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Variation in Reducing Sugar and Proline Content of *Saraca asoca* Due to Air Pollution

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Abstract:
Air pollution due to anthropogenic activities imparts superfluous stress to plants and compels it to respond in the same manner as other environmental stress conditions. In an attempt to investigate the effects of air pollution on the biochemical properties of plants, the proline and the reducing sugar content of *Saraca asoca* was analysed in this study which so far has not been determined in this species. Biochemical properties for two different samples from similar ecological statues but exposed to varying levels of atmospheric pollution were analysed in this study. *Saraca asoca* was chosen due to its high relative abundance in both the locations. The samples were collected from both the locations and labelled as control sample (unpolluted site) and polluted sample (near commercial and industrial area). The results indicated an increase in the proline content in the polluted sample, signifying its role in the defence mechanism when the plant is under environmental stress. On the other hand, the reducing sugar content in polluted sample declined which indicated photosynthetic inhibition. These variations in biochemical parameters occurring as a consequence of air pollution help the plant to make maximum use of its resources during stress. Thus, biomonitoring with plants assists in a low-cost and beneficial method to indicate the degree of air pollution in an area.

Keywords: Air pollution, proline, reducing sugar, *Saraca asoca*

1.0 Introduction:
The advent of industrialization and urbanization has brought about the desecration of the environment leading to pollution. Henceforth, pollution can be defined as fluctuation in the constituents of the biosphere from the quantities that would have existed prior to the human intervention. Micro industrialization, monetary development, urbanization and relative increment in vitality requests have brought about a significant decay of air quality in creating nations like India (Mate *et al.,* 2016; Saluja, 2017). Various types of pollution are air, water, soil, and sound. Among these types, air pollution has the highest effect on life (Khedhar and Gadge, 2014). The major leading contaminants responsible for causing air pollution are volatile organic compounds, oxides of Carbon, Nitrogen and Sulphur and suspended particulate matter of size less than 500µm in diameter. Airborne particles and gas molecules can be deposited when they pass close to the leaf surface. Most plant leaves have a large surface area per unit volume which increases the possibility of capturing such particulate emissions (Janhäll, 2015). These pollutants undergo some chemical reactions to form secondary pollutants and can further lead to additional outcomes which may project detrimental effects on plant and human health.

Plants being stationary are unremittingly imperilled to the pollutants in the atmosphere so air quality around them has a huge effect on their physiology (by showing visible damage to leaves) and health (Priyanka and Dibyendu, 2009; Dohmen *et al.,* 1990; Nowak *et al.,* 2017). Vehicular emissions can directly affect plants via leaves or indirectly via soil acidification (Steubing *et al.,* 1989). Plants have an important role in biomonitoring as they are known to capture formaldehyde, sulfur oxides and heavy metals such as mercury and lead from their immediate surroundings (Agarwal, 2017). Cadmium and lead are non-essential elements for plants and if these elements are accumulated in excess quantities then they drastically affect the reproduction, development and transport of important nutrients in the plant body (Dutta and Palathingal, 2017). However, they differ in their responses to the pollutants some are sensitive while others being hardy and tolerant (which is dependent on the content of various biochemical parameters such as proline content, reducing sugar content, ascorbic acid content total chlorophyll, relative water content and leaf extract pH). The sensitive species can be used as bioindicators of pollution (Agarwal, 2017). Hence this way biomonitoring with plants can serve as a low cost and valuable method to evaluate the effect of different air and environmental pollutants (Oliva *et al.,* 2007).

The catastrophic effects of air pollution menacing life on earth cannot be ignored. Consequently, an urgent need to curb the devastating repercussions of air pollution accentuates the need to explore and investigate the potential of plants as biomonitoring agents of air pollution. The present investigation attempts to examine the variations in the biochemical factors: Reducing sugar and proline in the leaf samples of *Saraca asoca* collected from polluted and control sites respectively.

2.0 Materials and Methods:

2.1 Collection of Samples:

Sample collection was done from two different sites, one was located adjacent to the Noida-Greater Noida Expressway in Uttar Pradesh, India, which is designated as polluted area and the other area near Okhla Bird Sanctuary in Gautam Budh Nagar, Uttar Pradesh which had similar ecological conditions, was the control site for the experiment, in 2017. The climatic condition in this area is hot and humid. Ten leaf samples of *Saraca asoca* were collected from the respective sites and taken to the laboratory to conduct the experiment.

