1985).

B : Stomatal behaviour and photosynthesis

Effect of SO₂

Absorption of air pollutants by leaf depends on concentration gradient from leaf exterior to leaf interior and on dimensions of stomata on the leaf surface. The stomata tend to close in the presence of higher concentrations of gaseous pollutants (Mansfield, 1988b), possibly in stress avoidance. However, lower concentration of pollutants may stimulate stomatal opening in some species (Mansfield and Freer-Smith, 1984). Low doses of SO₂ may induce an opening, while higher ones may cause a narrowing or closing of the stomatal pore as in the Ulmus americana seedlings (Noland and Kozlowski, 1979).

Stomates are the primary route of gas uptake into the interior of the leaf. They control the entry of gases into sub-stomatal cavity around which lies the mesophyll tissue. Once inside the tissue, the different gases dissolve in the water phase of the
cell walls and dissociate according to their dissociation constants and the prevailing pH values. The undissociated, neutral portion permeates via the plasmalemma into cytosol and from their following pH-gradients to the natural or alkaline compartments (chloroplasts, mitochondria, nuclei). There they get trapped and accumulate because of the anion trap function of those organelles (Pfanz, et al., 1987a). During dissociation, protons are formed that may lead to pH disturbances. The stomatal action is controlled by CO₂ concentration in the substomatal cavity, leaf water status, epidermal cell characteristics and the state of ion flux (Mansfield and Freer-Smith, 1984).

Environmental pollutants may alter the rate of photosynthesis through several mechanisms including:

- clogging of stomatal pores,
- altering the optical properties of leaves by changing the reflectance and decreasing the light intensity that reaches the leaf interior,
- inhibiting the photosynthetic process through break down of chlorophyll as well as changing the activity of carbon-fixing enzyme, the phosphorylation rate and pH buffering capacity,
- disrupting the integrity of membranes and ultrastructure of organelles, and
- inducing changes in leaf anatomy (Matyssek et al., 1995).

The photosynthetic pigments and many enzymes are associated with membranes of the chloroplasts. SO₂ can easily disrupt the fine structure of membranes (Wellburn et al., 1972), inactivate many enzyme systems by splitting their disulphide linkage (Bailey and Cole, 1959; Cecil and Wake, 1962) and activate some hydrolytic enzyme systems possibly by bringing about some conformational changes.
Although there is some debate about the specific mechanisms by which gaseous air pollutants, such as sulphur dioxide, cause injury to conifers (Ferenbaugh, 1979) it is generally agreed to that the pollutants enter the needle through the stomata and damage the photosynthetic tissue (Benedict, 1973).

It is known from fumigation studies that the degree of injury to plants varies with the concentration of pollutants and with the time and duration of exposure, but differences in responses to air pollutants have been noted among species and even among individuals of the same species (Brandt and Heck, 1968; Guderian, 1977). A reduction in the net photosynthetic capacity of a tree may affect the availability and storage of foods and the production of growth regulators, which may have a lasting effect on growth for periods of up to several years (Fritts, 1976).

Reduction in photosynthesis would be expected when leaves are injured or sored by exposure to the environmental pollutant, but photosynthesis is often inhibited long before a visible injury or growth reduction becomes apparent. The amount of reduction in photosynthesis and of the recovery following a pollution episode, varies with specific pollutants and their dosage/treatment and with the environmental condition (Kramer and Kozolwski, 1979; Kozolwski and Constantinidou, 1986). Photosynthetic suppression by pollutants may variously reflect changes with stomatal aperture and the biochemical fixation of CO$_2$ (Bennett and Hill, 1974; Dugger et al., 1963). Old healthy leaves and the severely injured leaves may have a lower photosynthetic rate than the young and less-injured leaves (Salam and Soja, 1995).

The effect of SO$_2$ depends on such factors as air humidity, light intensity, physiological activity of tissue and pH value inside the plant cells (Niewiadomask and Miszalski, 1995). Humidity influences the assimilation of SO$_2$ when stomata are closed and the gas enters the plant tissue through the cuticle (Lee et al., 1997).
The photosynthetic carbon assimilation decreases with increase of SO$_2$ concentration (Mudd, 1976; Chaphekar, 1982; Darrall, 1989; Gupta, 1992; Sharkey et al., 1994; Meng et al., 1995). Low concentration of SO$_2$ may sometimes promote photosynthesis (Ferenbaugh, 1979). The decline in photosynthesis was considerably greater at longer periods of exposure to $^{14}$CO$_2$. Total chlorophyll content declines with increasing SO$_2$ concentration, and the decrease in photosynthesis may be due to chlorophyll breakdown. Various enzymes of Calvin cycle are also likely to be affected by SO$_2$ fumigation (Ziegler, 1972; Gupta, 1992).

No effects on net photosynthesis were observed at SO$_2$ concentrations below a threshold of about 140 parts 10$^{-9}$ (400 µg m$^{-3}$), and the growth and yield did not then differ from clean-air control treatments in Vicia faba (Black and Unsworth, 1979). Sulphur dioxide inhibited leaf area index during canopy coverage before any effects on photosynthesis were detected in soybean (Lee et al., 1997). Stomatal resistance was not directly affected by SO$_2$ fumigation, but was indirectly affected due to a feed back loop between net photosynthesis rate and internal CO$_2$ concentration (Lee et al., 1997). Increasing levels of internal carbon dioxide (the regular substrate of photosynthesis) served as the potential acidic pollutant.

SO$_2$ can also have indirect effects on mechanisms such as nitrogen fixation (Sundstron and Hällgren, 1973), a reductive and energy requiring process. In nitrogen fixing algae, for example, the required energy is provided directly by the process of photosynthesis, whereas in other organisms such as nondulce bacteria, the connection to photosynthesis is indirect. The inhibitory effects of SO$_2$ on energy production by photosynthesis may be transmitted to have a similar inhibitory effect on nitrogen fixation which is completely inhibited at low pH conditions (such as these created by
H$_2$SO$_3$) (Malhotra and Hocking, 1976). The decrease in phosphoenol pyruvate (PEP) activity and concentration as a result of hydrolysis and mobilization from leaves may also inhibit photosynthesis (Joshi et al., 1993).

Inhibition of photosynthetic CO$_2$ fixation with low concentrations of sulphite added to the extracts of spinach and maize chloroplasts (Ziegler, 1972, 1973 and 1975) has shown that activities of RuBP and PEP carboxylase are inhibited by sulphite and that SO$_2$ may act by competing with CO$_2$ or bicarbonate for the binding sites in RuBP carboxylase (Black and Unsworth, 1979). Sulphate had a little effect on ATP formation in bean mitochondria (Ballantyne, 1973) even though it is known to inhibit photophosphorylation in chloroplasts (Malhotra and Hocking, 1976).

After 30 min of exposure to $^{14}$CO$_2$, the distribution of radioactivity decreased in phosphoglyceric acid (PGA), hexose phosphates and sucrose and increased in phosphoglycollate, phosphoenol pyruvate (PEP), serine, glycine, glycollate, malate and an unidentified amino acid due to SO$_2$ fumigation (Gupta, 1992). At each exposure to $^{14}$CO$_2$ the proportion of radioactivity decreased in PGA in the SO$_2$ fumigated plants; it is probably associated with the RuBP-C activity. Thus flux of carbon may be blocked by SO$_2$ fumigation even before the synthesis of PGA at carboxylation phase. $^{14}$C label in sugar phosphates decreased with the increasing SO$_2$ concentration and so did the RuBP-C activity (Gupta, 1992).

