Journal of Environmental Biology **January 2007,** 28(1) 127-132 (2007)

Biochemical parameters of plants as indicators of air pollution

A. K. Tripathi and Mukesh Gautam*

Ecology and Environment Division, Forest Research Institute, Dehradun-248 006, India

(Received: August 5, 2005 ; Revised received: December 22, 2005; Accepted: January 14, 2006)

Abstract: *In the present study, species like Mangifera indica, Linn., Cassia fistula, Linn., and Eucalyptus hybrid were exposed to different air pollution load for short duration (active biomonitoring). Variation in biochemical parameters like chlorophyll, protein, soluble sugar, free amino acid, ascorbic acid, nitrate reductase, superoxide dismutase and peroxidase in the leaves were found to be pollution load dependent. These variations can be used as indicators of air pollution for early diagnosis of stress or as a marker for physiological damage to trees prior to the onset of visible injury symptoms. Just by analyzing these biochemical indicators air quality can also be assessed.*

Key words: *Bioindicators, Superoxide dismutase, Nitrate reductase, Peroxidase*

Introduction

Air pollution is one of the severe problems world facing today. It deteriorates ecological condition and can be defined as the fluctuation in any atmospheric constituent from the value that would have existed without human activity. Various efforts have been done for environmental restoration in India but still it seems to be a formidable task. Dehradun valley is no exception. Its environment has undergone irreparable damage due to the population growth and its subsequent requirements in terms of housing and traffic density. Continuously increasing road traffic is a primary culprit. The changed ambient environment due to the air pollutants in urban area of Dehradun has exerted a profound influence on the morphological, biochemical and physiological status of plants, and therefore its responses. To assess the seriousness of the air pollution threat and to take effective actions, the components of an urban air quality management should also include a biological monitoring to complement the instrumental air quality monitoring. It will provide the necessary feedback information about receptor conditions in the face of regional pollutant emissions. National Forest Policy, 1988 clearly directs that forests be managed first as an ecological necessity, second as a source of goods for local populations and only third as a wood for industries. Since plants and trees are the ecological necessity and air pollutants cause large scale damage to these, therefore policy makers must consider the sensitivity of the plant receptors before prescribing the standards or framing the emission control policies in Indian air quality management system. Until now, data on pollutant effects on biological parameters at any level have almost never been used to set allowable levels of emissions in air quality monitoring programmes in Dehradun valley. Research needs to be expanded to encompass a greater variety of plant responses to interactive stresses caused by air pollutants in more realistic field conditions.

The main focus of this work is to provide an assessment of the use of biochemical parameters of plants as indicators of air pollution so that these biochemical indicators can be used for air quality monitoring in urban areas of Dehradun, the capital of Uttaranchal. The proposed study will provide a technical support to the air quality management in the city of Dehradun. The data generated will help us to find out the exact position *i.e.* success or failure of the regulatory measures which have been taken and also the corrective measures which are required to take up to bring the system to its normal or pristine stage.

Materials and Methods

Method selection: Active and passive biomonitoring are the two methods which can be applied to evaluate the applicability of the biochemical parameters of plants as indicators of air pollution. Here active biomonitoring method was opted which consists of exposing potted test plants to the polluted areas for short duration *i.e.* for three months (Klumpp, 2003).

Species selection: Species were selected on the basis of air pollution tolerance index (APTI). Species having APTI less than 10 are termed as sensitive species and can be used for the biomonitoring of air pollutants (Agrawal *et al.*, 1991). APTI of five forest tree species *(Mangifera indica*, Linn., *Cassia fistula*, Linn., *Eucalyptus hybrid, Grevillea robusta,* A. Cunn. *and Dalbergia sissoo,* Roxb.*)* was calculated by using the method given by Singh and Rao (1983). *Mangifera indica*, Linn (APTI 8.10), *Cassia fistula*, Linn. (APTI 7.56) and *Eucalyptus hybrid* (APTI 8.69) were selected to use their biochemical parameters as bio indicators of air pollution and were grown in polybags for one year. Four plants of each species were exposed to air pollution for three months (October to December, 2004) at selected bioindicator stations.

