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Physiological Studies on Effect of Air Pollution on *Saraca asoca* Plants

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Abstract

One of the significant global issues in recent times is the pollution of atmospheric air due to extensive industrialization and mechanization. Plants can be distinguished as sensitive or tolerant types based on their physiological and morphological response towards various air pollutants like compounds of nitrogen, sulphur (SO_x, NO_x) and particulate matter. The plants sensitive to these air pollutants serve as indicators for their presence in the environment. The present investigation aimed at examining the effect of pollutants in on *Saraca asoca* plants grown in two sites (Indraprastha, Delhi and River Yamuna Bank, Delhi) which have highly polluted air in comparison to plants grown in non-polluted (control) site (Botanical Garden, Noida). Plants were assayed with respect to pigment content, reducing sugar content, proline content and relative water content in the leaf. The leaves collected from *Saraca asoca* plants grown in polluted sites displayed an inverse relation to chlorophyll content, reducing sugar content and relative water content with the extent of air pollution, whereas a direct correlation of proline was observed with extent of air pollution. Therefore it was inferred that the above parameters are highly significant consistent in the plant-air pollution interactions and can be used as bio-indicators.

Keywords: Air pollution; *Saraca asoca*; Chlorophyll content; Proline content

Introduction

Air Pollution can be defined as the defilement of the atmospheric air which alters its quality and makes it unfit for survival of the living. Air pollution occurs when harmful substances including particulates and biological molecules are introduced into Earth's atmosphere. It is responsible for many diseases, allergies or death of humans; it may also cause harm to other living organisms such as animals and food crops, and damage the natural or built environment. Over the years, Urbanization has caused a significant increase in human population, automobile traffic and the number of industries, resulting in the increased concentration of gaseous pollutants and particulate matter in the atmosphere. Air quality has a huge impact on plants since they are stationary and are continuously exposed to variations in the environment. New Delhi, the capital city of India has a population of 18.6 million and population density of 29,259.12 people per square mile (Reported in 2016). The morphological features of plants therefore reflect the quality of air in the current surrounding. The presence of pollutants affects the health and physiology of plants [1]. Plants act as Bio-indicators and develop adaptive mechanisms for survival in the form of accumulation of organic molecules [2]. Chlorophyll content indicates healthy photosynthetic activity and nutritional state in a leaf. Chlorophyll pigment acts as the receptor of light in the process of photosynthesis where CO_2 is fixed to give out carbohydrates and oxygen. Exposure to pollutants such as SO_x and NO_x can cause the gradual death of chlorophyll causing yellowing of leaves which subsequently leads to decrease in the photosynthetic activity of plants. Sulphur dioxide is a byproduct of burning fossil fuels and is primarily responsible for causing chlorosis. Reducing sugars are synthesized in plants during photosynthesis and during breakdown reactions in respirations. They are known to impart osmoprotectant and cryoprotectant properties to leaves. Pollutants like SO_2, NO_2 and H_2S deplete the sugar content following there conversion to secondary pollutants. Proline forms an important component of several enzymes and proteins. The accumulation of proline in plants during abiotic stress reduces the degradation of other proteins. The increase in proline content ensures greater tolerance against salinity and drought stresses in plants. The water content at given water potential in leaves is indicative of healthy photosynthetic activities in plants. Since water is a main reactant for the process of photosynthesis, its content is highly monitored and regulated in leaves. Under stress by air pollutants, the rate of photosynthesis decreases drastically along with declining water potential. The underlying area between Indraprastha and Yamuna Bank regions of New Delhi are heavily Air Polluted due to a major Thermal Power Plant setup in the adjoining area that contributes to about 13% of the air pollutants along with two other power plants in Delhi [3]. There is a profound impact on the various parameters of plant leaves caused due to Air pollutants including cement dust, heavy metals and fly ash. The physiology of plants is highly organised and undergoes an array of physico-chemical changes in response to such stresses. Alteration of physiological parameters discussed, induced by air pollution have been studied and observed by other wokers [4-7]. The present study was planned to decipher the change in various parameters of leaves of *Saraca asoca* growing in this area and contrast them with those growing in a controlled region where air pollution was expected to be much lower. The results as expected showed a significant variation in the concentration of various parameters that were tested and follow the result trends of similar studies by other workers.

