Introduction
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Generation of energy by burning fossil fuels in power stations and engines, various types of industrial processes, the biodegradation of wastes, farming and forestry operations lead to the release of thousands of different chemicals into the atmosphere. Current causes of concern are for the increasing levels of carbon dioxide (CO$_2$) and other compounds related to 'green house effect' phytotoxic air pollutants (O$_3$, NO$_x$, NH$_3$, SO$_2$) and other gases (CH$_4$, CFCs), contamination of water bodies etc. All these gases are released in excess into the atmosphere due to anthropogenic activities. There is an alarming rise in these air pollutants, especially in highly industrialised nations. In Poland the annual emissions of SO$_2$ are 3 to 4 million tons (Oleksyn and Reich, 1994). In Europe, highest levels of sulphur deposition is found in the "Black Triangle", which includes South Western Poland, Germany and Czech Republic (Hettenlingh et al., 1993). In Scots pine (Pinus sylvestris L.) forests of Dutch region, deposition of atmospheric ammonia is 100-200 kg N ha$^{-1}$y$^{-1}$, while the critical load for nitrogen is 10-20 kg N ha$^{-1}$y$^{-1}$ (Perez-Soba, 1995). With the increasing levels of primary pollutants, the concentration of secondary pollutants particularly of ozone is increasing. The concentration of ozone in the troposphere continues to rise at a rate of $\approx 1$-2% per annum (Hough and Derwent, 1990).

Pollutants like O$_3$, NO$_x$, NH$_3$, SO$_2$ are known to have effects on human health, on plants, on animals and on natural and managed ecosystems. The impact of pollutants on vegetation is much apparent due to their statutory nature. Long term exposure of pollutants have resulted in widespread forest decline. In Poland, due to high SO$_2$ emissions, as many as 2500 plant species are endangered (∼25% of all species) and approximately 228 (∼20% of the tree species) have been extirpated (Oleksyn and Reich, 1994). Ozone is implicated as the factor contributing to the widespread decline of high elevated forests in central Europe and Eastern North-America (Barnes and Davison, 1988). Pollution is not only responsible for widespread forest decline, but also playing a major role in altering the ecosystem. Barnes et al., (1996) have
reported that excess of nitrogen deposition in the Netherlands has resulted in the transition of larger areas of *Calluna* dominated heathland into grassland dominated by nitrophilous species.

Thus with the widespread damage it becomes necessary to curtail the emissions at the source itself so as to protect the environment. Industrialised nations have set up air pollution acts and banned the production of hazardous chemicals. Results of such strict measures are already visible. In Britain and other parts of Europe, there has been a decrease in sulphur emissions over the past decade (Davison and Barnes, 1992). In India too, Air Pollution Act was laid down in 1986. Under this act, several industries violating the norms have been asked to shut down. In Gujarat, High court passed an order to close down 14 small scale industries at the Nandesari industrial estate. Some of the major industrial giants like the fertilizer company have been asked to slash production by 30-50% unless and until they curtail emissions.

**Interactions of Pollutants with Plants**

The vegetation present around the industries are affected the most. Trees, because of their perennial nature, are exposed to the pollutants most of the time and are damaged. Some of the common visible symptoms are chlorosis, necrosis, burnt tip of branches, defoliation etc. Infact the visual symptoms are the result of ongoing *subtle* changes within the plant system. Subtle changes start right from the entry of pollutants through the stomata and their direct interaction with the mesophyll cells. Distortion of stomata, disintegration of chloroplasts, enlargement of plastoglobuli are some of subtle injuries seen in plants on exposure to pollutants (Krishnayya and Bedi, 1989; Saastomoinen and Holopainen, 1989, Ballach et al., 1992). The other associated changes are alterations in antioxidants and carbohydrate levels. Experiments on natural sites have shown that antioxidative defense system is a reliable indicator of oxidative stress (Schmieden et al., 1993). Puccinelli et al., (1996) have
shown the use of peroxidase activity as a marker to edit maps of air quality. Hampp (1992), and Kainulainen et al., (1995) have suggested that carbohydrate levels indicate effects of pollution at an early stage and can be used to detect injury to the plants. The study of subtle injury indicates changes at the cellular level long before the appearance of visual symptoms. These subtle changes are further explicated in the overall growth of trees.