***Corresponding author:** E-mail: tripathiak@icfre.org, Tel.: 0135-2752674(O), 2750449 (R), Fax: 0135-2756865

Site selection/bioindicator station selection: Total three bioindicator stations including urban and suburban sites, close to streets with heavy and light pollution load were identified and Forest Research Institute (FRI) was treated as control site. Details of sites are :

Bioindicator station 1 - Darshan lal chowk (Near clock tower)
Bioindicator station 2 - Mohkampur (Haridwar road)
Bioindicator station 3 - Mohebewala (Dehradun-Delhi road)
Control site - FRI

Air quality analysis (SO_2, NO_x and SPM): During the exposure period, ambient air quality in terms of common air pollutants *i.e.* SO_2, NO_x and SPM was analysed at all the bioindicator stations. Sampling was done 24 hr and twice in a week during the exposure period. Average of 24 hr such sampling was taken for final calculation. For the collection of samples for SPM from ambient air, GF/A filter paper was used in high volume sampler (HVS) at the flow rate of 1.0 to 1.5 m^3/min. SPM was computed as per standard method. Filter paper was weighed before and after sampling. West and Gaeke method (1956) and modified Jacob and Hochheiser method (1958) were used for analysis of SO_2 and NO_x respectively.

Table-1: Ambient air quality and air pollution index for different bioindicator stations

Bioindicator stations	Pollutants (μg/m³)			Air pollution index
	SPM	SO_2	NO_x	
Station No. 1	395	19.20	23.90	117.66 (Severe air pollution)
Station No. 2	275	13.35	17.41	82.33 (Heavy air pollution)
Station No. 3	215	15.64	19.13	70.00 (Moderate air pollution)

(Ambient air quality standards taken for calculation of air pollution index 140 μg/m³ for SPM, 60 μg/m³ for SO_2 and 60 μg/m³ for NOx)

Table-2 : Rating scale for indices (ref)

Index value	Remarks
0-25	Clean air
26-50	Light air pollution
51-75	Moderate air pollution
76-100	Heavy air pollution
>100	Severe air pollution

Air pollution index (API): The average of the sum of the ratios of three major pollutant concentrations to their respective air quality standards were obtained. The average was then multiplied by 100 to get the index (Rao and Rao, 1989).

$$API = 1/3 \left[\frac{(SPM)}{(S_{SPM})} + \frac{(SO_2)}{(S_{SO_2})} + \frac{(NO_X)}{(S_{NO_x})} \right] X\ 100$$

where S_{SPM}, S_{SO_2} and S_{NO_x} represent the ambient air quality standards for SPM, SO_2 and NO_x.

Air pollution index of bioindicator stations were developed on the basis of ambient air quality analyzed at specified bioindicator stations through instrumental monitoring of SPM, SO_2 and NO_X and correlated with the variation in biochemical indicators. On the basis of air pollution index, bioindicator station 1 was categorized as severe air pollution site (air pollution index 117.66), station 2 as heavy air pollution site (air pollution index 82.33) and station 3 as moderate air pollution site (air pollution index 70.00) (Table 1 and 2).

Biochemical parameters: After three months of the exposure, plants were brought back to the institute and leaf samples were analysed for different biochemical parameters. Total chlorophyll was analysed following the method of Arnon (1949), ascorbic acid by Sadasivam and Balasubraminan (1987), protein by Lowry *et al.* (1951), total soluble sugars by phenol sulphuric acid method of Dubois *et al.* (1951), free amino acid by Moore *and* Stein (1948),

Table-3 : Biochemical Indicators of different species at different bioindicator stations