Effect of SO$_2$ and NO$_2$ combinations

Exposure of Scots pine trees to low concentrations of SO$_2$ and NO$_2$ during the growing season may have significant long-lasting effects on photosynthetic parameters. Small alteration in cellular components induced by these pollutants may
not result in functional disturbances when environmental conditions are favourable. However, these changes may delay the acquisition of freezing resistance in the autumn or modify the responses to environmental stresses (Strand, 1993).

The inhibition of photosynthesis by SO₂ + NO₂ is often greater than the effect of either gas alone (Darrall, 1989; Wellburn, 1990). The difference in photosynthetic characteristics between control trees and fumigated trees during early winter may be explained by an increased susceptibility to low and subfreezing temperatures in the needles of fumigated trees (Strand, 1995). Photosynthesis can be affected at low concentrations of SO₂ and NO₂ as a mixture, even though pigment concentration of the needles is unaffected (Strand, 1993; Rantanen et al., 1994). The effect is influenced by environmental factors like temperature and light (Kropff et al., 1990; Caporn et al., 1991).

Several authors have reported decreases in photosynthesis and increases in respiration after SO₂ and NO₂ exposure (Strand, 1993). Jung and Winter (1992) observed lower photosynthetic rates in needles of *Abies nordmanniana* stressed with SO₂ and low Mg in comparison with needles stressed only with SO₂.

Increasing the SO₂ or NO₂ alone did not affect the relationship between photosynthesis and increasing CO₂ in *Glycine max*. However, in the combined treatment (SO₂ + NO₂) as the level of either pollutant increased, the reduction in photosynthesis caused by any concentration of the other pollutant increased. At saturating CO₂ concentration, stomatal conductance of the *Glycine max* leaves decreased with increasing concentration of the pollutant mixture, but not as a function of concentration for each pollutant alone (Carlson, 1983).
Photosynthesis decreases during conditions of K deficiency (Marschner, 1986); exposure to SO$_2$ + NO$_2$ and limited availability of K affect photosynthesis and the quantity of the starch present more than the fumigation alone. The same was true for exposure to limited available K in presence of ozone or simulated acid rain (Holopainen and Nygren, 1989; Lütz et al., 1992).

Acidification strongly affects photosynthesis. Once inside the cytoplasm, acidification directly influences enzymatic reactions. The situation gets even more difficult when acidic air pollutants are additionally present in the cell wall phase. Depending on their ionic form (dissociated or undissociated as determined by the prevailing pH conditions), they will permeate more or less easily via the plasmalemma into the protoplast interior (Yin et al., 1991).

The Calvin cycle enzymes like fructose-bisphosphatase, sedoheptulose-bisphosphate or ribulose-bis-phosphate-carboxylase are extremely sensitive to pH-disturbances and are rapidly inactivated by acidification (Pfanz et al., 1998).

Effect of CO$_2$

The atmospheric CO$_2$ concentrations affect photosynthesis several ways. First, more CO$_2$ will tend to enter the leaves of plants exposed to high external CO$_2$ levels simply by mass action and the increased CO$_2$ gradient between leaf and air. This, however, is of relatively minor significance compared to the influence of CO$_2$ on the primary photosynthetic enzyme of C$_3$ plants, namely, ribulose bisphosphate carboxylase-oxygenase (Rubisco). Both CO$_2$ and O$_2$ are substrates of Rubisco, and so the two molecules compete for sites on the enzyme. The presence of CO$_2$ activates the enzyme, and CO$_2$ fixation initiates the photosynthetic carbon reduction cycle,
whereas \( \text{O}_2 \) fixation initiates the photorespiratory carbon oxidative cycle. An increase in the atmospheric ratio of \( \text{CO}_2 \) to \( \text{O}_2 \) thus favors photosynthesis and inhibits photorespiration. The net carbon gain rises as \( \text{CO}_2 \) increases, not only because more carbon is assimilated, but also because photorespiratory carbon loss is reduced (Wolfe, 1995).

As \( \text{CO}_2 \) is a limiting factor for photosynthesis of \( \text{C}_3 \) plants, it is generally assumed that a rise in \( \text{CO}_2 \) would stimulate photosynthesis and increase dry matter production. When \( \text{C}_3 \) plants are continuously cultivated in elevated \( \text{CO}_2 \), their total Rubisco activity (i.e., fully activated) and photosynthetic capacity (light-saturated rate of photosynthesis) often decrease (Besford et al., 1990; Bowes, 1991; Stitt, 1991; Woodrow, 1994) such that the rate of photosynthesis for plants grown and measured at high \( \text{CO}_2 \) is often just greater than that for plants grown and measured at 350 \( \mu \text{mol} \ \text{CO}_2 \ \text{mol}^{-1} \) (Stitt, 1991; Bowes, 1993). \( \text{CO}_2 \) will not inhibit photosynthesis, because its concentration are very seldom SO high as to hamper photosynthesis (Pfanz et al., 1998).

Elevated atmospheric \( \text{CO}_2 \) levels increase the rate of photosynthesis and reduce photorespiration in most plants, especially in \( \text{C}_3 \) plants (Mulchi et al., 1992; Rogers and Dahlman, 1993; Weber et al., 1994), resulting into enhanced plant biomass and overall yield (Kimball et al., 1990; Samarakoo et al., 1995; Slafer and Rowson, 1997). Roughly half of the carbon assimilated in photosynthesis (less photorespiration) is released to the atmosphere as \( \text{CO}_2 \) during plant respiration (Waring and Schlesinger, 1985; Amthor, 1989). Increases in photosynthetic products, such as starch, may be due to an imbalance between the activities of source and sink (Farrar and Williams, 1991; Arp, 1991; Stitt, 1991; Wulschleger et al. 1992; Baxter et al.,
1994). On the return of plants to the ambient CO$_2$, this accumulation of carbohydrate can decline (Sasek et al., 1985).

As the C$_3$ photosynthetic capacity is not saturated by the current ambient CO$_2$ (Ca), an increase in the net photosynthetic rate is the most expected effect of increasing CO$_2$. (Clifford et al., 1993; Idso and Kimbal, 1993; Gunderson and Wullschleger, 1994; Sicher et al., 1995; Volin and Reich, 1996; Miglitta et al., 1996; Roden and Ball, 1996; Polley et al., 1996; Manderscheid and Weigel, 1997; Sicher and Bunce, 1997; Stirling et al., 1997; Whitehead et al., 1997). With a doubling of the CO$_2$ concentration, average increase in photosynthetic rate was up to 52% in herbs (Cure and Acock, 1986) and 44% in trees (Gunderson and Wullschleger, 1994). Woody plants when exposed to elevated CO$_2$ for varying periods of time show an stimulated photosynthesis, and increased growth rate and biomass accumulation (Kramer, 1981; Ziska et al., 1991; Gunderson et al., 1993; Gunderson and Wullschleger, 1994; Ceulemans and Moussear, 1994; Atkinson et al., 1997; Saxe et al., 1998).

