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Impact Assessment of Air Pollution Stress on Plant Species through Biochemical Estimations

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Abstract**—**The present study was conducted to investigate the response of plants exposed to lignite-based thermal power plant emission. For this purpose, five plant species were collected from 1.0 km distance (polluted site) and control plants were collected from 20.0 km distance (control site) to thermal power plant. The common tree species *Cassia siamea* Lamk., *Polyalthia longifolia*. Sonn, *Acacia longifolia* (Andrews) Wild., *Azadirachta indica* A.Juss, *Ficus religiosa* L. were selected as test plants. Photosynthetic pigments changes (chlorophyll a, chlorophyll b and carotenoids) and rubisco enzyme modifications were studied. Reduction was observed in the photosynthetic pigments of plants growing in polluted site and also large sub unit of the rubisco enzyme was degraded in *Azadirachta indica A. Juss* collected from polluted site.

Abstract**—**Air pollution, Lignite-based thermal power plant, Photosynthetic pigments, Rubisco enzyme.

I. INTRODUCTION

INDUSTRIALIZATION, urbanization, economic growth and associated increase in energy demands have resulted in a profound deterioration of air quality in developing countries like India. Electricity demand in India has been increasing at an average rate of 8.8% per year in the past 35 years. Neyveli Thermal Power Stations are South Asia's first and only lignite-fired Thermal Power Stations and also the first pit-head power stations in India. It mines twenty-four million metric tonnes per annum (MTPA) of lignite, and produces 2,490 megawatts per annum (MW/year) of electricity from three open cast mines. A large percentage of the thermal electricity generated in Tamil Nadu comes from the power plants in Neyveli. Recent reports have shown, that air and soil quality around Indian thermal power plants are deteriorating at measurable rate [1,2,3]. Sulphur dioxide and fly-ash constitute major proportions of the gaseous and particulate emissions from thermal power plants. These pollutants when absorbed by the leaves cause a reduction in the concentration of photosynthetic pigments viz., chlorophyll and carotenoids, which directly affect the plant productivity. Chlorophyll is the principal photoreceptor in photosynthesis, the light-driven process in which carbon dioxide is "fixed" to yield carbohydrates and oxygen.

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Carotenoids are a class of natural fat-soluble pigments found principally in plants, algae and photosynthetic bacteria, where they play a critical role in the photosynthetic process [4,5] and also protect chlorophyll from photoxidative destruction [6]. When plants are exposed to the environmental pollution above the normal physiologically acceptable range, photosynthesis gets inactivated [7]. Ribulose-1,5-bisphosphate carboxylase / oxygenase (Rubisco; EC 4.1.1.39) is a bifunctional enzyme which catalyzes two competing reactions, photosynthetic CO_2 assimilation and photorespiratory carbon oxidation in the stroma of the chloroplasts, and is the most abundant protein in leaves. Environmental stress factors can cause reversible and irreversible inactivation of Rubisco [8,9]. The plants can be used as both passive biomonitors and biomitigators in the urban-industrial environment to indicate the environmental quality and to ameliorate the pollution level in a locality [10,11]. Present study examines the impact of lignite-based thermal power plant emission on photosynthetic pigments and enzyme of five tree species growing in the vicinity of thermal power plant. The chosen parameters were chlorophyll *a*, chlorophyll *b*, carotenoids and Ribulose bisphosphate carboxylase oxygenase (Rubisco) enzyme.

II.MATERIALS AND METHODS

A.Study Area and Sample Collection

Neyveli, situated in Cuddalore district of Tamil Nadu, at 11˚ 34'N, 79º 28'E, is about 190 km Southwest of Chennai. The Neyveli Lignite Corporation, a public-sector enterprise established in 1956. Presently, this power station has 6 units of 50 MW each and 3 units of 100 MW each. Thermal power station-II (TPS-II) has been a major source of power to all southern states of India. The 1470 MW capacity power station consists of 7 units of 210 MW each. Leaf samples were collected from five different plant species (*Cassia siamea* Lamk., *Polyalthia longifolia*. Sonn, *Acacia longifolia* (Andrews) Wild., *Azadirachta indica* A.Juss, *Ficus religiosa* L.) in triplicate from the surroundings of fly ash pond. Mean while the control plant leaves were collected from Vridhachalam, it is 20 km distance from Neyveli. The plant leaves were collected in a sterile polythene bags, kept in liquid nitrogen and then transported to the laboratory immediately. Then the samples were maintained at -20^0C until further analysis.