Materials and Methods

The present study is a comparative analysis of leaves collected from *Saraca asoca* plants grown in air polluted and control sites with respect

to four major parameters- chlorophyll content, reducing sugar content, proline content and relative water content.

Plant sample

Leaf tissue of *Saraca asoca* plants from three sites: two sites having highly polluted air (Indraprastha, Delhi and Yamuna Bank, Delhi) and one site having very limited polluted air (Botanical Garden, Noida). The site with limited polluted air served as control.

Determination of pigment content: 1 g of leaves was weighed accurately, chopped into fine pieces and taken in a test tube with 25 ml ethanol. 3 samples were prepared each for control and polluted leaves. The tubes were then incubated at 60 degrees for 30 minutes. The tubes were recovered and 2 ml of aliquot was taken from each sample. The optical density of the samples was evaluated by a spectrophotometer at 4 different wavelengths under UV-Visible range- 480 nm, 510 nm, 645 nm and 663 nm. Ethanol was taken as the blank solution. Mean ODs were taken for the control and polluted samples. The amount of various pigments was calculated using the given formulae Table 1 [8].

$$Total\ chlorophyll\ \left(mg/g\right) = \frac{20.2 \times OD_{645} + 8.02 \times OD_{663}}{25}$$

$$Chlorophyll\ a\ \left(mg/g\right) = \frac{12.3 \times OD_{663} - 0.86 \times OD_{645}}{25}$$

$$Chlorophyll\ b\ \left(mg/g\right) = \frac{19.3 \times OD_{645} - 3.6 \times OD_{645}}{25}$$

$$Carotenoid\ \left(mg/g\right) = \frac{7.6 \times OD_{480} - 1.49 \times OD_{150}}{25}$$

Parameter	Control (mg/g)	Polluted (mg/g)
Total chlorophyll	1.435	1.227
Chlorophyll a	0.663	0.532
Chlorophyll b	0.759	0.665
Carotenoid	0.373	0.296

Table 1: Calculated readings (by using formula).

Determination of reducing sugar content: The reducing sugar content was determined by DNSA method (3, 5- Dinitrosalicylic Acid Method). 1 g of leaf samples was weighed and crushed to smaller pieces. It was then incubated with 10 ml ethanol at 60 degree temperature for 30 minutes. The tubes were recovered and added 1 ml DNS. The solution was the placed in a boiling water bath until a color change was observed. The solution was diluted with 2 ml distilled water and its optical density was taken at 540 nm under UV-Vis range with the help of spectrophotometer. The amount of reducing sugar was calculated from a standard graph between concentration *vs.* optical density of glucose [9].

Determination of proline content: 0.2% solution of Ninhydrin was prepared by dissolving 0.2 g of Ninhydrin in 100 ml acetone. 25 ml of this solution was prepared by dissolving 50 mg Ninhydrin in 25 ml acetone. 0.5 g of leaf samples were weighed from both control and polluted samples and were grinded into a paste with 10 ml of 3% sulphosalicylic acid using a mortar and pestle. Three such samples were prepared each for control and polluted leaves. The solution was filtered using a filter paper. 2 ml of the aliquot was taken in a test tube, 2 ml glacial acetic acid and 2 ml of Ninhydrin solution were added. The reaction mixture was placed in a boiling water bath for 1 hour till a brick red color was observed. The reaction mixtures were cooled and 4 ml Toluene was added to it. The prepared solution was thoroughly mixed to separate the top layer of toluene and optical density was obtained at 520 nm using a spectrophotometer taking toluene as blank. The optical densities were compared to a standard curve of proline to estimate the amount of proline in the sample.

Determination of relative water content: 3 leaves each from control and polluted sites were taken and weighed accurately to obtain their Fresh Weight (F). The leaves were then immersed in water overnight, dried with a blotting paper and weighed to get there Turbid Weight (W). Next the leaved were placed in an incubator at 60° overnight and were weighed to obtain their Dry Weight (D) Table 2. The mean weight of the controlled and polluted samples was taken for all the 3 values. The relative water content of the leaf samples was estimated by the given formula.

$$RWC = \frac{F - D \times 100}{W - D}$$

Weights	Control	Polluted
	C1=0.378	P1=0.295
Fresh weight(F)	C2=0.232	P2=0.227
	C3=0.248	P3=0.163
Average	0.313	0.229
	C1=0.415	P1=0.345
Wet weight(W)	C2=0.270	P2=0.244
	C3=0.286	P3=0.184
Average	0.323	0.257
	C1=0.155	P1=0.186
Dry weight(D)	C2=0.099	P2=0.118
	C3=0.106	P3=0.089
Average	0.120	0.131

Table 2: Relative water content.

