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Biochemical changes in *Azadirachta indica* and *Tinospora cordifolia* by fly ash produced from coal based thermal power plant in Kota, India

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Abstract
Fly ash is made up of tiny, glass-like particles, and when it deposits on plant leaves, it prevents proper transpiration and photosynthesis. Fly-ash is highly basic, rich in many important and non-essential elements, but lack in both nitrogen and phosphorus, and it influences the physio-chemical properties of soil. Quality of air of Kota city is rich in fly ash content due to presence of coal based Thermal power plant in city and therefore it affects the primary and secondary metabolites of plants. In present study changes in biochemical traits of two medicinally important plants namely *Azadirachta indica* and *Tinospora cordifolia* were studied at and around Kota thermal power plant. Two experimental sites were selected at different distances from the power plant. Plant responses such as leaf pigments (Chlorophyll a, chlorophyll b) and total chlorophyll contents, protein and reducing sugars (Carbohydrates) were measured. Substantial decline in leaf pigments, total chlorophyll, protein and carbohydrate content was observed showing fly ash stress on plants. The study concludes that these plant species of the particular area are sensitive to damaging effect of various types of pollutions caused by flyash.

Keywords: *Tinospora cordifolia*, *Azadirachta indica*, carbohydrates

Introduction
From last few decades there is amplification in energy requirements in form of electricity due to increasing population and industrial expansions. Coal based Kota thermal power plants (KTPP) fulfills the energy demand of city but generates large number of particulates, gaseous pollutants and fly ash (Khandelwal,2015; Sharma and Tripathi, 2009) [6, 14]. Fly ash (FA) is a by-product from thermal power station causing pollution in local environment. The FA dust emitted by thermal power plants is predominantly alkaline in nature and generally have inhibitory effects on plant physiology forming water films. These surface films can have harmful effects, possibly by blocking stomata thereby reducing gaseous exchange and photosynthesis due to shading effect (Qadir *et al*; 2016; Ots *et al*., 2011) [12, 9]. Pollutants produced during coal combustion damage plant leaves, impair plant growth and limit primary productivity (Iqbal *et al*., 2010; Prusty *et al*., 2005) [5, 10]. These pollutants not only cause acute or chronic injury but also predispose plants to other biotic and physiological disorders.
Several studies have been conducted by authors to evaluate the effect of pollution on plant growth and survival, foliar morphology and biochemical changes and physiology at contaminated sites due to particulate dust pollution. The major infirmities caused by pollutants include chlorosis, necrosis and epinasty (Chaturvedi *et al*., 2013; Iqbal *et al*., 2010) [3, 5]. The influence or toxicity of pollutants is determined by their chemical composition, particulate size, the rate of deposition (Van Jaarsveld, 2008) [17] and exposure to different environmental factors (Weinstein and Davison, 2004; Chaturvedi *et al*., 2013) [18, 3]. These pollutants when combined together produce additive or synergistic effect on plant growth and physiology. Urban forests (trees, grasses and shrubs) are important because they provide a number of services and products to human societies. Some other services include improved human health, climate modification, recreational benefits and aesthetics. Nationally these urban forests offer the ability to remove significant amounts of air pollutants and consequently improve environmental quality (Livesley *et al*., 2016) [7]. However, these ecological services to humans provided by plants are not rendered without any cost, but they have to suffer the impairment caused by air pollution, as plants are exposed to pollutants throughout the year.

The air pollutants directly emitted through chimneys of thermal power station due to the combustion of coal is also spread in the local environment. Emission from thermal power station adversely affects the plant species and local vegetation directly or indirectly. As the cumulative effect of all these factors the biodiversity of that particular area is comparatively less and confined to limited species.
Therefore, present investigation is an attempt to find out effect of fly ash on biochemical parameters of two important medicinal plants namely *Azadirachta indica* (Family: Meliaceae)) and *Tinospora cordifolia* (Family: Menispermaceae) of Kota district from two pollution sites which are Hanging Bridge (Site I), area around Nanta (SiteII) and University campus was selected as control site. Analyses of the above-mentioned changes serve as a foundation for finding suitable parameters for objective assessment of FA stress on medicinal plant species.

Materials and Methods

Sampling sites

Two sites (I, II) selected for sampling situated in close proximity to dumping sites. University area was selected a control site situated about 12 km from the power plant. Tree species included for study are *A. indica* (Neem) and *T. cordifolia* (Giloy).

Methodology for Biochemical Procedures:

Leaves of both plants viz. were homogenized in 0.1 M phosphate buffer and centrifuged. Supernatant was used as plant extract and analyzed for different metabolites as per standard method described in following paragraphs. Absorptions of reaction mixtures were read with Spectrophotometer (visible, CSIM) at appropriate wavelength and quantitative estimations were made. Metabolites were expressed quantitatively in terms of mg per g fresh weight of plant tissues (mg/g fr. wt.). Blanks were prepared for each analysis by addition of plant extract to the reaction mixture.
The estimations were done as per the procedures detailed as under:

Carbohydrates

One gm of plant tissue was homogenized with the help of pestle and mortar in 10 ml of phosphate buffer (pH 6.0, 0.2M) and centrifuged at 3000 RPM for 15 minutes. Suitably diluted 1.0 ml of extract was mixed with 4 ml of 0.2% anthrone reagent (in conc, H2SO4) and placed on water bath for 5 min (Tandon, 1976) [15]. Per cent transmittance was read spectrophotometrically at 620 nm.