The other environmental factors kept unchanged, a doubling of average CO$_2$ concentration could lead to over 50% increase in potato and alfa-alfa yields and almost 30% increase in the yields of corn and soybeans (Rogers and Dahlman, 1990). The photosynthetic stimulation to elevated CO$_2$ seems to decline with long-term exposures (Shin, 1990). Although CO$_2$ is the substrate of photosynthesis it can inhibit photosynthesis at a certain (although very high) level by its acidifying properties.

High net photosynthesis rate observed under enriched CO$_2$ may be because of a reduced photorespiration (Machler and Nosberger, 1980). In evergreen trees growing under subfreezing temperatures the potential for photosynthesis gradually
declines during autumn and winter. Vu et al. (1983) and Cambell et al. (1988) have found that RuBP level in soybean leaves tends to decrease with increasing CO₂. No differences were found in RuBP-C activities of wheat leaves when expressed on a leaf area basis (Havelk et al., 1984).

Elevated CO₂ reduces the sensitivity of various plant species to SO₂ damage (Carlson and Bazzaz, 1985; Sandhu et al., 1992; Niewiadomska and Miszalski, 1995). The protection against SO₂ may be owing to stomatal closure as CO₂ is increased, but other internal factors owing to enhanced photosynthesis could also offer enhanced resistance through metabolic use of SO₂ or repair of damage that might be caused by SO₂ (Black, 1982; Allen, 1990). Enriched CO₂, with and without SO₂ treatments may increase the net photosynthesis rates, leaf area index and leaf dry weight, and may not significantly affect stomatal resistance, transpiration rate, leaf area, plant height, total biomass or grain yield (Lee et al., 1997).

In many short-term experiments in which other environmental conditions were maintained at optimum levels, a doubling of CO₂ decreased transpiration by 25-50% and increased water use efficiency per unit leaf area as much as two fold (Pearcy and Björkman, 1983; Cure and Acock, 1986). Nijs et al. (1989) measured an 87% increase in water use efficiency (WUE) of individual leaves of CO₂ enriched plants, though water use efficiency on a whole plant basis was increased by only 25%.

CO₂ can also affect stomatal densities, with fewer stomates per unit leaf area in plants grown with increased CO₂ levels (Oberbauer et al., 1985). The fall in stomatal density in elevated CO₂ condition reduces the maximum stomatal conductance that can be attained when stomata are fully open. There are thus changes occurring during leaf development that reflect the changing physiological priorities
brought about by variations in the atmospheric CO$_2$ concentration (Heath and Mansfield, 2000). Stomatal density under elevated CO$_2$ depends on leaf age and position in trees (Ceulemans et al., 1995; Drake et al., 1997). At times, stomatal index remain unchanged under elevated CO$_2$ (Estiarte et al., 1994).

Stomatal conductance has been observed to decrease with increasing CO$_2$ concentration (Morison, 1985, 1987). Stomata of most species close with increasing ambient CO$_2$ and this causes a decrease in stomatal conductance (gs), even in C$_4$ species (Owensby et al., 1997), commonly in the range 20 to 40% (Long, 1994; Knapp et al., 1994; Sage and Reid, 1994; Barnes et al., 1995; Pearson and Brooks, 1996; Volin and Reich, 1996; Picon et al., 1996). Variations in the intercellular CO$_2$ concentration (Ci) plays a major part in determining stomatal aperture, and reductions in intercellular CO$_2$ concentration as a result of mosopliyll photosynthesis partly account for increases in the stomatal aperture in light. The CO$_2$ concentration in the ambient air has been shown not to be directly in control of aperture; rather it is the substomatal concentration in contact with the inner surfaces of the guard cells that is important (Mott, 1988; Farquhar and Sharkey, 1982).

**Effect of O$_3$**

Effect of ozone on photosynthetic processes also depends on the duration of exposure and concentration of the gas. Ozone sensitivity is also related to the development age of the leaf (Lee, 1991). Ambient humidity during ozone exposure has major effects on stomatal response; high humidity may reduce the magnitude or speed of stomatal closure in response to ozone or reverse the direction of an ozone response (Darrall, 1989).
Stomatal conductance is the principal rate determining step in regulating ozone entry into leaves. In general, those species with higher stomatal conductances are more sensitive to ozone (Darrall, 1989).

**Effect of mixed pollutants**

The pollutants gases on entering the stomatal cavities, dissolving in extracellular fluid and reacting with plant material, produce ionic species and free radicals (Mansfield and Freer-Smith, 1981; Peleg, 1976) that are generally more reactive than the gases themselves. As a result of the reactions of these species with lipids and proteins in the cell walls and membranes, chain reactions are initiated giving rise to more free radicals. Unless this potentiality self-perpetuating process is blocked, it continues causing a widespread damage to membrane systems resulting in the breakdown of cell compartmentation and a severe metabolic disorder.

Photosynthetic rate is affected severely in the polluted air (Schmidt et al., 1990; Takahama et al., 1992; Chappelka and Freer-Smith, 1995; Sane et al., 1996); it may decline as much as up to 65% in *Peristrophe bicalyculata* and 75% in *Ruellia tuberosa* (Nighat et al., 1999, 2000).

Particulates such as cement kiln dust, some fluorides, soot, magnesium oxide, iron oxide, foundry dust and sulfuric aerosol, often inhibit photosynthesis (Keller, 1973). They also cause phototoxicity, generally by physically covering the leaves of the plant thereby possibly inhibiting plant respiration through stomata and/or reducing photosynthesis by limited absorption of light. Suspended particulate matter (SPM) can also damage the vital photosynthetic systems of plants by covering the leaves, plugging the stomata and reducing the absorption of CO₂ and sunlight (Yunus et al., 1996).
The chlorophyll, a pigment vital for photosynthesis, is the most sensitive parameter with regard to manifestations of air pollution and chlorophyll content is normally reduced under SO$_2$ stress which may be due to degradation of chlorophyll to phaeophytin (Varshney and Varshney, 1979). Chlorophyll is converted into phaeophytin when exposed to weak acids such as H$_2$SO$_3$. This conversion is brought about by the replacement of Mg$^{++}$ by two hydrogen atoms (Rao and Le Blanc, 1966). It is reported that SO$_2$ at moderate concentrations induces the conversion of chlorophyll to phaeophytin at pH 2.2 but not at pH 3.2 and above (Puckett et al., 1973; Malhotra and Hocking, 1976).

As the absolute concentrations of pigments depend on a wide range of environmental factors (Wolfenden et al., 1988) and also change with time, season and developmental status (Robinson and Wellburn, 1991), measurements of total amounts of pigment can not predict plant damage by air pollution (Hur and Wellburn, 1993).

Chlorophylls are more affected by SO$_2$ than carotenoids (Hur and Wellburn, 1993). Both chlorophylls ($a$ and $b$) are susceptible to SO$_2$ pollution that splits magnesium from the chlorophylls to form pheophytin. The loss of chlorophyll may decrease consistently from base to apex of the leaf. This may perhaps be due to structural and functional variation in different portions of the leaf and stomatal conductance could play a major role (Khan and Usmani, 1988).

