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Research Article

Analysis of some biochemical parameters of plants as indicator of air pollution

Preeti Pandey Pant[1] and A.K. Tripathi[2]

[1*]Ecology & Environment Division, Forest Research Institute, P.O. New Forest, Dehradun, Uttarakhand (India)-248006.

[2]Scientist-E, Ecology & Environment Division, Forest Research Institute, P.O. New Forest, Dehradun, Uttarakhand (India)-248006

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ABSTRACT

Plants are the only living organisms which have to suffer a lot from automobile exhaust pollution and industrial pollution because they remain static at their habitat. The present experiment was done to determine the impact of air pollution on species like Mangifera indica, Linn., Cassia fistula, Linn., and Terminalia arjuna, which were exposed to different air pollution load for short duration (Active biomonitoring). The biochemical parameters of the plants were studies in both the sites and compared with each other. The 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. The variations suggesting the activation of protective mechanism of these plants under air pollution stress, and also the plant make physiological adjustments to compensate for that environmental stress. Just by analyzing these biochemical indicators air qualities can also be assessed.

Keywords: Bioindicators, Superoxide dismutase, Nitrate reductase, Peroxidase.

INTRODUCTION

Environmental Pollution is one of the most serious problems world is facing today directly or indirectly. Ecological condition and can be define as the fluctuation in any atmospheric constituent from the value that would have existed without human activity[1]. In recent past, air pollutants, responsible for vegetation injury and crop yield loss, are causing increase concern[2]. Over the year there has been a continuous increase in human population, road transportation, vehicular traffic, and industries which has resulted a further increase in the concentration of gaseous and particulates pollutants[3]. The problems arising from the environmental cleaning and restoration has been facing serious challenges from many quarters with special reference to India. Dehradun valley is no exception. The city of Dehradun provides an example typical of any expanding city of the country. The air pollution problems of the city are expanding due to modernization, industrial

urbanization and with the expansion of the area of urban agglomeration. The changed ambient environment in 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 urban air pollution threat and to take effective actions, authorities need to set up an appropriate framework that will enable them to achieve and sustain healthy urban air quality.

The environment of Dehradun valley 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 Uttarakhand. 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.

EXPERIMENTAL DESIGN AND SET UP

***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**.**

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[4] APTI of three forest tree species (*Mangifera indica*, Linn., *Cassia fistula*, Linn., *Terminalia arjuna*) was calculated by using the method given by Singh and Rao[5] (1983). *Mangifera indica*, Linn (APTI 8.10), *Cassia fistula*, Linn. (APTI 7.56) and *Terminalia arjuna* 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, 2009) at selected bioindicator stations.

Site selection/ bioindicator station selection: Two sites have been selected for the development of air quality biomonitoring stations at Shatabdi Van Vigyan Kendra and Selaqui Industrial area. Forest Research Institute (FRI) was treated as control site.

These sites are:

Bioindicator station 1 - Shatabdi Van Vigyan Kendra (city centre) Dehradun

Bioindicator station 2 - Selaqui Industrial area (Dehradun- Chakrata road)

Control site – FRI

Air quality analysis (SO_2, NOx and SPM): During the exposure period, ambient air quality in terms of common air pollutants i.e. SO_2, NOx and SPM was analysed at all the bioindicator stations **(Table1).**

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 m3/min. SPM was computed as per standard method. Filter paper was weighed before and after sampling. West and Gaeke [6] method and modified Jacob and Hochheiser method [7] were used for analysis of SO_2 and NOx respectively.

Air pollution index (API): The averages 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 [8]

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$$API = 1/3 \left[\frac{(SPM)}{(S_{SPM})} + \frac{(SO_2)}{(S_{SO2})} + \frac{(NO_x)}{(S_{NOx})} \right] X\ 100$$

Where, SSPM, SSO_2 and SNOx represent the ambient air quality standards for SPM, SO_2 and NOx.