Species	Station/ sites	Chlorophyll (mg/g)	Protein (mg/g)	Total soluble sugar (mg/g)	Free amino acid (mg/g)	Ascorbic acid (mg/g)	N R (μmole NO_2 formed g^{-1} FW hr^{-1})	SOD (unit/g)	Px (changes in OD/30 sec/g)
M. indica	*Control*	*8.28*	*18.30*	*16.17*	*5.54*	*3.37*	*2.98*	*81.60*	*0.91*
	Station1	7.95	17.81	13.41	7.97	6.22	4.16	98.62	1.42
	Station2	9.34	18.12	15.38	6.23	5.12	3.25	88.73	1.30
	Station3	8.91	18.94	15.99	6.28	5.12	3.37	84.61	1.41
C. fistula	Control	9.91	13.96	10.96	3.51	2.13	1.46	58.25	0.34
	Station1	3.33	9.24	6.17	4.97	3.19	2.99	63.62	0.54
	Station2	6.43	10.15	8.13	4.15	2.14	2.23	61.80	0.57
	Station3	8.13	10.66	8.95	3.75	2.69	2.70	62.98	0.40
E. hybrid	Control	8.63	28.78	13.08	8.15	4.98	3.59	78.58	0.53
	Station1	6.73	30.17	11.55	10.27	6.62	5.80	87.24	0.83
	Station2	7.42	28.98	12.93	9.55	6.00	5.44	85.18	0.73
	Station3	8.31	32.16	12.15	9.16	5.19	5.13	82.59	0.73

Data represent mean of four replicates. Results are significant at 0.1% ($p<0.001$)

superoxide dismutase by Sangeetha *et al*. (1990), peroxidase by Malick *and Singh* (1980) and nitrate reductase by Jaworski's (1971) method. Results were statistically analysed and interpreted for drawing conclusions.

Results and Discussion

After three months of exposure, leaf samples of the plant species were analysed for chlorophyll, protein, soluble sugar, free amino acids and some of the enzymatic parameters like nitrate reductase, superoxide dismutase and peroxidase activity.

All the biochemical indicators exhibited significant variation ($p<0.001$) from species to species and station to station (Table 3).

***Mangifera indica*, Linn.:** At station 1, *Mangifera indica,* Linn. exhibited 3.6% reduction in chlorophyll content while increase of 12.8% and 7.6% was observed at stations 2 and 3 respectively. Significant reduction (2.7%) in protein content was observed at station 1, followed by a loss of 0.98% at station 2, whereas at station 3, gain of 3.5% was evident. All the stations showed significant reduction in soluble sugar ($p<0.001$). Maximum reduction (17.1%) in soluble sugar was exhibited at station 1 followed by 4.9% and 1.1% at stations 2 and 3 respectively. Free amino acid exhibited an increasing trend at all the stations. Maximum gain of 43.9% was evident at station 1 followed by 13.4% and 12.4% at stations 3 and 4. Ascorbic acid also showed increase over control at all the stations. Maximum enhancement of 84.6% was exhibited at station 1, followed by 51.9% each at stations 2 and 3. Nitrate reductase activity was also found to be increasing at all the stations. Maximum increase (39.6%) was observed at station 1, followed by station 3 (13.1%) and station 2 (9.1%). Superoxide dismutase activity varied positively at all the stations. Maximum stimulation of 20.9% was revealed at station 1 followed by 8.7% at station 2 and 3.7% at station 3. Positive trend was also observed in case of peroxidase activity at all the stations. Station 1 exhibited maximum increase (56%) followed by station 3 (55%) and station 2 (42.9%) (Table 3 and Fig. 1).

***Cassia fistula*, Linn.:** Biochemical indicators of *Cassia fistula,* Linn. at all the bioindicator stations varied significantly ($p<0.001$) (Table 3 and Fig. 2). Maximum reduction (66.4%) in chlorophyll content was observed at station 1 while at station 2 and 3, loss of 35.1% and 18% was observed. At all the stations, protein content showed significant reduction. Maximum loss of 33.8% was exhibited at station 1 followed by 27.3% and 23.6% at stations 2 and 3 respectively. Soluble sugar showed maximum loss of (43.7%) at station 1 followed by 25.8% at station 2 and 18.3% at station 3. Like *Mangifera indica,* Linn., *Cassia fistula,* Linn. also showed increasing trend of free amino acids at all the stations as compared to control values. Station 1 exhibited maximum enhancement (41.6%) in free amino acids followed by station 2 (18.2%) and station 3 (6.8%). Ascorbic acid was also found to be increasing at all the stations as compared to control. Maximum increase of 49.8% was evident at station 1 followed by 26.3% at station 3 and 0.5% at station 2. Stimulation in nitrate reductase activity was observed at all the stations as compared to control. Maximum stimulation (104.8%) was observed at station 1 followed by 84.9% at station 3 and 52.7% at station 2. Stimulating trend was also observed in case of superoxide dismutase activity at all the stations. At station 1, 9.2% stimulation was observed followed by 8.15% at station 3 and 6.1% at station 2. Like other enzymatic activities, peroxidase activity was also found be more at all the