Chlorophyll $b$ is more stable and less severely affected than chlorophyll $a$ (Ricks and Williams, 1975; Malhotra, 1977; Singh and Rao, 1980; Khan and Usmani, 1988; Rantanen et al., 1994). The greater loss of chlorophyll $a$ than $b$ may be ascribed to the inactivation of various enzymes associated with the synthesis and
action of chlorophyll $a$. A prolonged fumigation of SO$_2$ inhibits chlorophyllase (Khan and Usmani, 1988). At high SO$_2$ concentrations, chlorophyll is possibly degraded into phaeophytin due to pH-change in leaf (Singh and Rao, 1980).

SO$_2$ caused conversion of chlorophyll $a$ into phaeophytin $a$ (at 100-500 ppm of aqueous SO$_2$) and chlorophyll $b$ into chlorophyllide (at 10-50 ppm of aqueous SO$_2$). Pigment breakdown and retarded rate of photosynthesis could be due mostly to the specific direct actions of SO$_2$ and not a function of increased acidity (Malhotra, 1977). Total chlorophyll content and ribulose-1,5 bisphosphate carboxylase (RuBP-C) activity decline with the increasing SO$_2$ concentration (Gupta, 1992).

Chloroplasts have been reported to decrease in size in aged leaves of herbaceous species (Wittenbach et al., 1982). These are sites of both detoxification and the toxic effects of SO$_2$. Sulfite can change the activity of enzymes, disturb membranes by radical reactions, or inhibit the thylakoid membrane electron transport chain (Ziegler, 1975; Shimazaki, 1988; Covello et al., 1989). However, chloroplasts of mesophyll cells are able to metabolize sulfite in light-dependent reactions.

Hydration and dissociation of the hydration product sulfurous acid produces protons, HSO$_3^-$ and SO$_3$. In the cytoplasm of mesophyll cells, sulfite is taken up by the chloroplasts via the phosphate translocator (Hampp and Ziegler, 1977). Oxidation of sulfite to sulfate can proceed via a radical chain reaction (Asada and Kiso, 1973). Radicals may cause damage by their reaction with biomolecules. Sulfate is actively transported into the vacuoles (Kaiser et al., 1989), but can also be reduced slowly in the so called ‘bound pathway’ of cysteine synthesis (Aderson, 1980). The other pathway involves a direct reduction of sulfite (Hennies, 1975). The product is a highly toxic intermediate, hydrogensulfide, which is metabolized to the amino acid.
cystein, although a small part of it is released into the atmosphere (Hällgren and Fredriksson, 1982).

Photosynthetic pigments and many enzymes are associated with the membranes of chloroplasts. These membranes, being very fine structures, can be easily disrupted by SO$_2$ (Wellburn et al., 1972). Significant thylakoid swelling observed near the pulp mills can be probably associated with SO$_2$ emission (Kärenlampi and Houpis, 1986; Fink, 1989; Schiffens-Gruber and Lütz, 1992). Starch grains are absent from chloroplasts in mesophyll cells exhibiting fully developed winter hardiness. However, occasional remnants of starch present in needles collected from industrial sites (polluted areas), probably indicates a disturbance in carbohydrate metabolism in winter hardening (Wulff et al., 1996).

Senescence-related ultrastructural symptoms include increase in number and size of plastoglobuli, in large lipid accumulation, and in number of dark grains in the cell wall, as observed with increasing needle age. A poor differentiation of granal membranes and an increase in small cytoplasmic lipid bodies and irregularly shaped lipid material were the symptoms typical of needles in the industrial areas. The ageing symptoms appeared in younger needles and were more evident in industrial areas than at the control site, suggesting an accelerated ageing process in the industrial areas (Wulff et al., 1996).

Acute level of SO$_2$ have been shown to cause plasmolysis of the cells (Brandt and Heck, 1968). Active mesophyll cells collapse upon prolonged exposures of plants to SO$_2$. One of the earliest signs of SO$_2$ toxicity appears in chloroplast structure (Wellburn et al., 1972.).
Effect of SO₂ and NO₂ combinations

Exposure to SO₂ (Sutinen, 1986), or K deficiency (Marschner, 1986) reduces the amount of starch in chloroplast, whereas NO₂ enhances starch content (Schiffgens-Gruber and Lütz, 1992). However, decreased length of starch grains was most apparent under the combined effect of SO₂ + NO₂ with limited availability of K, possibly indicating an altered carbohydrate metabolism. In cases where photosynthesis is reduced, the energy stored in starch is essential for continued growth, detoxification processes and repair mechanisms (Black, 1982).

The most conspicuous symptom of Mg, K and Ca deficiency has been the collapse of the phloem (Fink, 1989, 1991). Other symptoms observed are large starch grains, reduction of thylakoids and an increased number of plastoglobuli in Mg-deficient conditions (Fink, 1988), separation of the thylakoid membranes and increases in the size of starch grains in needles with low Ca concentrations (Fink 1988, 1989), and increased formation of vacuoles, vesiculation of the tonoplast and increasing number of lipid bodies in the cytoplasm in needles of K-deficient seedlings (Holopainen and Nygren, 1989).

Effect of CO₂

Mature leaves from plants exposed to increased CO₂ show a decline in the chlorophyll a/b ratio indicating that the ratio of light-harvesting to reaction-centre protein is increased (Van Oosten et al., 1994, 1995). When acclimation occurs, annuals and perennials show similarities in their long-term response to CO₂, such as loss of RubisCo protein activity, lower chlorophyll content, chl a/b ratio, and accumulation of carbohydrates (Besford et al., 1990, Rowland-Bamford et al., 1991, Woodrow, 1994, Tissue et al., 1993, Wilkins et al., 1994).
Effect of O₃

Ozone has a great potential for disruption of chloroplast metabolism. In *Pinus ponderosa* fumigated with O₃, the chloroplasts aggregate in branches of mesophyll cells. The mesophyll cells become abnormally folded and twisted following the aggregation of chloroplasts, and eventually collapse. Among the components of the functioning chloroplast that are sensitive to ozone are ascorbate, ATP, nitrate reductase, DNA and lipids. The most widely observed change induced by ozone is chlorophyll destruction. Metabolic processes in photosynthesis may also be affected in non-specific ways through effects on integrity of the chloroplast envelope and thylakoid membranes that disrupt the chemiosmotic balance (Runeckle and Chevone, 1992).

The chlorophyll content, as well as the needle length and needle dry weight may be reduced by ozone treatment (Yang et al., 1983).

Mixed pollutants

Chlorophyll measurement in leaves is a useful diagnostic tool for subtle pollutant effects (Malhotra, 1977; Agrawal and Agrawal, 1989a). Chloroplast damage due to coal-smoke pollutants might cause reduction in chlorophyll content in the polluted leaves (Nighat et al., 1998, 2000). A decrease in chlorophyll content in the leaves of different tree species growing around Thermal Power Plants, aluminium factories and cement factories has been reported (Vij et al., 1981; Pandey 1983; Agrawal and Agrawal, 1989a; Agrawal and Khanam, 1989; Narayan and Agrawal, 1992; Agrawal et al., 1993; Nighat et al. 1998, 2000).
Reduction in chlorophyll content at sites with a high dust accumulation rate is attributed to alkalinity caused by the excessive soluble salts on the leaf surface (Elseewi et al., 1980) and also to increased foliar temperature which retards chlorophyll synthesis (Mark, 1963). It is suggested that coal-particle pollution also causes induction of chlorophyllase or breakdown of chlorophyll to form phaeophytin (Beckerson and Hofstra, 1979; Mudd, 1975). In Hibiscus abelmoschus, cement dust was more damaging to chlorophyll than the coal dust, while fly ash slightly increased the chlorophyll content (Pawar et al., 1982). Continuous application of particulate matters clogs the stomata and interferes with the gaseous exchange. This may raise leaf temperature which in turn may hamper synthesis of chlorophyll (Singh and Rao, 1981).