**Deposition and entry of Pollutants**

Pollutants and their interactions with plants are process-mediated, from their deposition to entry. Deposition of the pollutants on the receptive surface (vegetation) takes place by dry, wet (rain) or occult (fog/dew) deposition. Meteorological factors affecting the deposition of air pollutants are radiation, temperature, dew, wind speed and wind direction (Erisman, 1992). The height of the chimney also plays a role in the dispersal of pollutants. For taller chimneys, the deposition nearer to the source is less and increases downwind from the source until it reaches a maximum and decreases again. For chimneys with lesser height, dry deposition with high concentrations takes place near the source and gradually decreases downwind.

Once deposited on the foliar surface the entry of the pollutants into the leaves occur either via stomata or by permeating through the cuticular layer (Wellburn, 1988; Riederer, 1989). Selective uptake of ions through the cuticle has also been suggested (Evans et al., 1985). The ability of leaf surface to absorb substances varies between species, depending on epicuticular wax content, chemistry and other cuticular characteristics (Turunen and Huttunen, 1991). Embedded intracuticular waxes produce a non permeable barrier for solutes (Lendzian and Kerstein, 1991). Rennenberg and Polle (1994) have suggested the difference in concentration gradient between the atmosphere and substomatal cavity as the diffusive flux for water soluble pollutants like $\text{SO}_2$. Sulphur dioxide may pass the plasmalemma and enter the
symplasm either in the form of sulphate mainly by active, carrier mediated transport (Cram, 1990) or by diffusion (Spedding et al., 1980).

**Fate of sulphur dioxide**

Sulphur dioxide due to its higher solubility enters rapidly into the cell. Inside the cell hydrated \( \text{SO}_2 \) is dissolved into \( \text{H}^+ \), \( \text{HSO}_3^- \) and \( \text{SO}_3^{2-} \). Chloroplasts of the mesophyll cells are capable of oxidising (Asada and Kiko, 1972) and reducing (Schwenn et al., 1976) sulphur dioxide during light phase. Oxidation of \( \text{SO}_3^{2-} \) leads to the formation of sulphuric acid and finally into sulphate anions. Accumulation of sulphate occurs in the vacuoles (Polle and Rennenberg, 1993). Reduction of \( \text{SO}_2 \) leads to the formation of sulphur-containing amino acid which is finally incorporated into proteins and glutathione (Diltrich et al., 1992). Reduction also results in the release of \( \text{H}_2\text{S} \) gas which accounts for 10% of the influx of \( \text{SO}_2 \) (Kindermann et al., 1995). Oxidation of \( \text{SO}_2 \) into sulphate is a fast process compared to the reduction, and is preferred (Takahama et al., 1992). Kaiser et al., (1993) also stressed that 68% of \( \text{SO}_2 \) taken up through the leaves were oxidized to sulphate. The remaining 38% detoxified by reduction either to \( \text{H}_2\text{S} \) or organic sulphur compounds.

The presence of sulphate within the plants would signify the presence of higher ambient \( \text{SO}_2 \) and its entry. Raitio et al., (1995) found that total sulphur content in Scots pine needles showed a high correlation with the estimated mean \( \text{SO}_2 \) concentration in the air. The amount of sulphate accumulated in the shoot is dependent on \( \text{SO}_2 \) concentration applied, the duration of exposure, the species analyzed and the sulphur nutritional status (De Kok, 1990). Thus plants detoxify \( \text{SO}_2 \) either into oxidised or reduced form.
Free radicals and its generation

The entry of pollutants escalates the formation of free radicals in plant cell. Oxidation of sulphite into sulphate in chloroplast can give rise to the formation of superoxide anion ($O_2^-$) (Asada, 1980). Superoxide being a powerful nucleophile can react with protons to form the more toxic hydroperoxyl radical. In the hydrophobic environment of membranes, $O_2^-$ can react non-enzymatically with $H_2O_2$, thereby forming hydroxyl radical (Sgherri et al., 1996) Hydroxyl radicals lead to lipid peroxidation. The toxic superoxide anion is removed catalytically in the presence of enzymes and results in the formation of $H_2O_2$. Further, $H_2O_2$ can react with superoxide to form dangerous radicals (Cakmak et al., 1993). Thus the generation of radical continues. The active oxygen species are part of an alarm signalling process in plants (Foyer et al., 1994).