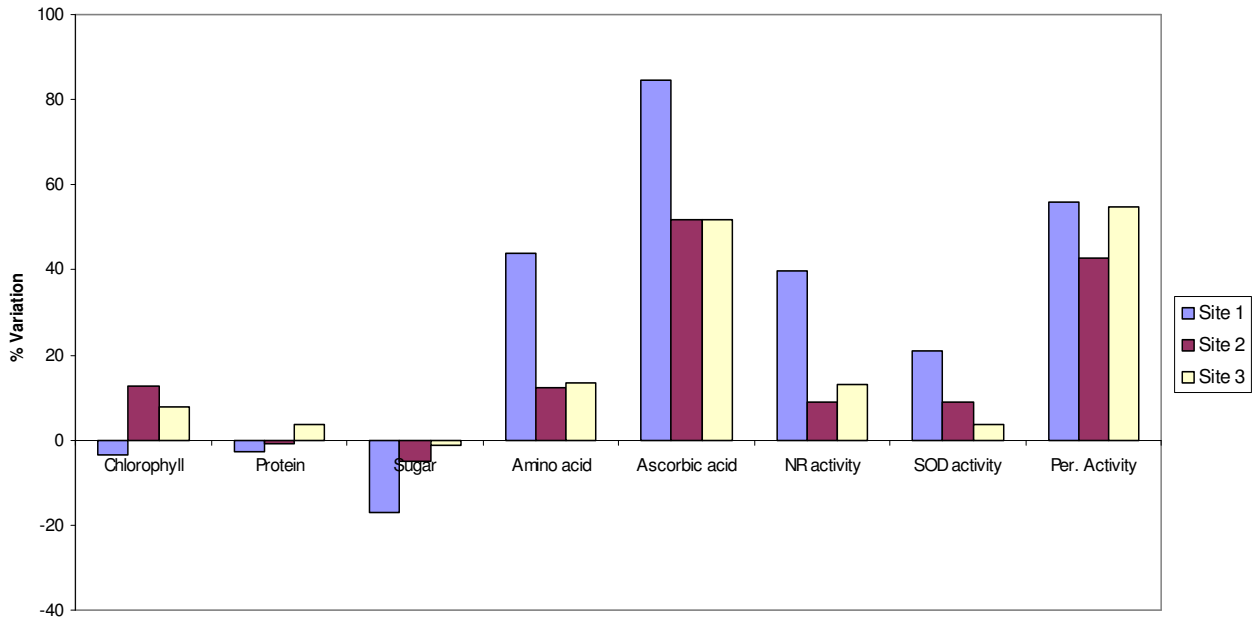

Fig. 1: Variation in biochemical indicators of *Mangifera indica* at different bioindicator stations