Results and Discussion

Pigment content

In leaves from control region, the total chlorophyll was found to be 1.435 mg/g fresh wt, Chlorophyll a 0.663 mg/g fresh wt, chlorophyll b 0.759 mg/g fresh wt, and Carotenoids 0.373 mg/g fresh wt. In leaves from polluted region, the Total Chlorophyll was found to be 1.227 mg/g fresh wt, Chlorophyll a 0.532 mg/g fresh wt, Chlorophyll b 0.665 mg/g fresh wt, and Carotenoids 0.296 mg/g fresh weight (Figure 1 and Table 3).

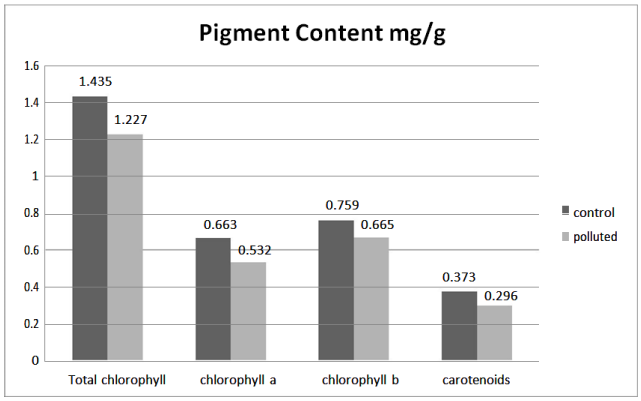

Figure 1: Concentration of various pigments in control and polluted leaf samples.

Wavelengths	Control	Polluted
480 nm	C1=1.408	P1=1.103
	C2=1.425	P2=1.112
	C3=1.399	P3=1.115
Average	1.41	1.11
510 nm	C1=0.927	P1=0.701
	C2=0.908	P2=0.655
	C3=0.931	P3=0.723
Average	0.922	0.693
645 nm	C1=1.205	P1=0.009
	C2=1.222	P2=0.105
	C3=1.198	P3=0.068
Average	1.208	0.06
663 nm	C1=1.465	P1=1.184
	C2=1.398	P2=1.127
	C3=1.437	P3=1.159
Average	1.433	1.156

Table 3: Spectrophotometer readings for chlorophyll content in control and polluted leaf samples.

The variation in the amount of different pigments in leaves from Control and Polluted areas occurred due to the intensity of Air Pollution in Polluted sites. Air pollutants like SO_X and NO_X along with fly ash have a significant impact on the Photosynthetic leaves are responsible for reduced chlorophyll content and therefore photosynthetic activity. The results obtained are very similar to the experimentation results of [4]. The various photosynthetic pigments are highly sensitive to pollutants and exist in an organized manner. Pollution stress can cause these pigments to undergo certain chemical reactions like oxidation, reduction, bleaching etc; leading to their structural alteration. This can cause changes in the Physiological, Morphological and Biochemical nature of the plant [10-12]. Chlorophyll in response to the effect of air pollutants get degraded into phaeophytin by losing Mg ions depending upon the time and intensity of pollutants.

Reducing sugar content

The Reducing Sugar Content was found to be 697 µg/g in leaves from Control region whereas 475 µg/g in leaves from Polluted region (Figure 2 and Table 4).

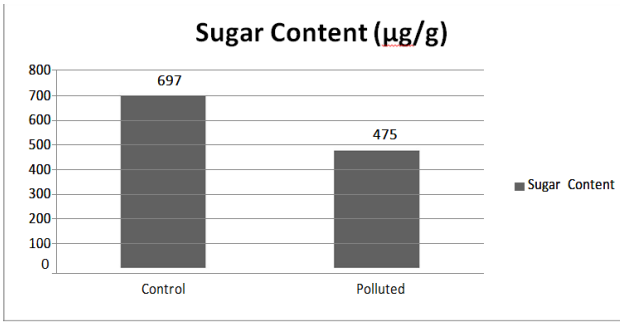

Figure 2: Reducing sugar content in control and polluted leaf samples.