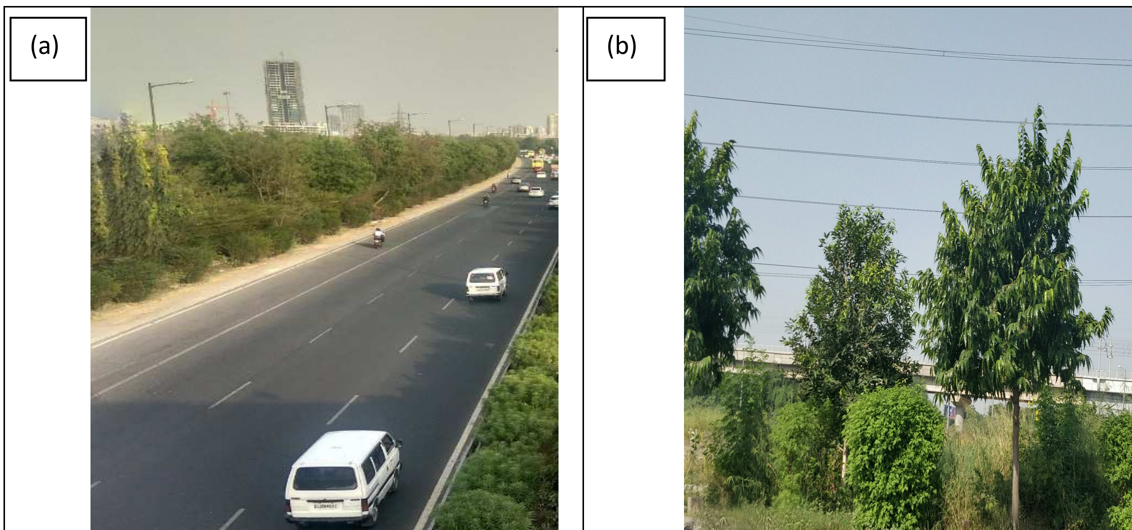
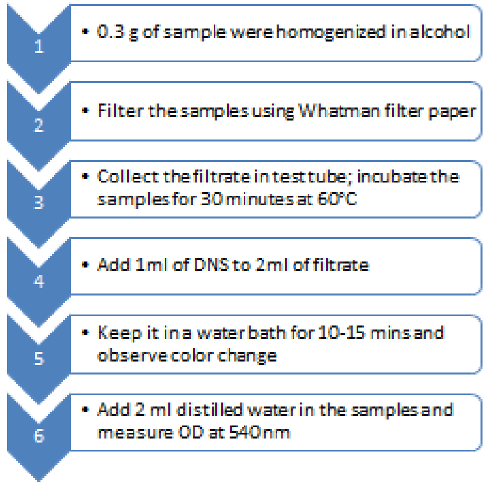


Fig A: Sample sites (a) Polluted site (Noida-Greater Noida Expressway, Uttar Pradesh) (b) Control Site (near Okhla bird sanctuary in Gautam Budh Nagar, Uttar Pradesh)

2.2 Protocols:

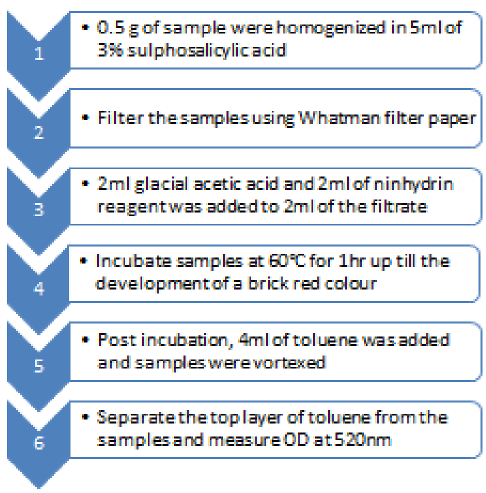
2.2.1 Reducing Sugar:

The Reducing sugar content was determined in accordance with the method as suggested by DuBois, 1956. 0.3g of the leaf samples were homogenized in alcohol and filtered with Whatman filter paper. Samples were left for incubation at 60°C for 30 minutes. To 2ml of the filtrate 1ml of Dinitrosalicylic acid (DNS) was added and the samples were kept in a water bath for 10-15 minutes till colour change was observed. 2ml of distilled water was added to the sample solution and OD was measured at 540nm.