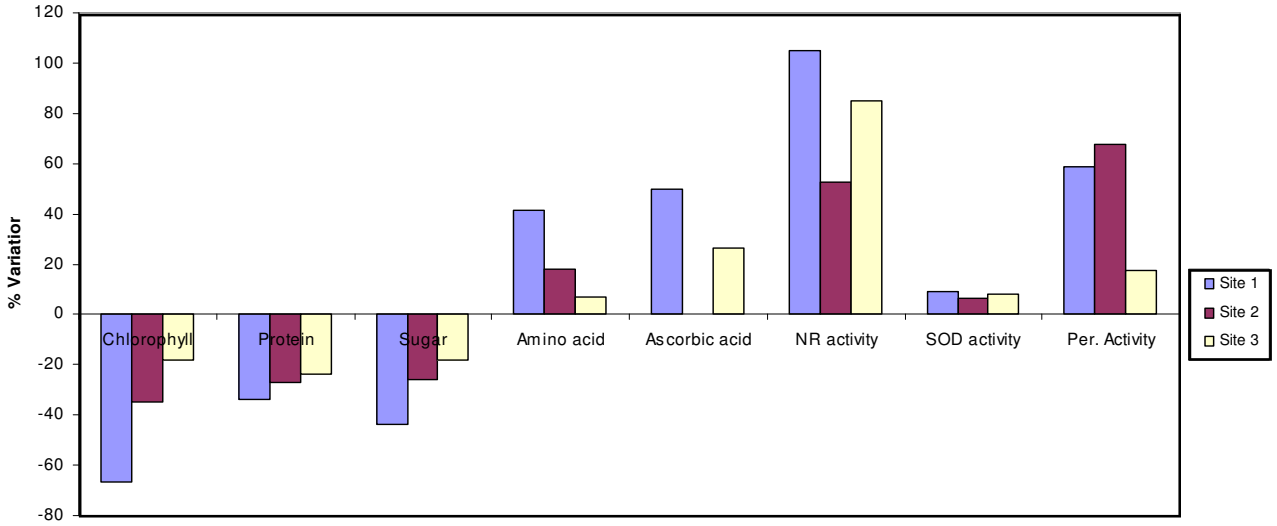

Fig. 2: Variation in biochemical indicators of *Cassia fistula* at different bioindicator stations

stations, as compared to control. Maximum stimulation of 67.6% was exhibited by the species exposed at station 2, followed by 58.8% at station 1 and 17.6% at station 3 (Table 3 and Fig. 2).

Eucalyptus hybrid: Chlorophyll content at station 1 showed 22% reduction, followed by 14% reduction at station 2 and 3.7% at station 3, as compared to control ($p<0.001$). Increase in protein content was exhibited at all the stations. Maximum increase (11.7%) was observed at station 3 followed by station 1 (4.8%) and station 2 (0.7%) at all the stations. Soluble sugar was found to be significantly reduced ($p<0.001$). Maximum reduction (11.6%) was revealed at station 1 followed by station 3 (7.1%) and station 2 (1.15%). Free amino acids were found to be more at all the stations as compared to control. Maximum enhancement (26%) in free amino acid was exhibited at station 1 followed by 17.25% at station 2 and 12.3% at station 3. Ascorbic acid content was significantly increased at all the stations and again maximum gain (32.9%) was observed at station 1 followed by station 2 (20.48%) and station 3 (4.2%). Enzymatic activities like nitrate reductase, super oxide dismutase and peroxidase were found to be higher than respective control values. Maximum increase (61.6%) in nitrate reductase activity was evident at station 1 followed by station 2 (51.5%) and station 3 (43.0%). Super oxide dismutase activity increased 11.0% at station 1, 8.3% at station 2 and 5.1% at station 3 as compared to control. Peroxidase activity exhibited maximum stimulation (56.6%) at station 1, followed by 37.7% each at station 2 and 3 (Table 3 and Fig. 3).

Although all the species showed significant variation in all the biochemical indicators, the extent up to which plant species were affected varied from species to species and station to station. Almost all the species showed maximum variation in biochemical indicators at station 1, which is found to be severe air pollution site. A considerable loss in total chlorophyll, in the leaves of plants exposed at station 1 (severe air pollution site) supports the argument that the chloroplast is the primary site of attack by air pollutants such as SPM, SO_2 and NO_X. Air pollutants make their entrance into the tissues through the stomata and