B. Estimation of Chlorophyll and Carotenoids

Chlorophyll and carotenoids were estimated by the method of Arnon (1949) [12].

C. Protein Isolation and SDS-PAGE

Protein was isolated from plant leaf as described by Andreas Bahl & Gunter Kahl (1994) [13]. SDS-polyacrylamide gel electrophoresis was performed as described by Laemmli (1970) [14].

III. RESULTS

TABLE I
CONCENTRATION OF DIFFERENT PHOTOSYNTHETIC PIGMENTS (mg g-1) IN THE LEAVES OF SELECTED TREE SPECEIS COLLECTED FROM POLLUTED AND CONTROL SITES

Tree species	Parameters											
	Chlorophyll 'a'			Chlorophyll 'b'			Total Chlorophyll			Carotenoids mg g-1		
	P	C	%R	P	C	%R	P	C	%R	P	C	%R
***Cassia siamea* Lamk.**	1.29	2.13	39.44	0.97	1.15	15.65	2.26	3.28	31.10	0.56	0.84	33.33
***Polyalthia longifolia.* Sonn**	1.57	1.61	2.48	0.81	1.07	24.30	2.38	2.68	11.19	0.77	0.88	12.5
***Acacia longifolia*(Andrews) Wild**	0.63	0.92	31.52	0.31	0.43	27.91	0.94	1.35	30.37	0.25	0.36	30.56
***Azadirachta indica* A.Juss**	0.82	1.03	20.39	0.48	0.60	20	1.29	1.63	20.86	0.38	0.53	28.30
***Ficus religiosa* L.**	0.78	1.46	46.58	0.41	0.61	32.79	1.19	2.07	42.51	0.23	0.53	56.60

(P= Polluted, C= Control and %R= Percentage of reduction)

A. Photosynthetic Pigments Changes

Cassia siamea Lamk.: The concentration of chlorophyll 'a' content in the leaves of *Cassia siamea* Lamk. at polluted site was recorded as 2.13mg g^{-1}, which was 1.29mg g^{-1} at control site. Thus a reduction of 39.44% in chlorophyll 'a' content was recorded in the samples from polluted site in comparison to control site. The concentration of chlorophyll 'b' content was 1.15mg g^{-1} in the leaf samples collected from polluted site while it was 0.97mg g^{-1} in the samples from control site. The polluted site samples thus had 15.65% less chlorophyll 'b' content. Total chlorophyll content 2.26mg g^{-1} and 3.28mg g^{-1} in the leaf samples collected from polluted and control site respectively. Thus there was a reduction of 31.10% in the concentration of total chlorophyll content in the samples from polluted site. The concentration of carotenoid pigment in the leaf samples from polluted and control site were recorded as 0.56mg g^{-1} and 0.84mg g^{-1}, respectively with a reduction of 33.33% in the leaf samples from polluted site (Table 1).

Polyalthia longifolia. Sonn: This plant showed 2.48% reduction in chlorophyll 'a' content, 24.30% reduction in chlorophyll 'b' content, 11.19% reduction in total chlorophyll content and 12.5% reduction in carotenoid content in the leaves sampled from polluted site in comparision to leaves sampled from control site.

Acacia longifolia (Andrews) Wild: In case of *Acacia longifolia* (Andrews) Wild. the reduction recorded was 31.52% in chlorophyll 'a' content, 27.91% in chlorophyll 'b' content, 30.37% in total chlorophyll and 30.56% in the carotenoid content in the leaf samples collected from polluted site.

Azadirachta indica A.Juss: The reduction recorded in the leaves of *Azadirachta indica* A.Juss sampled from polluted site was 20.39, 20, 20.86 and 28.30% for chlorophyll 'a', 'b', total chlorophyll and carotenoids, respectively.

Ficus religiosa L.: In *Ficus religiosa* L., chlorophyll 'a' was 46.58%, chlorophyll 'b' was 32.79%, total chlorophyll was 42.51% and carotenoid content was 56.60% less in the leaf samples collected from polluted site.