Proteins

Total proteins were extracted by homogenizing 1 g of plant tissue in 10.0 ml of phosphate buffer (pH 6.0, 0.2M) and centrifuged at 3000 RPM for 20 minutes. Protein content was estimated by suitably diluting the crude centrifuged extract to 1 ml and adding 5 ml of Coomassie Brilliant Blue G-250 (Bradford, 1976) [2]. The per cent transmittance of resultant blue solution was measures at 595 nm and protein contents expressed as mg/g fresh weight of tissues.

Chlorophyll Estimation

Fresh leaves (1.0g) were cut into small pieces and homogenized with the help of pestle and mortar in excess of 80 per cent acetone (Arnon, 1949) [1]. The homogenized extract was filtered through Whatman No. 42 filter paper. Extraction was again repeated by adding sufficient quantity of 80 per cent acetone. Once again the contents were filtered and brei was washed with acetone until colorless. The filtrate were pooled and made up to 100 ml by 80 per cent acetone in a volumetric flask. The absorbance was measured at 645 and 663 nm for the determination od chlorophyll a and b and total chlorophyll. The chlorophyll contents were calculated in fresh weight basis by the following formulae:

Total chlorophyll (mg/g tissue) = 20.2 (A_{645}) + 8.02 (A_{663}) X V/1000 X W
Chl a (mg/g tissue) = 12.7(A_{663}) – 2.69 (A_{645}) X V/1000 XW
Chl b (mg/g tissue) = 22.9 (A_{645}) – 4.68 (A_{663}) X V/1000 X W

$$\text{Total chlorophyll mg/g tissue} = \frac{20.2\ (\text{O.D } 645\lambda.) + 8.02\ (\text{O.D } 663\lambda.)}{a \times 1000 \times W}$$

Where
A = absorbance at specific wavelength
V = Final volume of chlorophyll extract in 80% acetone and
W = fresh weight of tissue extracted

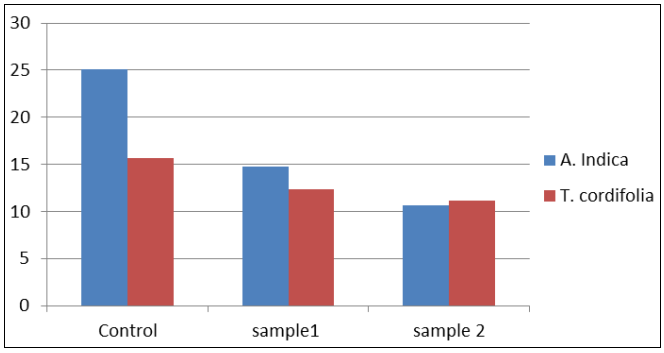
Results and Discussion

Carbohydrate contents

In present study reduction in carbohydrate content was observed. In case of *A. indica* it was found maximum in control (25.06 mg/g of leaf) and minimum at site II (10.66). Similarly, *T. cordifolia* also showed reducing pattern in which very low value of 11.12 mg/g of plant tissue was recorded at Site II (Table 1; Graph 1). The results are similar to various studies reported by Qadir *et al*., 2016 [12], Sharma *et al*., 2009 [14]. Carbohydrate in plant samples specifies that pollutants are accountable for condensed plant output (Qadir *et al*., 2016) [12].

Table 1: Estimation of Carbohydrate (Reduced)

Plant type	Control mg/g	Site I mg/g	Site II mg/g
A. indica	25.06	14.73	10.66
T. cordifolia	15.66	12.38	11.12



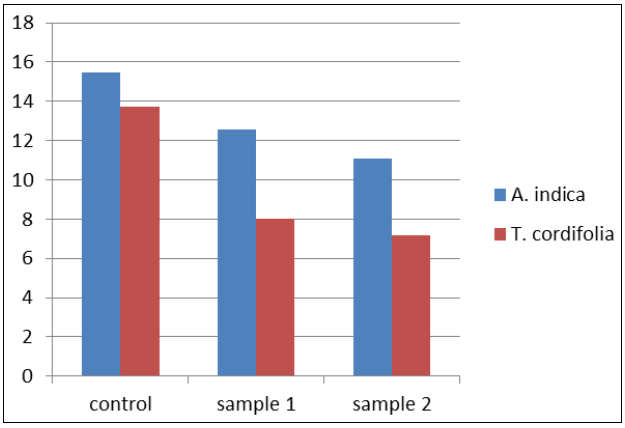
Graph 1: Estimation of carbohydrates (reduced) in *A. indica* and *T. cordifolia*