D: Other biochemical responses

Effect of SO₂

Activity of the enzyme nitrate reductase (NR) is adversely affected by SO₂ exposures. The NR activity inhibition in SO₂-exposed plants may be due to a reduction in enzyme synthesis or to the non-availability (for enzyme activity) of electron donor NADH⁺, produced during the conversion of glyceraldehyde-3-phosphate to phosphoglyceric acid in the cytoplasm by the enzyme glyceraldehyde-3-phosphate dehydrogenase; reduction of NAD to NADH⁺ furnishes the electron for reduction of nitrate to nitrite (Varshney and Varshney, 1979).

SO₂ initially increases the NR activity in the youngest fully expanded wheat leaves, but later it decreases. The decrease is more in older leaves than in younger
leaves of wheat (Huang et al., 1993). SO$_2$ fumigation also changes pool sizes of nitrogen compounds (soluble protein and amino acids) in plants tissues. Total sugar levels, protein content, NR activity and inorganic nitrogen/organic nitrogen ratio declined with a significantly high specific leaf area in Dalbergia sissoo, Eucalyptus rostrata and Mangifera indica at the polluted sites, compared to the reference site (Rao et al., 1993). Thomas and Runge (1992) have proposed that nitrate reduction is involved in the neutralization of portons generated by SO$_2$ uptake. NR activity increased in the leaves of nitrate-grown oaks exposed to 160 nl l$^{-1}$ SO$_2$ (Huang et al., 1993).

In herbaceous plants, nitrate is reduced mainly in the leaves, whereas in woody species, nitrate reduction is assumed to occur mostly in the roots (Pate, 1980; Andrews, 1986). Recent investigations, however, have demonstrated nitrate reductase activity in the foliage of the deciduous as well as the coniferous trees (Al-Gharbi and Hipkin, 1984; Smiroff et al., 1984; Wingsle et al., 1987).

Sulphur dioxide fumigation (68 nl l$^{-1}$ for 20 weeks) did not have a direct impact on nitrate reductase activity though it prevented the stimulatory effect of NO$_2$ on nitrite reductase in the leaves (Murray and Wellburn, 1985; Wellburn, 1982). However, nitrite reductase activity in leaves was decreased by a continuous long-term exposure of barley plants to SO$_2$ (15-38 nl l$^{-1}$) (Borland and Lea, 1991). Exposure of Triticum aestivum to 441 nl l$^{-1}$ SO$_2$ decreased the total nitrogen content in mature leaves, but increased it in immature leaves (Huang et al., 1993).

Exposure to low levels (< 100 nl l$^{-3}$) of SO$_2$ either increased leaf protein concentration or had no effect on it but exposure to higher levels of SO$_2$ (> 100 nl l$^{-3}$) usually decreased leaf protein concentration in crops such as soybean (Sardi, 1981;
Saxe, 1983). Clover (Murray, 1985) barley (Borland and Lea, 1991). Ranieri et al. (1990) have observed that fumigation with up to 110 nl l⁻¹ SO₂ decreased leaf soluble protein concentration in maize.

Increases in total amino acid concentration in plant tissues subjected to SO₂ pollution may be because of stimulated amino acid synthesis, inhibited oxidation, impaired protein synthesis, an enhanced protein hydrolysis or combinations of some or all of these factors (Rowland et al., 1988; Ranieri et al., 1990). Concentrations of total amino acids and soluble protein also increase with NaCl salinity in leaves (Huang et al., 1993).

Faster incorporation of inorganic nitrogen ions into proteins could possibly reduce the toxicity towards the photosynthetic phosphorylation in *Azadirachta indica*, *Acacia auriculiformis* and *Bombusa arundinaceae*, thus enabling the plants to maintain sufficient total sugars to provide energy for the incorporation of ammonium nitrogen into protein. It appears that the above mentioned mechanisms which exhibit differential response in different plants constitute a defense mechanism developed by plants to cope with the excess amount of ammonium deposition (Rao et al., 1993). *Azadirachta indica*, *Acacia auriculiformis* and *Bombusa arundinaceae* could successfully incorporate the excess deposited ammonia into protein while plants like, *Dalbergia sissoo*, *Eucalyptus rostrata* and *Mangifera indica* are lacking this ability (Rao et al., 1993). Protein contents increased more in the older leaves than in the younger leaves under SO₂ stress (Huang et al., 1993).

The concentration of soluble sugars (sucrose, glucose, fructose) is indicative of the physiological activity of a tree and varies during the active season (Puls and Rademacher, 1988). In the case of spruce, the concentration seems to the highest in
January, March and then in October. Defoliation causes a decrease of starch and soluble sugars (Gregory et al., 1986) as do the pollutants.

*Dalbergia sissoo, Eucalyptus rostrata* and *Mangifera indica* could not incorporate the excess $\text{NH}_4^+$, mainly due to decline in sugars. The results indicate the ability of a plant to undergo species-specific metabolic changes in order to cope with the excess nitrogen deposition which may ultimately result in increasing or decreasing the tolerance to $\text{SO}_2$ (Rao et al., 1993).

Sucrose and sugar phosphates were estimated in $\text{SO}_2$-fumigated and unfumigated plants. The movement of carbon through Calvin cycle to the hexose phosphates appears to be the main reason for decrease of sucrose in fumigated plants. Unlike sucrose, $^{14}$C-distribution markedly increased in amino acids and organic acids (malic acid) (Gupta, 1992).

Plants like *Azadirachta indica, Acacia auriculiformis* and *Bombusa arundinaceae* maintained enhanced total sugars and NR activity, and incorporated excess $\text{NH}_4^+$ into proteins, thus enabling the plant to compensate/ alleviate $\text{SO}_2$-induced injury. *Ficus benghalensis* and *Ficus religiosa* maintained an unaltered total sugar and NR activity and could partly incorporate $\text{NH}_4^+$ into proteins, thus modifying the $\text{SO}_2$ impact to some extent (Rao et al., 1993). Malhotra and Sarkar, (1979) stated that the increased concentration of reducing sugar caused by $\text{SO}_2$ might be due to an increased biosynthesis of sugars or to a break down of reserve polysaccharides rich in the reducing sugar.

Sulphur is available to roots as sulphate in the soil and to shoots as the gaseous sulphur compounds in the atmosphere. Sulphate influx into the roots is mainly an active, carrier-mediated process (Rennenberg, 1984; Cran, 1990; Clarkson
et al., 1993) controlled by sulphur nutrition of the plant (Herschbach and Rennenberg, 1991; 1994). An increased foliar S concentration or an increased S/N ratio in needles of red spruce (*Picea ruben* Sarg.) treated with acid mist containing sulphate was correlated with a decrease in frost hardiness (Sheppard, 1994).