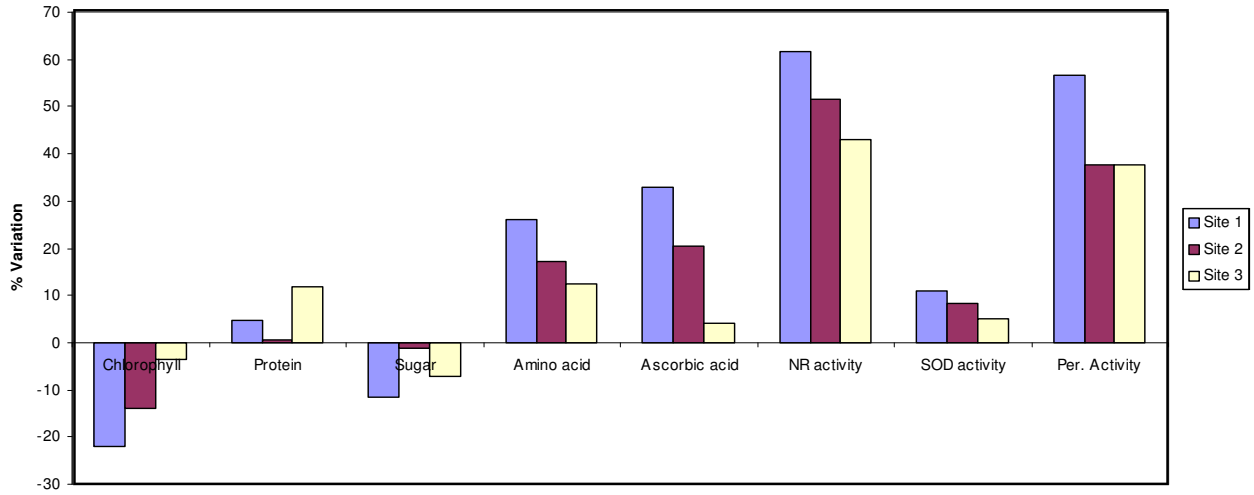

Fig. 3: Variation in biochemical indicators of *Eucalyptus hybrid* at different bioindicator stations

cause partial denaturation of the chloroplast and decreases pigment contents in the cells of polluted leaves. Rao and Leblanc (1966) mentioned that high amount of gaseous SO_2 causes destruction of chlorophyll and that might be due to the replacement of Mg^{++} by two hydrogen atoms and degradation of chlorophyll molecules to phaeophytin. In *Cassia fistula,* Linn. and *Eucalyptus hybrid*, maximum depletion in chlorophyll content at station 1 may be due to the maximum pollution load at this site whereas station 2 and 3 showed less depletion due to lower pollution load.

Reduction in protein content in *Cassia fistula,* Linn. at all the stations while station 1 in case of *Mangifera indica,* Linn. might be due to the enhanced rate of protein denaturation which is also supported by the findings of Prasad and Inamdar (1990). Constantinidou and Kozlowski (1979) found enhanced protein denaturation and breakdown of existing protein to amino acid as the main causes of reduction in protein content.

Soluble sugar is an important constituent and source of energy for all living organisms. Plants manufacture this organic substance during photosynthesis and breakdown during respiration. Our study revealed significant loss ($p<0.001$) of soluble sugar in all the species at all the stations. All the species showed maximum loss at severe air pollution site *i.e.* at station 1, followed by heavy air pollution site (station 2) and moderate air pollution site (station 3). The concentration of soluble sugars is indicative of the physiological activity of a plant and it determines the sensitivity of plants to air pollution. Reduction in soluble sugar content in polluted stations can be attributed to increased respiration and decreased CO_2 fixation because of chlorophyll deterioration. Davison and Barnes (1986) mentioned that pollutants like SO_2, NO_2 and H_2S under hardening conditions can cause more depletion of soluble sugars in the leaves of plants grown in polluted area. The reaction of sulfite with aldehydes and ketones of carbohydrates can also cause reduction in carbohydrate content.

All the species showed increased free amino acids at all the stations but it varied with the air pollution load. Severe air pollution site *i.e.* station 1 exhibited maximum increase of free amino acids as compared to control and other stations. More free amino acids at severe air pollution site may be due to more nitrate reductase activity or may also be due to more protein denaturation at this station.