The Detoxification Mechanism

To counteract the damaging effects of free radicals, plants possess natural scavengers which remove the free radicals at various steps to minimise the damage. Tolerance mechanisms and chemical constituents have been identified which scavenge free radicals. These comprise of low molecular weight antioxidants (glutathione, ascorbate, $\alpha$-tocopherol) and protective enzymes (ascorbate peroxidase, superoxide dismutase). This system operates in the cytoplasm and chloroplast of plant cells in a series of reactions.

Foyer and Halliwell (1976) successfully devised a mechanism to show how the entire enzyme system functions (Fig. 1). The enzyme system involves detoxification of $H_2O_2$ to $H_2O$ by ascorbate peroxidase, regeneration of ascorbic acid catalysed either by monodehydroascorbate or dehydroascorbate reductase at the expense of NADH or
Fig. 1: $\dot{O}_2^-$ and $H_2O_2$ detoxification pathway (after Foyer & Halliwell, 1976)
reduced glutathione respectively. For the regeneration of reduced glutathione, glutathione reductase functions by using NADPH.

**Free radical scavengers**

Ascorbate, reduced glutathione and peroxidase are important superoxide scavengers in the chloroplast. The significance of each of these scavengers is dependent on their concentration and rate constant for the conversion of superoxide radicals (Rennenberg and Polle, 1994). Role of those scavengers which were chosen for the present study are described briefly.

**Glutathione** (GSH) is a tripeptide (γ-L-glutamyl L-cysteinyl glycine). It is widely distributed in plant cells. Through its particular structure glutathione renders its-SH group less susceptible to any attack by oxidants than other -SH group containing macromolecules (Smith *et al*., 1989). It functions in concert with ascorbate. It acts to protect labile macromolecules from the attack of free radicals and hydrogen peroxide. One of the main functions of GSH in a cell is to protect -SH groups in enzymes against oxidation to sulphide, and structural proteins either by acting as a scavenger for the oxidising agents or by repairing oxidised -SH group via the GSH disulphide exchange reaction (Alscher, 1989).

**Ascorbic acid** (AA) is a major metabolite in plants. It is an antioxidant and in association with other components of the antioxidant system, protects plants against oxidative damage resulting from aerobic metabolism, photosynthesis and a range of pollutants (Smirnoff, 1996). It occurs in the cytosol, chloroplasts, mitochondria and apoplastic (Anderson *et al*., 1993 and Rautenkranz *et al*., 1994). In addition to its role in photoscavenging cycle, AA can act directly as a scavenger of hydroxyl radicals. Ascorbate inhibits chain initiation by scavenging phenoxy radicals, sulphite radicals and superoxide radicals, which can be formed by reactions of sulphite radical with
oxygen (Takahara et al., 1992). It also functions in the regeneration of α-tocopherol, a compound of the thylakoid membrane which acts to scavenge toxic molecular species formed as a result of oxidative damage (Kunert and Ederer, 1995).

**Peroxidase (POD)** are ubiquitous enzymes which can oxidize donor molecules with the consumption of H$_2$O$_2$. Peroxidase have been implicated in a broad range of metabolic functions such as cell elongation, lignification, pathogen defense, wound healing etc. Ascorbate peroxidase exists both in chloroplast and cytosol. Peroxidase uses ascorbic acid as an electron donor in the detoxification of H$_2$O$_2$. Peroxidase is labile in the absence of ascorbic acid (Polle et al., 1990)

**Impact of air pollutants on levels of antioxidants**

The entry of air pollutants lead to an increase in cellular oxidants that induce increased synthesis of non enzymic antioxidants and antioxidant enzymes (Foyer et al., 1997). Substantial amount of work has been carried out on the changes in the antioxidants level of conifers exposed to pollutants (Madamanchi and Alscher, 1991; Van Hove et al., 1992; Polle et al., 1993; Fangmeier et al., 1994, Polle et al., 1994b).

