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Morphological and biochemical changes in *Azadirachta indica* from coal combustion fly ash dumping site from a thermal power plant in Delhi, India

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ABSTRACT

The foliar and biochemical traits of *Azadirachta indica* A. Juss from fly ash (FA) dumping site in Badarpur thermal power plant (BTPP) New Delhi, India was studied. Three different experimental sites were selected at different distances from the thermal power plant. Ambient suspended particulate matter (SPM) and plant responses such as leaf pigments (chlorophyll a, chlorophyll b, and carotenoids), total chlorophyll, net photosynthetic rate, stomatal index (SI), stomatal conductance (SC), intercellular carbon dioxide concentration $[CO_2]_i$, net photosynthetic rate (NPR), nitrogen, nitrate, nitrate reductase activity, proline, protein, reducing sugar and sulphur content were measured. Considerable reduction in pigments (chlorophyll a, chlorophyll b and carotenoids), and total chlorophyll was observed at fly ash dumping site. Fly ash stress revealed the inhibitory effect on Nitrate reductase activity (NRA), Nitrate, soluble protein, and reducing sugar content, whereas stimulatory effect was found for the stomatal index, nitrogen, proline, antioxidants and sulphur content in the leaves. Under fly ash stress, stomatal conductance was low, leading to declining in photosynthetic rate and increase in the internal CO_2 concentration of leaf. Single leaf area (SLA), leaf length and leaf width also showed a declining trend from control to the polluted site. Antioxidant enzymes increased in leaves reflecting stress and extenuation of reactive oxygen species (ROS).

1. Introduction

In order to meet the domestic, agricultural and industrial energy requirements of a very large population of 1.27 billion in India, during the past few decades, new thermal power plants (TPPs) have been commissioned and the capacities of the existing ones are being increased to cater to the increasing energy requirements. Documented reports have shown, that plant, soil and air quality around thermal power plants are deteriorating at a measurable rate. Coal combustion in thermal power plants adds a composite mixture of pollutants into the ambient air (Sharma and Tripathi, 2009). The chief pollutants released by these power plants includes Fly ash (FA), Sulphur dioxide, carbon and nitrogen oxides, noncombustible hydrocarbons and toxic heavy metals (Pandey, 2005). The major constituents of gaseous and particulate emissions from these power plants include sulphur dioxide and FA. The FA dust emitted by thermal power plants is predominantly alkaline in nature and its influence on plant physiology may be favourable as well as unfavourable because alkaline dust commonly forms a surface crust on plant materials with the formation of water films. These surface films can have harmful effects, possibly by blocking stomata thereby reducing gaseous exchange and photosynthesis due to shading effect (Ots et al., 2011; Grantz et al., 2003). Plants are continuously affected by physiochemical effects of FA, coupled with antagonistic environmental conditions primarily caused by a dark grey coloured FA and its sandy texture. The major factors that limit plant growth on FA dumping sites are lack of indispensable plant nutrients, mostly Nitrogen (<0.05), available phosphorus (0.05–0.2%), high boron content and concentration of some potentially toxic elements including Pb, Cd, Hg, Mo, Se and Cr in ash (Pavlovic et al., 2007; Pandey et al., 2010, 2016). Pollutants produced during coal combustion damage plant leaves, impair plant growth and limit primary productivity (Iqbal et al., 2010; Prusty et. al., 2005). These pollutants not only cause acute or chronic injury but also predispose plants to

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other biotic and physiological disorders. Intensive and continuous deposition of pollutants causes disorder in plant's physiological developments, perceptible impairment, the decay of plant species, and even disappearance of sensitive plant species from sites exposed to pollution, while tolerant species flourish and dominate the vegetation (Prusty et al., 2005).

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 (Sharma and Tripathi, 2009; Gavali et al., 2002; Chaturvedi et al., 2013; Iqbal et al., 2010). The influence or toxicity of pollutants is determined by their chemical composition, particulate size, the rate of deposition (Van Jaarsveld, 2008) and exposure to different environmental factors (Weinstein and Davison, 2004; Chaturvedi et al., 2013). 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 of the 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); as some plant species have been recognized to absorb, detoxify and tolerate high levels of pollution (Ninave et al., 2001). 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. These plant-pollutant interactions transform the plant metabolism and leaf architecture, to acclimatize to new ecological habitats. Thus, plants can be used as both subservient biomonitors and bio-mitigators for environmental pollution (Akguc et al., 2010).