Present investigation revealed a great deal of variation in the levels of ascorbic acid in all the species at all the stations. Pollution load dependent increase in ascorbic acid content of all the species may be due to the more rate of production of reactive oxygen species (ROS) such as SO_3^-, HSO_3^-, OH^-, O_2^- etc. during photooxidation of SO_3^- to SO_4^- where sulfites are generated from SO_2 absorbed. The free radical production under SO_2 exposure would increase the free radical scavengers, such as ascorbic acid, super oxide dismutase, peroxidase etc. (Pierre and Queirz, 1981) based on dosage and physiological status of plant. Increased level of ascorbic acid may be due to the defense mechanism of the plant.

Nitrate reductase is a metalloflavoprotein inducible enzyme which catalyses the reduction of nitrate to nitrite. It acts as a rate limiting step and regulatory enzyme in the pathway $NO_3 \longrightarrow NO_2 \longrightarrow NH_4 \longrightarrow$ amino acids, and its activity often controls the overall assimilation rate of nitrate. There are two distinct pools for nitrate in plant tissues *i.e.* storage and metabolic pools, only nitrate of the metabolic pool functions as a substrate for NR and contributes to organic nitrogen. In the present investigation air pollution load dependent increase in NR activity may be due to the more availability of nitrate in the metabolic pool of the plants at more polluted site. The source of the nitrate may be the NOx pollutants in the atmosphere. Zeevaart (1974) found induction of nitrate reductase activity in plants by atmospheric NO_2.

Superoxide radicals (O_2^-) are less toxic than other potential secondary oxy radicals, thus removal of superoxide radicals is a detoxification process or indirect protective action. SOD, catalase and peroxidase serve as interlinked primary protection mechanism. SOD along with catalase and peroxidase that acts on the end product (H_2O_2) of SOD activity can interact to regulate injurious oxy radicals and peroxyl concentrations in cells and organelles and determine equilibrium rates (Bennett *et. al.,* 1984). SOD increases and can protect cells against free radicals produced by air pollutants by catalyzing following reaction to form H_2O_2 (Scandalios, 1993).

$$2O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$$

H_2O_2 is the end product and is broken down by peroxidase into H_2O and O_2. In the present study SOD and peroxidase activities in all the species were found to be maximum at severe air pollution station than other stations. This may be due to the more interlinked primary protection mechanism offered by SOD and peroxidase in plants to protect themselves at severe air pollution station as compared to the less polluted sites. Increased resistance of plants may be correlated with increased SOD activity (Tanaka *et al.,* 1982).

In view of the data obtained in present investigation it seems reasonable to conclude that SOD play significant role in protecting living cells against the toxicity of active O_2^- species. Varshney and Varshney (1985) reported increase in peroxides activity in plant cells under a variety of stresses, such as mechanical injury and attack by pathogen or an influence of environmental pollution. The increase in peroxides activity varies with the plant species and the concentration of pollutants. Khan and Malhotra (1982) reported that leaves of the resistant plants might have high peroxidase activity.

Data on ambient pollutant concentrations do not allow direct conclusions to be drawn on potential impacts on plants and the environment. Evidence of effects can only be provided by using

plants itself as monitors. These types of plant bioindicators integrate the effects of all environmental factors including interactions with other pollutants or climatic conditions. This permits the risk of complex pollutant mixtures and chronic effects occurring even below threshold values to be assessed. Therefore use of plants, as bioindicators is inexpensive and easy technique. Merely by analyzing the present parameters, an early diagnosis of the extent of pollution can be done in the absence of visible injury.

Acknowledgments

Authors thank Dr. (Mrs.) P. Soni, Head, Ecology and Environment Division for her valuable suggestions and Forest Research Institute, Dehradun for financial support.

References

Agrawal, M., S.K. Singh, J. Singh and D.N.Rao : Biomonitoring of air pollution around urban and industrial sites. *J. Environ. Biol.,* **12**, 211 (1991).

Arnon, D.I.: Copper enzyme in isolated chloroplast. *Plant Physiol.,* **24,** 1-15 (1949).

Bennett, J.H., E.H. Lee and H.E. Heggestad : Biochemical aspects of plant tolerance to ozone and oxyradicals : Superoxide dismutase. *In* : Gaseous air pollutants and plant metabolism (*Eds* : M.J.Koziol and F.R. Whatley). Butterworth, London. pp. 413-424 (1984).