Their studies revealed an increment in antioxidants level. Ballarin-Denti (1996) estimated ascorbic acid, peroxidase, superoxide dismutase and glutathione reductase to study different degrees of forest decline in Italy. Rabotti and Ballarin-Denti (1996) concluded that leaves of beech extend life span of their tissues by increasing the levels of different antioxidants. Nast et al., (1993) reported increased levels of peroxidase and glutathione in Norway spruce exposed to single pollutant and combination treatments of SO$_2$ and NO$_2$. Studies on the tropical trees have shown an increase in peroxidase and ascorbate in plants growing in polluted environment (Krishnayya and Bedi, 1990; Rao and Dubey, 1990). Klumpp et al., (1994) reported reductions in ascorbic acid, increase in glutathione and peroxidase content in broad leaved species near industrial area of Cubato, Brazil. Terry et al., (1995) interpreted that the
increased activity of enzymes and subsequent contents of substrates are to scavenge the free radicals generated. Contrary to the findings that antioxidants level have increased to the pollution stress, Wingsle and Hallgren (1993) reported no significant changes in membrane bound or water soluble antioxidants such as α-tocopherol, carotenoids and glutathione on exposure of SO₂ and NO₂ to pine needles. Polle et al., (1994a) failed to record significant differences in the activities of superoxide dismutase and total peroxidase in the needles of Scots pine from three SO₂ polluted sites. Antioxidants level increased only when the levels of pollutants were high.

Polle and Rennenberg (1993) suggested that to avoid damage, it is necessary that plants contain sufficient concentrations of antioxidants in those sub-cellular compartments in which reactive oxygen species are generated. The regeneration rates for reduced antioxidants cope up with the production of free radicals. Injury is observed in the absence of maintenance of antioxidants level. Rabotti and Ballarin-Denti (1996) reported higher injury levels in beech leaves with lower ascorbate peroxidase activity. Likewise May and Lever (1993) observed that in the absence of large reduced GSH pool, hydrogen peroxide accumulates in the cytosol and causes oxidative damage. Thus, when the influx of pollutants exceeds the detoxification capacity and repair mechanism by enzymatic and non-enzymatic systems in the cell, several types of injury are conceivable (Wingsle and Hallgren, 1993). The radicals attack the proteins and lipids leading to their peroxidation. Lipid peroxidation as measured by malondialdehyde content serves as an indicator of damage to cellular membrane system (Weigel et al., 1989). Increase in lipid peroxidation due to exposure of plants to SO₂ & NO₂ has been reported (Sandmann and Gonzales, 1989; Price et al., 1990; Navari-Izzo et al., 1992). The higher rate of lipid peroxidation leads to destruction of membranes. Weckx and Clijsters (1996) suggested that lipid peroxidation leads to an increase in phase transition temperature of the membranes, resulting in an enhanced membrane leakiness.
The antioxidants level has a direct role in the tolerance of a species (Weigel et al., 1989). Pfanz et al., (1996) reported that the sensitivity of the trees to the pollutants differed greatly between species, mainly by their detoxification capacity. Rao and Dubey (1993) studied response of two soybean cultivars (CV-Punjab 1 and CV-JS 7244) to SO$_2$ exposures. Their results indicate a relation between the ability of a plant to maintain reduced glutathione and ascorbate contents, and SO$_2$ tolerance. The species with elevated antioxidants could tolerate the stress of SO$_2$ induced free radicals. Madamanchi and Alscher (1991) on a similar work on Nugget concluded that resistant variety had higher glutathione content then the sensitive variety. Thus the resistant species can prevent the oxidative stress by increasing the antioxidants level.