Sulphur originating from SO$_2$ and absorbed by the leaves was found to be translocated to the roots and to appear in various sulphur fractions of the roots including sulphate. Part of the SO$_2$, assimilated into organic sulphur compounds in the shoot, was also transported to the roots as glutathione and cysteine (Dekok, 1990). Since both sulphate and glutathione are mobile in the phloem and xylem (Schupp et al., 1992; Schneider et al., 1994), circulation of oxidized and or reduced sulphur can be assumed in the vascular system of the stem (Rennenberg and Herschbach, 1996).

Simultaneous exposure of soybeans to SO$_2$ and salinity has shown that SO$_2$-induced leaf injury was severe in the non-saline plants than in the saline plants, with leaf sulphur concentrations being significantly decreased by soil salinity. In summer, the meteorology and the photosynthetically active stage of vegetation influence the rate of dry deposition of sulphur from SO$_2$ (Zunckle, 1999).

The capacity to metabolize SO$_2$ is inextricably linked with nitrogen metabolism due to close functional relationship between sulfur and nitrogen metabolisms (Murray et al., 1994). Wet deposited SO$_2$ (as sulphate) may not exert such marked influences on chemical contents of soils and litters as an equivalent input of dry-deposited SO$_2$ can do (Newsham et al., 1992).

The deposition of environmentally realistic concentrations of SO$_2$ on Sycamore leaf litter can be assumed to have led to the solubilization of SO$_2$ in water on moist
leaf surfaces (Newsham et al. 1992). $\text{SO}_2$ would then have been oxidized via sulphurous acid, bisulphite and sulphite to sulphate, resulting in significant increases in the levels of sulphate - S in the leaf litter leachates. Protons released in the oxidation of $\text{SO}_2$ were significantly increased in leachates by $\text{SO}_2$ treatments and, as a result, pH values of the leachates declined. These protons could then have displaced calcium, magnesium, and potassium ions from the leaf litter tissue, resulting in decreased concentrations of these cations in leachates and tissues (Newsham et al., 1992). The rate of sulphate formation in leaf litters and calcium and magnesium contents of leaves were linearly related to the atmospheric $\text{SO}_2$ concentration (Newsham, 1991). Calcium and magnesium are important elements for plant growth, and hence their removal from leaf litters could have serious implications for the primary productivity of the forest ecosystem as leaf-litter fall is a major pathway by which these elements are returned to higher plants.

Depending largely on pH, dissolved $\text{SO}_2$ undergoes a series of dissociation reactions forming sulphurous acid, bisulphite and sulphite, which ultimately is oxidized to sulphate. $\text{SO}_2$ deposition on leaf surfaces should, therefore, enhance sulphate leaching from the leaves. More sulphate was leached from the leaf litters exposed to environmentally-realistic concentrations of $\text{SO}_2$ (Newsham et al., 1992).

**Effect of NO$_2$**

After foliar entry, the gaseous NO$_2$ reacts with the extracellular water, forming nitrite and nitrate ions which are incorporated in the N metabolism of the cell by the action of nitrite and nitrate reductases. The gaseous NO$_2$ may thus serve as an additional nitrogen source. Nitrates are present in all plants and are an essential
source of nitrogen for normal growth (Spink and Parson, 1995; Fytianos and Zarogiannis, 1999). Nitrate reductase activity may be induced by atmospheric NO\textsubscript{2} as well as by soil-borne nitrate (Zeevart, 1974, 1976; Yoneyama et al., 1980; Murray and Wellburn, 1985).

After uptake into the leaf, NO\textsubscript{2} participates in the normal pathway of nitrate reduction followed by synthesis of amino acids and proteins (Wellburn, 1990). The synthesis of amino acid and protein provides a means to partially detoxify excess NO\textsubscript{2} derivates in the leaves (Murray and Qifu, 1994). The pollutant can react with either extracellular water or cell sap to produce equal amounts of nitrate and nitrite ions and as such its assimilation by the cell is initiated by the activities of nitrate reductase and nitrite reductase.

Nitrate reductase is believed to be the rate-limiting enzyme in nitrate assimilation, as nitrite and ammonium normally do not accumulate in plant tissues (Beevers and Hageman, 1969). Even when plants are exposed to low levels of NO\textsubscript{2} for several days, there is no build up of nitrite although nitrate concentration increases several fold (Srivastava and Ormrod, 1984, 1986).

The nitrate concentration also increases when plants are exposed to higher concentrations of NO\textsubscript{2} for several hrs (Zeevart, 1976). Low levels of NO\textsubscript{2} increase NR activity when plants are raised without nutrient nitrogen (Srivastava and Ormrod, 1984). However, in the presence of adequate nitrogen nutrition, the pollutant either has no effect or inhibits the enzyme activity (Srivastava and Ormrod, 1984; Takeuchi et al., 1985). NO\textsubscript{2} assimilation is related to the nitrate reductase, and perhaps nitrite reductase activities of the leaf (Thoene et al., 1991). Dependence on these enzyme activities, which occur in the cytoplasm and chloroplasts of the leaf, respectively,
might underline the mesophyllic resistance to NO\textsubscript{2} uptake (Hereid and Monsoon, 2001). Many factors such as nitrate availability, photosynthetic energy supply, removal of intermediate nitrate reduction products (e.g. amino acids) and other environmental factors may affect leaf NR activity (Delvin and Witham, 1983).

Whereas many studies have dealt with the impact of NO\textsubscript{2} fumigation on nitrogen metabolism in plants, information about the effects of pollutant combinations on nitrogen assimilating enzymes is deficient. Welburn et al. (1981) have shown that induction of nitrate reductase by NO\textsubscript{2} was prevented when SO\textsubscript{2} was applied simultaneously.

The pollutant NO\textsubscript{2} may be absorbed and assimilated by plants to serve as a source of nitrogen (Amundson and MacLean, 1982; Cowling and Kozoiol, 1982; Okano et al., 1985). Because the majority of foliar 'N' is associated with proteins participating in photosynthetic reactions, a strong positive correlation usually exists between foliar N content and light-saturated rate of photosynthesis. The excess of nitrogen, arising from either fertilizer or from air pollutants causes growth disturbances and apical shoot death in tree species (Dijk and Roelofs, 1988; Ferm et al., 1990). Water soluble substances present on foliar surface of trees indicate an enhanced NH\textsubscript{4}\textsuperscript{+} deposition which leads to enhanced foliar protein content (Rao et al., 1993). During the enhanced protein synthesis and increased organic/inorganic nitrogen ratio in plants the deposited ammonium is incorporated into proteins (Rao et al., 1992).

The mechanisms of the disturbances caused by nitrogen excess at the cellular level are still largely unknown, although severe imbalances in amino acid concentrations and protein biosynthesis have been observed (Ferm et al., 1990).
Effect of mixed pollutants

The total soluble sugar content may be higher in plants growing near the emission sources and receiving the maximum pollution load (Agrawal, 2000). The accumulation of sugar results either from its disturbed transportation and reduced utilization for repair processes or from inhibition of synthesis of other metabolites.

The adverse impact of air quality on carbohydrate metabolism, as evident from changes in the pool size of free carbohydrates in the leaf tissue, can be successfully used as a bioindicator of air pollution stress. Changes in carbohydrate pool may also give an insight of changes in the photosynthetic process. Koziol and Jordon (1978) and Prakash et al. (1989) have correlated reductions in photosynthesis with reductions of starch.