Constantinidou, H.A. and T.T. Kozlowski : Effect of SO_2 and O_3 on *Ulmus Americana* seedling. 1. Visible injury and growth 2. Carbohydrate, proteins and lipids. *Can.J. Bot.,* **57,** 170-184 (1979).

Davison, A.W. and J.D. Barnes : Effects of winter stress on pollutant responses. *In*: How are the effects of air pollutants on agricultural crops influenced by the interaction with other limiting factors ? CEC, Brussels. pp. 16-32 (1986).

Dubois, M., K. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith : A colorimetric method for the determination of sugars. *Nature,* **168,** 167 (1951).

Jacob, M.B. and J.B. Hochheiser: Continuous sampling and ultramicrodetermination of nitrogen dioxide in air. *J. Analy. Chem.,* **30,** 426-428 (1958).

Jaworski, E.G. : Nitrate reductase assay in intact plant tissues. *Biochem. Res. Comm.,* **43**, 1274-1279 (1971).

Khan, A.A. and S.S. Malhotra : Peroxidase activity as an indicator of SO_2 injury in jack pine and white birch. *Biochem. Physiol. Pflanzen*, **177,** 643-650 (1982).

Klumpp, A., W. Ansel and G. Klumpp: European network for the assessment of air quality by the use of bioindicator plants, Report by University of Hohenheim. Ecomed publishers, D-86899, Landsberg, Germany (2003).

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.S. Randall : Protein measurement with Folin reagent. *J. Biol. Chem.,* **193**, 265 (1951).

Malick, C.P. and M.P. Singh : *In*: Plant enzymology and histoenzymology. Kalyani Publishers, New Delhi, **53**, pp. 286 (1980).

Moore, S. and W.H. Stein : Photometeric method for use in the chromatography of amino acids. *J. Biol. Chem.,* **176,** 367-388 (1948).

Pierre, M. and Q. Queiroz : Enzymic and metabolic changes in bean leaves during continuous pollution by necrotic levels of SO_2. *Environ. Pollut.,* **25**, 41-51 (1981).

Prasad, M.S.V. and J.A. Inamdar : Effect of cement kiln dust pollution on black gram (*Vigna mungo*). *Proc. Indian Acad. Sci. (Plant Sci.),* **100(6),** 435-443 (1990).

Rao, D.N. and F. Leblanc : Effects of SO_2 on the lichen algae with special reference to chlorophyll. *Biologist,* **69**, 69-95 (1966).

Rao, M.N. and H.V.N. Rao : Air pollution. Tata McGraw-Hill publishing company limited, New Delhi. pp. 271-272 (1989).

Sadasivam, S and T. Balasubraminan : *In* : Practical manual in biochemistry. Tamil Nadu Agricultural University, Coimbatore. pp. 14 (1987).

Sangeetha, P., U.N. Das, R. Koratkar and P. Suryaprabha : Increase in free radical generation and lipid peroxidation following chemotherapy in patients with cancer. *Free Radic. Biol. Med.,* **8,** 15-19 (1990).

Scandalios, J.G. : Oxygen stress and superoxide dismutase. *Plant Physiol.,* **101**, 7-12 (1993).

Singh,S.K. and D.N. Rao : Evaluation of plant for their tolerance to air pollution. *In*: Proceedings of symposium on air pollution control. Indian Association for Air Pollution Control, New Delhi (1983).

Tanaka, K., N. Kondo and K. Sugahara : Accumulation of hydrogen peroxide in chloroplasts of SO_2 fumigated spinach leaves. *Plant Cell Physiol*., **23,** 999-1007 (1982).

Varshney, S.R.K. and C.K. Varshney : Response of peroxidase to low levels of SO_2. *Environ. Exp. Bot.,* **25,** 107-114 (1985).

West, P.W. and G.C. Gaeke : Fixation of sulphur dioxide as sulfitomercurate (II) and subsequent colorimetric determination. *J. Analy. Chem.,* **28,** 1816-1819 (1956).

Zeevaart, A.J. : Induction of nitrate reductase by NO_2. *Acta. Bot. Neerl.,* **23,** 345-346 (1974).