**Effects of pollutants on photosynthesis and related activities**

Sulphur dioxide acts directly on the chloroplast (Alscher et al., 1997). Chloroplast is the most sensitive organelle and the damage is due to the reactivity of SO$_2$ or anions formed during its hydration, and such reactions are irreversible (Veljovic-Jovanovic et al., 1993). Chlorophyll $a$ is more sensitive to aqueous SO$_2$ than chlorophyll $b$. Any destruction to the chloroplast ultimately effects the pigment content and rate of photosynthesis. The contents of photosynthetic pigments decreased in chronic and acute treatments compared to control in *Hedera helix* L. exposed to O$_3$ (Della-torea et al., 1996). Heimler et al., (1989) found that on 2 years of treatment to *Picea abies* with pollutants (SO$_2$ + NO$_2$ + O$_3$) had decreased the chlorophyll content considerably without the appearance of visible injury. Khan and Khan (1994) reported reduction in pigments exposed to pollutants. Similar results were observed by Tiwari and Bansal (1993) in *Mimusops elengi* exposed to SO$_2$. In a study by Pandey and Agrawal (1994), the chlorophyll concentration in tomato plants increased initially but declined after longer period of exposure (SO$_2$ + NO$_2$). The decline in the photosynthetic pigments has an impact on the net photosynthesis. The photosynthesis was
progressively inhibited as the SO$_2$ concentration increased (Veljovic-Jovanovic et al., 1993). Ashenden et al., (1995) reported lower rate of net photosynthesis in white clover exposed to gaseous pollutants.

Contrary to the ill-effects of pollutants on chlorophyll pigments, the presence of nitrogen in pollutant mixture often has a positive effect. Wingsle and Hallgren (1993) reported increase in chlorophyll content on NO$_2$ exposure and suggested that increment is due to the nutritional effect of NO$_2$. Van Hove et al., (1989) reported higher photosynthetic rates in poplar trees exposed to 100 µg NH$_3$ m$^{-3}$. Kelly et al., (1993) have reported increase in photosynthetic rates per unit leaf area with increasing O$_3$ exposure.

The reduction in photosynthetic capacity has a direct effect on carbohydrate metabolism. Photooxidants shift the activation of CO$_2$ fixation enzymes to oxidised state and thus decrease the fixation capacity for CO$_2$ in light (Hampp, 1992). Alscher and Amthor (1988) have proposed two possible mechanisms to explain the altered carbohydrate metabolism: (1) Limit the ability of the photosynthetic mechanism to fix carbon (2) Carbon fixed will be more rapidly catabolised to support the maintenance and repair processes. The diminished levels of assimilates (starch and sucrose) will result in diminished export to growing and storage tissues.

Alteration in the carbohydrate levels have been reported (Katzel and Loffler, 1996; Nerg et al., 1996). Changes in carbohydrates under the influence of SO$_2$ are very diverse and depend on the SO$_2$ concentration and fumigation durations, as well as on the sensitivity of different tree species and provenance to SO$_2$ (Kainulainen et al., 1995). Schmieden and Wild (1994) reported that trees growing nearest to the source contained significantly higher amounts of starch and sugar than control. Steubing et al., (1989) found accumulation of starch content in beech exposed to a mixture of O$_3$, SO$_2$ and NO$_2$ with a significant decrease in glucose content. Higher starch contents
were also reported by Anttonen et al., (1996). The alteration in leaf carbohydrates is dependant on leaf age with older leaves responding more strongly than younger leaves (Bucker and Ballach, 1992).

Sulphur dioxide has been reported to affect the translocation of carbohydrates by blocking the phloem loading, thus leading to starch accumulation in the chloroplast of mesophyll cells (Steubing et al., 1989). Likewise, ozone also induces changes in the flux of photoassimilates, by the impairement of translocation within the assimilation tissue, impairement of phloem loading, increased allocation towards locations with increased repair activity (Schmieden and Wild, 1995). Forschner et al., (1989) reported impairement of the transport of photoassimilates in damaged spruce trees.

Depleted carbohydrate reserves has an impact on growth rates either by reducing the ability to withstand environmental extremes or by reducing the ability to resist disease and insects or both. This ultimately exacerbates the susceptibility of plants to multiple stresses (Kelly et al., 1993). Kainulainen et al., (1993) reported higher occurrence of conifer aphids in polluted environments. The increased pest attack is due to an increase in the concentrations of reducing sugars and free amino acids which are important nutrients for aphids.