Cambial activity and wood production

Plants grow in height (primary growth) and diameter (secondary growth) through the activity of meristematic tissues which comprise a very small fraction of the total mass. Increase in the diameter of trees occurs primarily from meristematic activity of the vascular cambium, a cylindrical lateral meristem located between the xylem and phloem in the stem, branches, and roots. The cambium produces xylem inwards and phloem outwards; the new annual increments of xylem and phloem are inserted between old layers of these tissues (Iqbal, 1990, 1994, 1995; Romberger et al., 1993).

The cambium normally originates from the derivatives of apical meristem. It may derive, however, from parenchyma or callus also. Unlike the apical meristem, this lateral meristem produces specific tissues rather than entire organs. Structure of
cambium differs with species in respect of shape, size, length, width and arrangement of its constituent fusiform cells and ray cells. Based on the arrangement of fusiform cambial cells, the cambia are recognized as being storied and nonstoried, the latter being of more common occurrence in plants than the former (Iqbal and Ghouse, 1990; Silva et al., 1990; Iqbal, 1995). The rate of cambial cell division as well as the duration of cambial activity is important in reference of the total quantum of the secondary tissues produced in a growth year. The vascular cambium varies greatly in its activities during different seasons of the year, in different plants, and in different parts of the same plant. In most species it exhibits alternate active and dormant phases during a growth year with few exceptions of tropical species in which the meristematic activity may continue all the year around. Most tropical plants, unlike the temperate ones, do not show a sharply rhythmic and cyclic growth pattern. However, even in such cases, a close correlation exists between apical growth and radial growth as it does in the temperate species (Iqbal, 1981, 1990, 1994; Iqbal and Ghouse, 1985a, 1990).

The cambium is active usually in seasons that are sufficiently hot and humid. On the contrary, reactivation of dormant cambium through its exposure to exceedingly cold temperature is also on record (Mellerowicz et al., 1992). In the temperate species, the cambium is active usually during spring and summer. This is followed by a period of dormancy that extends to the following spring. In the tropics where environmental conditions are usually less limiting the growth season tends to be longer than in the temperate zones where it hardly exceeds 4-5 months (Iqbal, 1995).

Fahn et al. (1981) have observed that (a) in the mediterranean plants, cambial activity starts in the spring and ceases at the end of summer or the beginning of
autumn, thus behaving as the temperate zone plants; (b) in some desert shrubs, cambial activity starts with the rainy season (between November and December) and ceases in the beginning of the dry season (late April-early June); and (c) in the tropical and subtropical trees, the cambium is generally active throughout the year but some species also form growth rings. During long dry season, deciduous trees cannot store reserve materials sufficient for leaf development and a concurrent start of cambial activity, whereas in evergreen trees the quantities of reserve materials might suffice for a simultaneous start of the two processes (Fahn and Werker, 1990).

In many Indian trees, cambial activity begins around June-July and lasts until October-November. The cambium of tropical trees remains active either for a major part of the year (Iqbal, 1993), or in some cases, for the whole year (Paliwal et al., 1993). In some species, growth span may be as short as in temperate species (Iqbal, 1994). In temperate regions the annual radial growth generally displays a typical sigmoid growth curve normally depicting a rapid increase in activity in the beginning, followed by a constancy and then a gradual decline (Iqbal, 1994).

Any correspondence between the cambial activity and an external factor does not necessarily indicate a simple and direct relation between the two. However, if the factor becomes limiting, any change in the factor would directly affect the cambial activity (Munting and Willemse, 1987). In many tropical trees, the commencement of cambial activity and the increased summer temperature are closely correlated (Paliwal et al., 1975; Ghouse and Hashmi, 1978, 1979; Ajmal and Iqbal, 1987; Siddiqi, 1991). The cambium can reactivate at a moderately high temperature, but a further increase in temperature and a heavy rainfall would accelerate the rate of the cambial growth. Once begun, the cambial activity persists even at relatively low

Cell divisions in the vascular cambium may start several weeks before sprouting and cease at about the same time as the elongation growth of the shoots (Sennerby-Forsse, 1986). The first few divisions in the cambial zone take place slowly and a period of 3 to 4 weeks may elapse between the first 3 to 4 divisions; later, cell division occurs more rapidly (Brown, 1971). In white Cedar (Thuja occidentalis) the time lapse between successive divisions of xylem-mother cell during rapid earlywood formation ranges from 4 to 6 days (Bannan, 1962). This period of time is much longer than the interval between successive divisions of cambial cells as reflected by the length of time required for the growing phragmoplasts to reach the tips of the long cambial derivatives. In Aesculus hippocastanum fusiform initials divide two weeks after the first division in the precursor phloem (Barnett, 1992).

The periclinal cell divisions leading to accumulation of secondary tissues follow closely the cambial swelling. Once initiated, the process of division may continue for many months (Philipson et al., 1971). The growth period tends to shorten in higher latitudes and lengthen towards the equator, and is generally longer in conifers than in dicotyledons (Studhalter et al., 1963). In ray cells, the periclinal division begins relatively later, attains the maximal frequency later but stops earlier than in fusiform cells (Evert, 1963; Catesson, 1964). Most anticlinal divisions in the cambium of mature stems occur towards the end of the growth season. However, in the fast-growing young shoots, where rate of periclinal division is relatively high, anticlinal division may prevail throughout the tenure of the radial growth (Iqbal, 1994, 1995). In late spring and early summer, during the period of most frequent cell
divisions, the radial enlargement of the xylem mother cells in conifers is rapid but on reaching 10 to 15 micron they again divide. Cells that do not divide further may attain radial diameter of 40 to 50 microns (Bamman, 1955). In final, radial growth pattern depends largely on the seasonal environmental conditions imposed upon the trees during this period (Brown, 1971). On the outer side of the cambium, phloem-mother cells are cut off from the initiating layer at a slower rate than the xylem mother cells on the inner side. Difference in sensitivity of the meristem to the stimuli may also affect xylem-phloem relationship in young and old organs (Aloni, 1988). Increased root growth provides an effective store for carbon but, it would be of limited value to the production of timber outside a coppicing system (Atkinson, 2000).

Secondary growth in trees is commonly considered to be indirectly controlled by leaf and bud formation through the levels of growth substances (Wareing, 1958; Iqbal, 1995). Indoleacetic acid (IAA) is known to activate dormant cambia in hardwoods and conifers (Catesson 1974, 1984; Savidge and Wareing, 1981; Riding and Little, 1984). However, early in dormancy (during the first 2-4 weeks), the cambium cannot be induced to produce xylem even when indole-3-acetic acid (IAA), a naturally occurring stimulator of cambial activity, is supplied under environmental conditions favourable for cambial activity (Riding and Little, 1984, 1986; Sundberg et al., 1987). This inability of the resting cambium to produce xylem cells in response to IAA could be due to various factors including high abscisic acid content, low level of IAA, gibberellin and cytokinin, and impaired basipetal IAA transport (Little, 1981; Little and Waring, 1981; Savidge and Wareing, 1984; Sundberg et al., 1987; Lachaud, 1989). High concentrations of IAA together with low concentrations of GA₃
(gibberellic acid) produce greater amount of xylem, whereas reverse combinations give rise to a maximum amount of phloem. The role of water potential and sugar concentration in cambial growth is significant. Auxin levels kept constant, sucrose content may alter the balance of phloem/xylem production. At lower concentration xylem forms, at higher concentration phloem results (Iqbal, 1995).