Leaf sample	Optical density	Standard graph reading(ug/g)
Control	C1=0.985	
	C2=1.202	
Average	1.093	697
Polluted	P1=0.928	
	P2=0.651	
Average	0.789	475

Table 4: Reducing sugar content in control and polluted leaf samples.

The concentration of reducing sugars is reduced in leaves from polluted region. These leaves were exposed to air pollutants like NO and SO along with particulate matter. The probable reason for this decrease is the inhibition of photosynthetic pigments as sugar is a product of photosynthesis. The alteration of respiration rate is also responsible for the decline in sugar content. Heavy metals present in the atmosphere interact with Ribulose Bisphosphatase Carboxylase and stimulate carbon metabolism [13]. Exposure to Ozone caused an immediate decline in the concentration of soluble sugars [14]. The starch content declined under scute concentration of oxidants [15,16].

Proline content

The Proline Content in leaves from Control region was found to be 2.42 µg/g whereas as in leaves from Polluted region it was 3.07 µg/g (Figure 3 and Table 5).

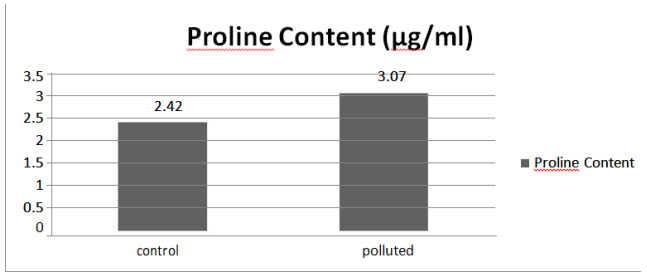

Figure 3: Proline content (µg/ml) in control and polluted leaf samples.

Leaf sample	Optical density	Standard graph
		Reading (ug/g)
Control	C1=0.061	
	C2=0.010	
	C3=0.008	
Average	0.026	2.42
Polluted	P1=0.030	
	P2=0.042	
	P3=0.038	
Average	0.036	3.07

Table 5: Proline Content in leaves.

The amino acid Proline reportedly accumulates under stress condition in plants [17,18]. The amount of proline was found to be significantly higher in plants growing in polluted regions. The reason for this is reported to be an adaptive measure [19]. The amount of Proline is being used to differentiate stress tolerant species since they possess a higher Proline content [20]. Under stress, Proline accumulates as a result of inverse behavior of the two enzymes-proline digesting and proline synthesizing [21]. The amount of Proline accumulation varies from species to species and at different locations in the plant [22].

Relative water content

The leaves from Control region have about 95.39% Relative Water Content whereas those of Polluted region have about 77.77% Relative Water Content (Figure 4). The percentage of water present in plant leaves varied in leaves from Control and Polluted areas. It was found that leaves from Control areas possess a higher Relative Water Content compared to those of Polluted areas. The Control leaves contain about 95% water which is reduced to about 75% in polluted leaves. Higher Relative Water Content helps a plant to cope stress conditions. Due to air pollution, the permeability of the cells increases [23] leading to loss of water and nutrients from the cell cause premature senescence of leaves. Plants that possess high Relative Water Content under pollution are known to be sensitive species. However none of the leaves under study depicted such results.

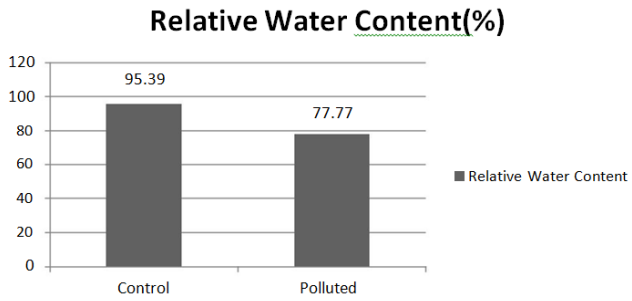

Figure 4: Relative Water Content (%) in control and polluted leaf samples.

Therefore it can be concluded that these plants are sensitive species. The study suggests that the change in the physiological features of *Saraca asoca* plants is caused due to pollutants such as NO_x and SO_x along with particulate matter and fly ash produced by industries and automobile smoke. There is a need of installing air filtering devices and developing a belt of green resistant plant species for the betterment of plant and animal species. It can be concluded that industrial and automobile exhaust is a significant environmental factor causing the defilement of our environment. The effects can be severe on other plant and animal species which require a further scope of study.

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