2.2.2 Proline:

Proline content in the samples was determined in accordance with the method as suggested by Bates *et al.,* in 1975. 0.5g of leaf sample was homogenized in 5ml of 3% sulphosalicylic acid, and the samples were filtered with Whatman filter paper. 2ml glacial acetic acid and 2ml of ninhydrin reagent was added to 2ml of the filtrate. The samples were incubated at 60°C for 1hr up till the development of a brick red colour. After cooling the samples, 4ml of toluene was added and samples were vortexed. The top layer of toluene was separated from the samples and OD was measured at 520nm.



3.0 Results and Discussions:

3.1 Reducing Sugar:

Reducing sugar is an important component and source of energy for living beings. It is produced by plants during photosynthesis and is broken down during respiration. It serves as a crucial indicator of the physiological activity of plants as it shows plant sensitivity to air pollution (Tripathi and Gautam, 2007). In this study, we had observed a lower level of reducing sugar content in leaf samples obtained from the polluted site as compared to the control site. The results are strongly in agreement to the previous studies (Kameli and Löselδ, 1993; Keller and Ludlow, 1993; Naya *et al.,* 2007, Shvaleva *et al.,* 2005; Assadi *et al.,* 2011; Karmakar *et al.,* 2016).

Table 1: Mean values of standard amounts of reducing sugar in control and polluted leaf samples

Mean Control standard amount (µg/g of fresh weight)	Mean polluted standard amount (µg/g of fresh weight)
684.7	462.1

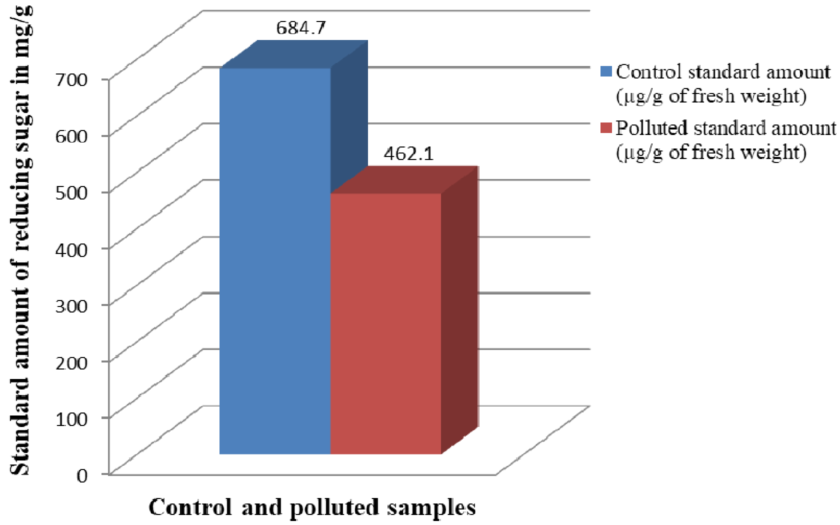


Fig B: Graphical representation of reducing sugar in control and polluted samples.

Pollutants such as NO_2, SO_2, H_2S, SPM (suspended particulate matter) and airborne heavy-metal under hardening conditions cause degradation of the reducing sugar content in plants. Under the polluted condition, its concentration decreases because of the increased respiration rate and decreased rate of carbon fixation as sulfites react with the aldehydes and ketones of carbohydrates and reduce the carbohydrate content (Seyyednejad and Koochak, 2013; Stambulska *et al.,* 2018). Using ANOVA test our F value was greater than the critical F value ($p<0.05$) by which we can reject our null hypothesis which says that the polluted sample has more reducing sugar.

Hence, the reducing sugar content decreases as a consequence of the lower level of photosynthetic rate and high energy demand (Tripathi and Gautam, 2007). It has been observed from previous researches that more resistant plant species show accumulation of reducing sugar while less stress-resistant plants show less accumulation solution sugar (Kameli and Löselδ, 1993; Keller and Ludlow, 1993).