Effects on foliar surface and internal structures

Foliar surface is the direct phase of contact between the trees and the atmosphere. Foliar surfaces are directly exposed to the pollutants and are vulnerable to damage. Foliar surface comprises of cuticle made up of cutin and waxes. Other appendages include trichomes. The functions of surface structures of higher plants are protection against dessication, frost and pathogen infestation.
The cuticular wax layer of leaf surface represents the primary point of attack for air borne pollutants. Direct effects occur on surfaces and inside the mesophyll tissue of leaves. This leads to the deterioration of surface wax structure, disturbances in stomatal functions and visible injury (Raddi et al., 1992). Percy et al., (1990) have reported that acid deposition induces delayed cuticular development. It damages the tubular structures of epicuticular waxes in epistomatal chambers of needles. Delayed development of the epistomatal waxes and four types of deformed stomatal closure were found in Scots pine needles exposed to simulated acid rain treatment (Turunen and Huttunen, 1991). Erosion of epicuticular waxes have been reported by Schmieden and Wild (1995). The erosion of wax and structural changes to stomata can change needle wettability and alter cuticular and stomatal diffusion resistance to the needles leading to altered transpiration (Paoletti et al., 1996). Significant changes in the chemistry of epicuticular waxes in plants exposed to nitric acid were found indicating that nitric acid could enter plants through cuticle in addition to stomatal uptake (Bytnerowicz et al., 1996).

Trichome density and trichome length were increased in plant populations of *Cercis canadensis* of the polluted habitats as reported by Sharma (1996). The increased trichome density increases the receptive area (Bitterlich and Upadhyaya, 1990). Prasad and Inamdar (1990) reported decrease in stomatal frequency and increase in trichome frequency in *Pisum sativum* exposed to pollution. Similar results were described by Sharma (1992). Paoletti (1996) concluded that xeromorphic adaptations, such as thick cuticle and dense pubescence further promote self-protecting mechanism in holm oak. Studies have shown that pollutants alter the foliar internal structures. Some of the changes include reductions in mesophyll cell size (Sutinen et al., 1990), decline in the intercellular spaces of spongy layer (Back, 1994) and increase in epidermis thickness (Antonelli et al., 1996; Laakso et al., 1996). Percy et al., (1992) have reported an increase in cuticular membrane thickness following exposure to O₃ in red spruce needles.
From the above review of literature it is observed that pollutants alter the plant metabolism in various ways. Higher production of antioxidants leads to an early aging of leaves (Gilliemaut et al., 1992) and finally to early senescence (Nie et al., 1993). Further, the decline in photosynthetic activity results in reduced plant productivity (Dann and Pell, 1989; Monk and Murray, 1995). Hampered translocation of photosynthates results in the accumulation of reserve food within the leaves and thus is not available for growth of the plant. Long term exposure to pollutants inevitably leads to death of the trees and ultimately gets explicit in forest decline.

**Importance of the present study**

Present study was conducted in Baroda industrial region, where trees are naturally exposed to mixtures of pollutants emitted continuously by the industries. Brown and Bell (1990) stressed that interactions with air pollutants is best served in realistic environment than in a closed chamber. Even the open top fumigation chambers (OTCs) used extensively in crop loss assessment programmes in USA and Europe have been criticised. Sanders et al., (1995) viewed that in OTCs there is alteration in the microclimate resulting in higher influx of pollutants, thereby showing greater damage as compared to ambient conditions. Schmieden and Wild (1995) concluded that physiological and biochemical reactions of damaged and preconditioned trees in natural systems frequently differ from those of non-preconditioned trees. Hence field studies were chosen to address the objectives of this work. Ample amount of work was done on conifers and temperate trees. Few studies were conducted on tropical trees. The proposed study of tropical trees will enrich the available information. From the review of literature it is evident that subtle changes manifest early in plants exposed to pollutants. In order to assess these changes in tropical trees before the magnitude of pollution damage becomes manifold, this study was planned. To rate the performance of trees and to make a comprehensive biochemical study, the following parameters were chosen - (1) Total chlorophyll, malondialdehyde and total
sulphhydryl group levels to record the extent of damage (2) Peroxidase, ascorbic acid and glutathione to monitor the changes in antioxidant pool (3) Starch, soluble sugars, and exudate study to know the variation in carbohydrate levels. The data were supplemented with few anatomical studies. The entire generated data would be used to classify the selected tree species as sensitive or tolerant ones.

**Objectives of the study are**

(1) To record biochemical changes in 12 tropical tree species growing in Baroda industrial areas.
(2) To study the changes occurred to external and internal features of the leaf.
(3) To categorise the selected tree species as sensitive and tolerant ones.