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 NOx and correlated with the variation in biochemical indicators. On the basis of air pollution index, bioindicator station 1 was categorized as heavy air pollution site (air pollution index 95.33), station 2 as severe air pollution site (air pollution index 123.88) **(Table-1 and Table-2).**

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

Bioindicator stations	Pollutants (µg/m3)			Air pollution index
	SPM	SO_2	NOx	
Station No. 1	310	17.58	21.74	95.33 (heavy air pollution)
Station No. 2	378	28.83	32.18	123.88 (severe air pollution)

(Ambient air quality standards taken for calculation of air pollution index 140 µg/m3 for SPM, 60 µg/m3 for SO2 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	Severe air pollution

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 by following method of Arnon [9] , ascorbic acid by Sadasivam and Balasubraminan [10] , protein by Lowry *et al*[11]., total soluble sugars by phenol sulphuric acid method of Dubois *et al* [12]., free amino acid by Moore and Stein [13], superoxide dismutase by Sangeetha *et al* [14], peroxidise by Malick and Singh [15] and nitrate reductase by Jaworski's [16] 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).**

Table-3: Biochemical Indicators of different species at different bio indicator stations

Species	Station/ site	Chlorophyll (mg/g)	Protein (mg/g)	Total Soluble Sugar (mg/g)	Free amino acid (mg/g)	Ascorbic Acid (mg/g)	NR (µmole NO2 formed g-1 FW hr-1)	SOD (unit/g)	Px (changes in OD/30 sec/g)
C. fistula	control	0.49	20.90	28.20	1.01	8.88	2.21	69.82	0.21
	Station1	0.21	18.92	16.02	1.69	13.32	4.19	78.15	0.42
	Station2	0.44	26.59	20.22	1.92	13.42	4.62	91.45	0.74
T. arjuna	control	1.22	18.90	29.17	0.56	4.99	6.13	68.72	0.35
	Station1	1.58	16.68	26.01	1.93	11.10	7.79	80.45	0.48
	Station2	1.38	20.12	31.91	1.22	6.52	9.92	98.12	0.54
M. indica	control	1.29	44.50	26.46	0.72	17.76	4.26	68.81	0.81
	Station1	1.22	22.50	21.16	0.93	19.42	5.79	87.92	1.21
	Station2	1.11	32.33	28.82	0.99	22.88	8.12	72.33	0.81

Cassia fistula, Linn: Biochemical indicators of Cassia fistula, Linn., at all the bioindicator stations varied significantly ($p<0.001$) **(Table 3 and Fig. 1).** Maximum reduction (57.14%) in chlorophyll content was observed at station 1 while at station 2, loss of 10.2% was observed. At stations 1, protein content showed significant reduction of 9.47% while higher protein content of 27.22% was observed at station 2. Soluble sugar showed maximum loss of (43.19%) at station 1 and 28.29% at station 2. *Cassia fistula*, Linn showed increasing trend of free amino acids at all the stations as compared to control values.

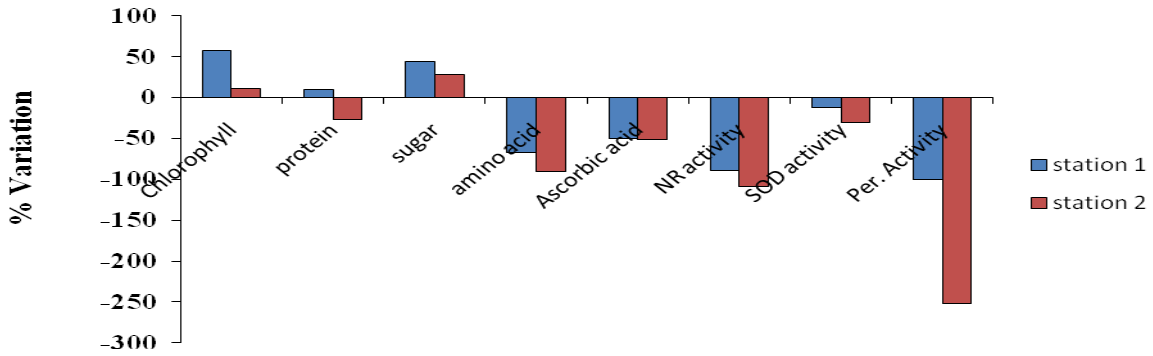

Fig. 1: Variation in biochemical indicators of *C. fistula* at different bioindicator stations