These pollutants at suboptimal conditions become phytotoxic and result in the production of reactive oxygen species (ROS). It is well known that pollutants may induce oxidative stress with overproduction of reactive oxygen species such as superoxide radicals (O_2^-), hydroxyl radicals (OH^-) and hydrogen peroxide (H_2O_2).These reactive oxygen species very rapidly react with DNA, lipids and proteins and results in cell destruction (Tauqeer et al., 2016; Pandey et al., 2010; Goswami and Das, 2016). Plants respond to ecological stresses in a number of ways, which encompasses changes at physiological, cellular and transcriptome levels. By applying these complex means, plants may avoid impairment and safeguard persistence under stressful environments, but often at the cost of reduced growth and yield (Ali et al., 2016).

Tree leaves such as Neem (*Azadirachta indica* A. Juss) can efficiently accumulate dust because of substantial leaf area (Lu et al., 2008). The effect of dust is shown in the physiological functions of trees, as a result, of which variations occur in plant morphology expressed as high defoliation levels (*abscission)*, changes in size and mass of leaves, shoots and falling vigour of plants (Ots et al., 2011).

Considering the role of plants in indication, abatement and control of pollution, the present study was conducted to clarify the morphological, biochemical and antioxidant responses of Neem (*A. indica*) to FA dust pollution at BTPP ash dumping site. Analyses of the above-mentioned changes serve as a foundation for finding suitable parameters for objective assessment of FA stress on vitality, growth and other biochemical parameters of this medicinal plant species.

2. Materials and methods

2.1. Geography and weather

Located in the subtropical belt. Delhi, the capital of India, lies between 28°24′17″ and 28°53′00″N Lat., 77°45′30″ and 77°21′30″E long., approximately 216 m AMSL, with a geographical area of about 1483 sq. km (47% urban, 53% rural) on the western end of the Gangetic plain, drained by river Yamuna. The climate of Delhi mainly influenced by its inland position and the prevalence of climate of continental type during the major part of the year. The climate of Delhi is characterized by extreme dryness with an intensely hot summer and cold winter with four major well-defined seasons namely winter (Dec–Feb) pre-monsoon (March–May) Monsoon (June–August) and post-monsoon (Sep–Nov).

2.2. Selection of experimental sites

Three sites, (I), (II) and (III), were selected for sampling situated at a distance of about 0.5, 4 and 18 km from Jamia Millia Islamia (JMI) to fly ash dumping site of (BTPP) in downstream wind direction because of recurrent wind flow in this direction. This zone was free from other pollution units of anthropogenic nature such as vehicular and industrial. University area Jamia Millia Islamia (JMI), Site (I) served as a control, because of being a residential and academic area, there was no other pollution-causing sources i.e. neither fugitive or anthropogenic nor any fly ash generating unit. The vehicular traffic is low as compared to the rest of Delhi because JMI falls under the rural area of Delhi. Further from air quality monitoring data (SPM), JMI showed a significantly low concentration of pollutants as compared to other sites. All the sites possessed sandy loam type of soil, coarse texture, with pH value 6.69, 7.12 and 7.16 respectively, due to the obvious seasonality of air pollution concentration differences in the study area because of the occurrence of significantly wet and dry periods. The winter season was selected (Oct–Feb) for the present study when the concentration of pollutants are is maximum for investigating the effect of fly ash particulate pollution on the leaf characteristics of the tree species growing at the three different sites (Chaturvedi et al., 2013; Prusty et al., 2005).

2.3. Sampling of leaves

Principal tree species designated for the study include *A. indica* A. Juss belonging to the family *Meliaceae.* At each sampling site, five individual plants having an identical diameter at breast height were marked, and the age was calculated with the formulae of Moser et al. (2015). On each tree, two twigs having full sun exposure, containing fully expanded and healthy leaves (five leaves) were sampled, thus, 75 leaves were used for this study. Marked leaves were sealed in plastic bags and carried to a laboratory for further analysis.

2.4. Measurement of pH and suspended particulate matter (SPM)

Leaf-extract pH was calculated according to the procedure of Singh and Rao (1983) 0.5 g of leaf material was ground to paste in pestle and mortar, dissolved in 50 ml distilled water and the pH was measured by using standardized digital pH metre. Whereas pH of soil and fly ash was determined by, dissolving known amount of soil and fly ash in distilled water and the pH was recorded accordingly. The concentration of SPM in the atmosphere was calculated using High Volume Air Sampler.