3.2 Proline

Proline is an amino acid, is a strong reductant and protects cellular components against SO_2 and OH^- accumulation and protects the enzymes of the Calvin cycle (Krishnaveni and Kumar, 2017). It was observed in our study that the proline content was high in the leaf samples obtained from the polluted sites as compared to the control site. The results are strongly in agreement to the previous researches (Seyyednejad *et al.,* 2009; Mafakheri *et al.,* 2010; Seyyednedjad and Koochak, 2011; Seyyednejad and Koochak, 2013; Khedhar and Gadge, 2014).

Table 2: Mean values of standard amount of proline content in control and polluted samples.

Mean control amount (µg/ml)	Mean polluted amount (µg/ml)
2.453	3.048

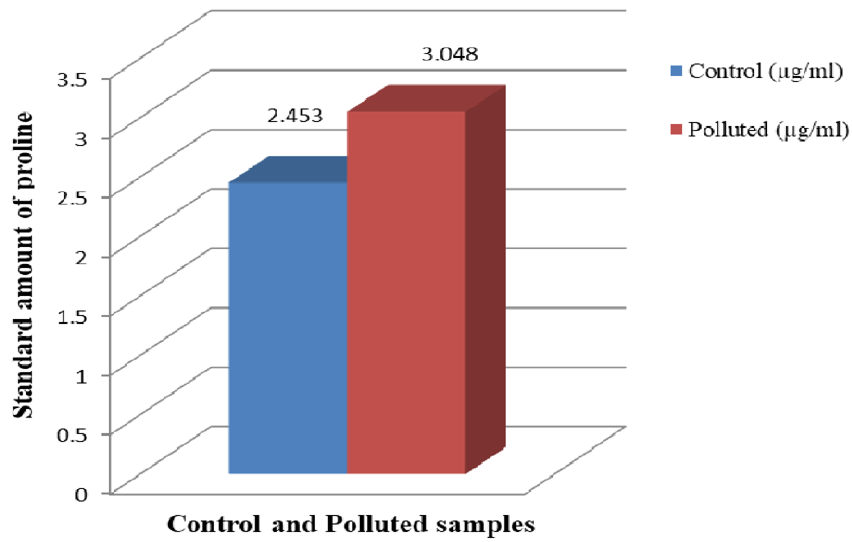


Fig C: Graphical representation of proline in control and polluted leaf samples

Continuous exposure of plants to the environment forces them to absorb, accumulate and integrated pollutants impinging on their foliar surfaces (Agarwal, 2017). Plants generally accumulate some kind of compatible solute such as proline or polyols in cytol to raise osmotic pressure and thereby maintain both turgor and driving gradient for water uptake and protect membrane and proteins (Agarwal, 2017).

The concentration of proline is known to affect the physiological activity of the plants and is known to depict plants sensitivity to air pollution. In plants, proline is the universal osmolyte and has a defensive action against the stress condition in the environment. It is known to accumulate during any kind of stress condition as an adaptation to environmental stress. Due to the enhanced amount of protein denaturation and the breakdown of existing protein into the amino acids, there is a decrease in the protein content. As a consequence of the increased generation of reactive oxygen species (ROS) such as $S0^{3-}$, HSO_3, OH^-, O_2 during the photo-oxidation reaction for the formation of SO^{4-} from SO^{3-}, sulphites are generated from the SO_2 absorbed. The pollutants cause the production of ROS in plants causing the peroxidative destruction in cellular constituents. To combat this in such situations proline gets accumulated and acts as a scavenger to protect plants from the damages caused due to oxidative stress. Proline gets accumulated mainly because of the reciprocal regulation of two pathways which are the increased expression of proline synthetic enzymes and repressed expression of proline degradation enzyme (Delauney and Verma (1993); Peng *et al.,*1996). The accumulation of proline under stress conditions could be used to select stress tolerant species since proline concentrations has been reported to be higher in stress tolerant species (Yancy *et al.,* 1982 Ashraf and Foolad; 2007; Jaleel *et al.,* 2007). Using ANOVA test our F value was greater than the critical F value ($p<0.05$) by which we can reject our null hypothesis which says that the control sample has more proline.

4.0 Conclusions:

The results of the present investigation report that there is a decrease in the reducing sugar content and increase in the proline content of the leaf extract samples obtained from the polluted sites compared to the ones obtained from the control site. This may suggest that the plants are sensitive to stress caused due to air pollutants and develop adaptive mechanisms to combat the stress levels. Hence, biomonitoring with plants can be used as a valuable tool to examine the effects of air pollutants.

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