Station 1 exhibited maximum enhancement (67.32%) in free amino acids followed by station 2 (90.09%). Ascorbic acid was also found to be increasing at two of the stations as compared to control. Maximum increase of 50% was evident at station 1 and 51.12% at station 2. Stimulation in nitrate reductase activity was observed at all the stations as compared to control. Maximum stimulation (109.04%) was observed at station 2 and 89.59% at station 1 Stimulating trend was also observed in case of superoxide dismutase activity at both the stations. At station 1, 11.93% stimulation was observed followed by 30.97% at station 2. Like other enzymatic activities, peroxidase activity was also found be more at all the stations, as compared to control. Maximum stimulation of 252.38% was exhibited by the species exposed at station 2 and 100% at station 1 **(Table 3 and Fig. 1).**

Terminalia Arjuna : Chlorophyll content at station 1 showed 29.5% enhancement, followed by 13.11% at station 2, as compared to control ($p<0.001$). Increase in protein content was exhibited at stations 2 (6.45%) and reduction of 11.74% was observed at station 1. Soluble sugar was found to be reduced at station 1(10.83% and increased at station 2 9.39%. Free amino acids were found to be more at all the stations as compared to control. Maximum enhancement (244.64%) in free amino acid was exhibited at station 1 followed by 117.85% at station 2. Ascorbic acid content was significantly increased at the stations maximum gain (122.44%) was observed at station 1 and station 2 (30.66%). Enzymatic activities like nitrate reductase, super oxide dismutase and peroxidase were found to be higher than respective control values. Maximum increase (61.82%) in nitrate reductase activity was evident at station 2 followed by station 1 (27.07%). Super oxide dismutase activity increased 17.06% at station 1 and 42.78% at station 2 as compared to control. Peroxidase activity exhibited maximum stimulation (54.28%) at station 1 and 37.14% at station 2 **(Table 3 and Fig. 2).**

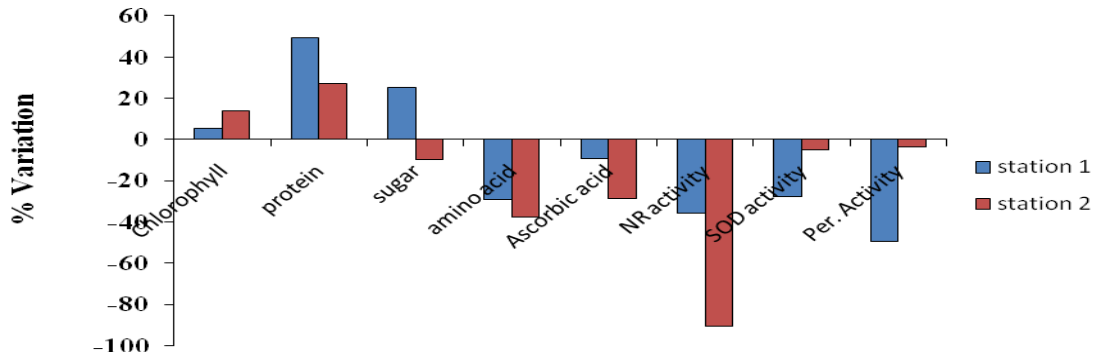

Fig. 2: Variation in biochemical indicators of *T. arjuna* at different bioindicator stations

Mangifera indica Linn. : At station 1, Mangifera indica, Linn., exhibited 5.42% reduction in chlorophyll content and 13.95% was observed at stations 2. Significant reduction (49.43%) in protein content was observed at station 1, followed by a loss of 27.34% at station 2. The stations 2 showed significant reduction of 8.92% in soluble sugar while reduction of 25.04% was exhibited at station 1. Free amino acid exhibited an increasing trend at all the stations. Maximum gain of 37.5% was evident at station 2 followed by 29.2% at stations 1. Ascorbic acid also showed increase over control at all the stations. Maximum enhancement of 28.82% was exhibited at station 2 and 9.34% at station 1. Nitrate reductase activity was also found to be increasing at both the stations. Maximum increase (90.61%) was observed at station 2 and station1 (35.91%). Superoxide dismutase activity varied positively at all the stations. Maximum stimulation of 27.77% was revealed at station 1 and 5.11% at station 2. Positive trend was also observed in case of peroxidase activity at all the stations. Station 1 exhibited maximum increase of 49.3% and 3.7% at station 2 **(Table 3 and Fig. 3).**

Adverse effects of air pollution on biota and ecosystem have been demonstrated worldwide. Much experimental work has been conducted on the analysis of air pollution effects on crop and vegetation at various levels ranging from biochemical to ecosystem level levels[17]. Plants that are constantly exposed to environmental pollutants absorb, accumulate and integrate these pollutants into their systems. It reported that depending on their sensitivity level, plant shows visible changes which would include alteration in the biochemical process and accumulation of certain metabolites[18].