Protein contents

In our study protein content showed significant negative correlation with FA stess. Protein content showed reduced trend between polluted site and control site. Protein content in case of *A. indica* was found to be maximum at control (15.45) and minimum (11.1) at sampling site II and varied from 13.72 to 7.16 in case of *T. cordifolia* (Table 2 and Graph.2). Reduction in protein in plants occurs due to stress or reduced rate of photosynthesis. Similar results have been observed in leaves of *D. sisso* and *P. longifolia* (Qadir *et al*., 2014) [11].
Soluble protein content diminished due to fly ash stress is also reported by Qadir *et al*., 2016 [12]. Decrease in protein content could be due to break down of present proteins (Saha and Padhy, 2011) [13]

Table 2: Estimation of Protein

Plant type	Control mg/g	Site I mg/g	Site II mg/g
A. indica	15.45	12.56	11.10
T, cordifolia	13.72	8.02	7.16



Graph 2: Estimation of Proteins (reduced) in *A. indica* and *T. cordifolia*

Chlorophyll Contents

The present study showed changes in pigment level (Ch a,Ch b) and total chlorophyll content in the plant species exposed to FA dust. Shift specific variation in cast of biochemical parameters of both plants at different sites is present in graph. 3&4 and Table 3 & 4.

As per the findings of present investigation, the negative response was observed between FA stress and pigment levels. The results showed that Ch b in *A. indica* is present in higher concentration in polluted site. In case of *T. cordifolia* both Ch a and Ch b showed considerable reduction at both sites as compared to control. Total chlorophyll content was higher at control site as compared to the pollution sites. Therefore, in present study total chlorophyll ranged from 2.7 at control and 1.41to 1.46 at polluted site I and II in case of *A. indica*. And same pattern was observed in *T. cordifolia* with 2.16 value at control and sampling sites varied to 1.75 to 0.92.

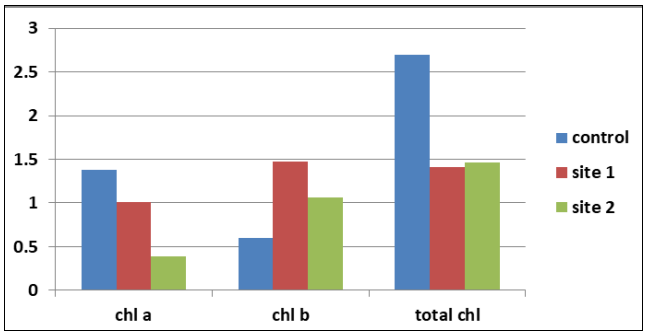
The decline in total chlorophyll in pollution exposed leaves compared to that of control site may be due to alkaline environments developed by solubilization of chemicals present in dust particulate in cell sap which is accountable for degradation. Moreover, the evidence of reduced chlorophyll synthesis and degradation is due to deposition of FA and oxidation of chlorophyll to free radicals. A close relation between pollution density and photosynthetic activity, total chlorophyll content and untimely leaf senescence has been reported by Honour *et al*., 2009 [4].

Table 3: The Spectrophotometric determination of Chlorophyll content (mg/g) of *A. indica*

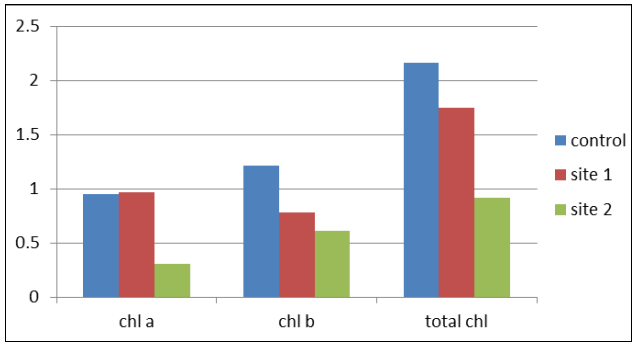
Sampling Site	Chl-a (mg/g)	Chl-b (mg/g)	Total chlorophyll (mg/g)
Control	1.38	0.595	2.7
Site I	1.01	1.471	1.412
SiteII	0.391	1.06	1.46

Table 4: Spectrophotometric determination of Chlorophyll content (mg/g) of *T. cordifolia*

Sampling Site	Chl-a (mg/g)	Chl-b (mg/g)	Total chlorophyll (mg/g)
Control	0.952	1.213	2.164
Site I	0.967	0.784	1.75
Site	0.306	0.614	0.92



Graph 3: Total chlorophyll, Chl a and Chl b of *A. indica*



Graph 4: Total chlorophyll, chl a and chl b of *T. cordifolia*

Conclusion

The present study concludes that leaves of both *A. indica* and *T. cordifolia* can efficiently accumulate dust because of substantial leaf area (Lu *et al*., 2008). The effect of dust is shown in the physiological and biochemical functions of trees, as are result, of which variations occur in plant morphology expressed as high defoliation levels (abscission), falling vigour of plants and reduced carbohydrate, protein and chlorophyll contents.

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