Wareing (1958) and Wareing et al. (1964) have demonstrated the interacting role of IAA with GA in cambial reactivation and xylogenesis. Since GA enhances cell expansion, it plays an important part during vessel element differentiation (Robards and Kidwai, 1969). Both IAA and GA affect the cambial division and the differentiation of the cambial derivatives quite significantly. Radial auxin distribution probably has an influence on latewood production in Pinus species (Jenkins, 1974; Nix and Wodzicki, 1974). The tracheid diameter in Pinus resinosa is enlarged by auxin supplements (Larson, 1962).

Trees are considered as active bioindicators of air pollution (Brawn and Leward, 1986), because they react sensitively to external interferences. The interference may severely reduce carbohydrate contents of the roots and disturb cell differentiation in the secondary xylem (Gregory et al., 1986). Air pollution has caused extensive damage to forest ecosystem (Smith, 1981; Kim, 1982; Freer-Smith, 1996). Having entered the needle/leaf normally through the stomata, pollutants cause damage to photosynthetic tissue and hence the net photosynthesis without any visible injury to tree foliage (Smith, 1974). A decrease in the photosynthetic capacity of a tree may affect the availability and storage of foods and the production of growth regulators, which may have a lasting effect on growth for periods of up to several years (Fritts, 1976).
Studies of the wood anatomy of trees grown in heavily polluted industrial areas suggest that there are changes in wood quality due to industrial emissions (Halbwachs and Kisser, 1967; Grill et al., 1979; Halbwachs and Wimmer, 1987; Kartusch, 1988; Wimmer 1993; Pozgaj et al., 1996). Most changes in wood structure and quality, however, may not lead to changes in wood properties (Bauch, 1986).

Exposure to SO$_2$ hampers CO$_2$ uptake in young spruce and reduces photosynthetic activity (Keller, 1980), which in turn retards the cambial activity and consequently the wood production. The influence of air pollution on cambial activity in tree species growing in the vicinity of smoke-emitting factories may result in a retarded production of secondary xylem and phloem (Jabeen and Abrahm, 1998).

With the activity of the vascular cambium over a time period, a large amount of secondary xylem (wood) and a relatively less amount of secondary phloem (bark) are produced each year. The amounts of the tissues produced vary widely depending upon tree species, growth vigour, and environmental conditions. Ratios of xylem and phloem can range from a high of 15:1 to a low of 2:1, with a ratio of 4:1 being most common (Dickison, 2000). The xylem-phloem ratio often declines under the environmental stress.

The processes that influence cambial differentiation determine the fate of the dividing cambial initials and physical structure of their derivatives (e.g., lumen size and wall thickness). These features are important in determining the capacity of a stem to transport water to its leaves and its ability to withstand reductions in the availability of soil water and to avoid xylem cavitation and embolism (Wullschleger et al., 1992). In the very few cases where these effects have been studied, increases in CO$_2$ have produced quantitative and qualitative changes in xylem structure (Tauz...
et al., 1996; Mjwara et al., 1996). The width of sapwood band varies greatly among species, being relatively wider in stems of young trees and narrower in old trees. Sapwood proportion also increases with the height of the stem (Bosshard et al., 1986). The change in sapwood portion may give an idea of the rate of radial growth in plants and can thus be a good indicator of the influence of air pollution on wood formation (Pozgaj et al., 1996). Sapwood portion in spruce trees decreases with loss of needles; a little loss of needles may bring about evident changes in sapwood width (Bosshard et al., 1986). Thus, sapwood width will decrease with increasing damage to trees. This should hamper water condition and then transpiration in the affected trees. The reduction of sapwood is relatively low in hardwood species. It is inversely proportional to the degree of damage in beech trees (Aszmutat et al., 1986) and oak trees (Bues and Schulz, 1990). A reduction of the sapwood width and sapwood portion in transections illustrates an interaction between photosynthesis, cambial activity and sapwood physiology.

Vins (1965) and Ashby and Fritts (1972), who used dendrochronological techniques to date and analyze ring width series, revealed that in years with high levels of air pollution, the average ring width was smallest in trees near the pollution source, and that average ring width increased with the distance of trees from the pollution source. The decrease in ring width due to air pollution is greater in the coniferous trees than in the dicot trees (Aufsess 1981; Eckstein et al., 1981; Bauch and Frühwald, 1983; Bosshard et al., 1986; Fink, 1986; Frühwald, 1986; Schweingruber, 1986; Torelli et al., 1986; Wagenführ, 1987; Sell et al., 1989; Pozgaj et al., 1996).
The pollution affects the radial growth much before any visible symptoms appear on the needles (Poleno, 1982). De Kort (1986) categorized trees as healthy, diseased, very diseased, dying and dead depending on crown appearance which is determined by the density and condition of the needles. Differences in the annual-ring width may not be significant along tree height (Wahlmann et al., 1986). Changes in ring width of declining trees might correlate to the density of wood (Keller, 1980; Knigge et al., 1985; Bauch et al., 1986; Kucera and Bosshard, 1989). The annual rings in the temperate zone trees consist of earlywood as well as latewood, and the upward transport of water solution in the coniferous/dicotyledonous trees occurs through the tracheids/vessels of earlywood. The proportion of latewood is correlated to the width of the annual rings (Ohta, 1978; Grosser et al., 1985; Yokobori, 1985; Bues et al., 1989).

The influence of air pollution on size of tracheary elements varies with site, plant species and pollutants, concentration and may be positive or negative. The wall thickness of late tracheids remains nearly unchanged (Sell et al., 1989; Kucera and Bosshard, 1989) though it is affected in early tracheids under heavy air pollution (Grosser et al., 1985; Bauch, 1986; Bauch et al., 1986). Radial diameter of the earlywood tracheids may also change (Göttsche-Kühl et al., 1988; Kartusch, 1988). The fine structure of cell walls and pits of diseased conifers remain unaffected (Fengel, 1985). However, frequency of tracheids increases in diseased fir trees. Environmental stresses caused by pollutants may not only reduce the radial wood increment (the annual ring width) but also seem to disturb the normal cell division (Grosser, 1986).
Density of wood may be a fundamental factor to evaluate the physical properties of wood. Different species vary enormously in their wood density which can be a good quantitative pointer of the influence of air pollution on radial growth. The density of the latewood may be as higher as three times the density of the earlywood. Ring width and wood density in healthy coniferous trees are negatively correlated; the density decreases with increasing ring width (Bernhard, 1964). This relation is not confirmed in the very diseased spruce trees where the reverse seems to apply (Evertsen et al., 1986; Bodner, 1988).

Air pollution decreases wood density as, for instance, in certain oaks (Hughes and Woods, 1986) and spruce trees (Pozgaj and Kurjatho, 1986). The density of latewood may either decrease (Keller, 1980; Bauch, 1986; Lewark, 1986) or go unchanged (Eckstein et al., 1981).

The toxic effects of pollutants may interfere with cellular functions, especially in the presence of poor and acid soil, affecting several parts of the plants including leaves and also the wood (Leitao-Filho et al., 1993). The quantitative aspects of wood structure may be affected (Jagels, 1986; Eckstein, 1988; Kort, 1990; Mayer et al., 1993).