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 NOx. Air pollutants make their entrance into the tissues through the stomata and cause partial denaturation of the chloroplast and decreases pigment contents in the cells of polluted leaves. [19] Pandey *et.al.*mentioned that high concentration of metal reduced the chlorophyll content in tree species 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. maximum depletion in chlorophyll content at station 1 may be due to the maximum pollution load at this site whereas station 2 showed less depletion due to lower pollution load.

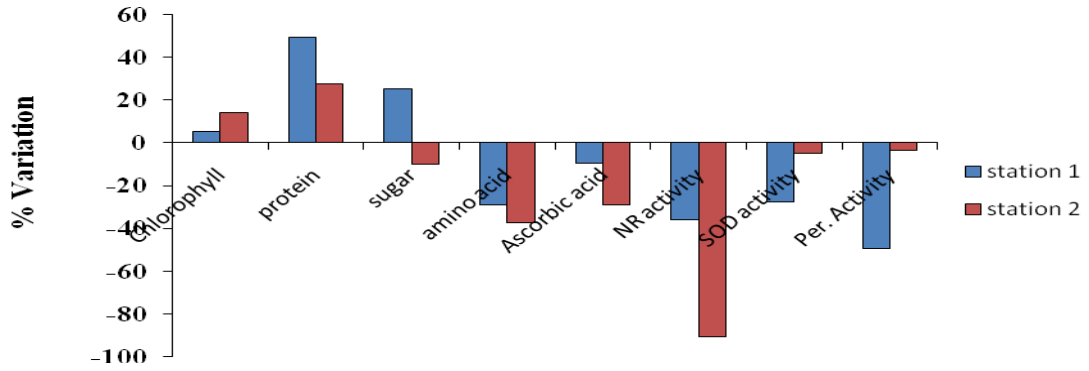

Fig. 3: Variation in biochemical indicators of *M. indica* at different bioindicator stations

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 [1,20,,21,] also supported the similar finding on *M. indica*.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 [22]. Our study revealed significant loss ($p<0.001$) of soluble sugar in all the species at all the stations accept station 2 for *T. Arjuna*. All the species showed maximum loss at heavy air pollution site i.e. at station 1 and severe air pollution site (station 2). 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, which is also supported by the finding of [23] Thanbavani,and Prathipa[23] .

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. The ascorbic acid is natural detoxicant, which may prevent the damaging effects of air pollutant in plant tissues[24] . Present investigation revealed an increase 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 photo oxidation 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. [25] based on dosage and physiological status of plant. Increased level of ascorbic acid may be due to the defence 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 NO_2 NH_4 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.

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 peroxidise 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 [26]. SOD increases and can protect cells against free radicals produced by air pollutants by catalyzing following reaction to form H_2O_2 $2O_2$ - + 2H+ H_2O_2 + O_2 (Tripathi and Gautam)[1]. 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 higher 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 [1](Tripathi and Gautam).

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 [27]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. [28] Kuddus *et al.,* reported that leaves of the resistant plants might have high peroxidise 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.

CONCLUSION

Trees are planted around industries and along roadside to absorb pollutants in air including particulate matter so as to reduce air pollution. Although tree poses some stress tolerant mechanism within them, considerable amount of damage of is caused to them which are evident from this study. The study reveals that biochemical indicators of air pollution which responds to air pollution in predictable way can be used for the bio-monitoring of the air pollution level of that particular region. In the present study we are using biochemical indicators of *M. indica* and *C. fistula* for bio-monitoring the air quality of the surroundings of two bio-monitoring Stations (Shatabdi Van Vigyan Kendra and Selaqui industrial area). It is speculated that *M. indica* and *C. fistula* were used for monitoring the air quality. This type of bio-monitoring is useful as it provides assessment of the impact of air pollution on plant's biochemical indicators and also for monitoring of air quality simultaneously.

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***Correspondence Author: Preeti Pandey Pant;** Ecology & Environment Division, Forest Research Institute, P.O. New Forest, Dehradun , Uttarakhand (India)-248006.