B. Rubisco Enzyme Modifications

M-Marker; C1-Control Site plant 1; C2 -Control Site Plant 2; P-Polluted site

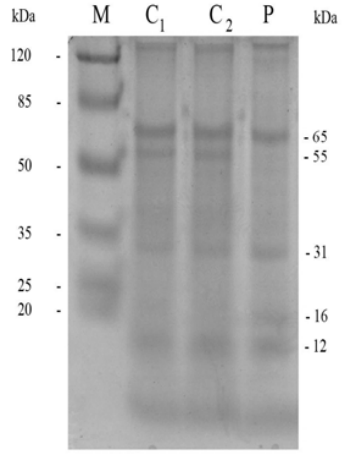

Fig. 1 SDS-PAGE of total protein from *Azadirachta indica A.Juss* from control and polluted environment.

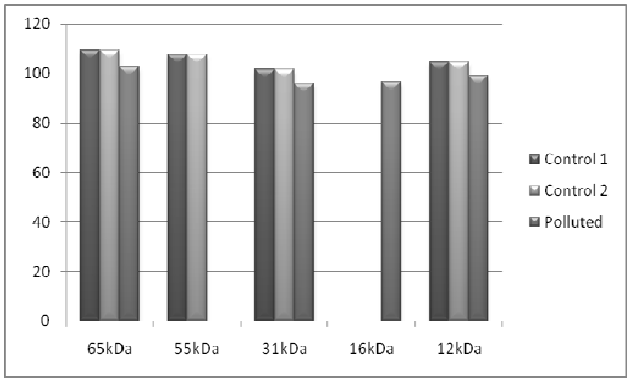

Fig. 2 Densitometry analysis of total protein from *Azadirachta indica A.Juss* from control and polluted environment.

Azadirachta indica A.Juss. was further taken for the study of rubisco enzyme changes by comparing the plant from polluted and control site. SDS-PAGE was performed and the results were indicated that there is considerable change in the rubisco enzyme isolated from the leaves of polluted plant. The 65 kDa protein is supposed to be Rubisco binding protein (RBP). It was reduced 6% in the plant leaves collected from polluted environment compared to control plant leaves. Major change was observed in the rubisco large subunit (LSU), a 55 kDa protein, which was completely lost in the leaf of polluted plant. In the unknown 31 kDa protein a 6% reduction was observed in the polluted plant leaves. A unknown 16 kDa protein was expressed only in the plant collected from polluted environment and not in the control plants. In the case of rubisco small subunit (SSU), a 12 kDa protein, 5% reduction was observed in the leaves collected from polluted environment compared to the control environment.

III. DISCUSSION

The photosynthetic pigments are the most likely to be damaged by air pollution. Chlorophyll pigments exist in highly organized state, and under stress they may undergo several photochemical reactions such as oxidation, reduction, pheophytinisation and reversible bleaching [15]. Hence any alteration in chlorophyll concentration may change the morphological, physiological and biochemical behaviour of the plant. Air pollution-induced degradation in photosynthetic pigments was also observed by a number of workers [16,17,18]. In both the plants chlorophyll a and chlorophyll b content were reduced significantly at polluted site.

Azadirachta indica A.Juss was selected for further study of rubisco enzyme changes. Protein was isolated from the leaf tissue and SDS-PAGE was performed for the plant collected from polluted and control site. From the results, major change was the loss of large subunit of rubisco enzme, a 55 kDa protein, in the plant collected from polluted site compared to the control plant. And also degradation was observed in some unknown protein of 65 kDa, which is supposed to be rubisco binding protein. A 16 kDa protein was expressed in the plant from polluted site. This protein may be the degraded fragment of rubisco large subunit. Similarly, the degradation of rubisco large subunit (LSU) was studied by Hiroyuki Ishida et al., (1997) [19] in which they demonstrated that the large subunit was fragmented into 37 kDa containing the N-terminal end of the LSU and 16 kDa containing the C-terminal end of the LSU, by active oxygen species. Such altered proteins are more susceptible to degradation by intracellular proteases [20].

IV. CONCLUSION

In all the plants collected from polluted environment the photosynthetic pigments were reduced and the percentage of reduction was studied. Then *Azadirachta indica A. Juss* was further taken for the study of rubiso enzyme modifications because of their medicinal importance and aesthetic value. Results of this study shows that there is negative impact on plants by thermal power plant pollution and plant species differ in their response to air pollution. Until now there is no comparable data on impact of pollution on rubisco enzyme modifications in any other plant species, collected from polluted environment at field level.

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