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Pigment Degradation of Higher Plants near Sugar Mill

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Abstract

Sugar industry generate a significant large amount of waste during the manufacture of sugar and contains a high amount of production load particularly in items of suspended solids, organic matters, press-mud, bagasses and air pollution. Air pollutants from sugar mill can directly affect plants via leaves or indirectly via soil acidification. When exposed to air pollutants, most plant experience physiological changes before exhibiting visible damage to leaves. Leaf pigment content can provide valuable insight into the physiological performance of leaves. In the present study, the extraction of chlorophyll and their derivatives were studied in the five tree species such as Ficus benghalensis, Delonix regia, Ficus religiosa, Azadirachta indica and Pongamia pinnata. The study area was polluted with organic pollutants which results in reduced pigment levels in the leaves. The pheophytin a and b were formed greater than hundred percent in Delonix regia Azadirachta indica and Pongamia pinnata. For Ficus benghalensis, pheophytin a was found to be 27.17% and pheophytin b was more than hundred percent. In Ficus religiosa, pheophytin a was formed by 41.09% where as pheophytin b was produced less than the residential area. These results indicate that the degradation of chlorophyll was higher near sugar mill because of the emission of gases. Among the five tree species growing near sugar mill, Ficus religiosa showed minimum decomposition of chlorophyll and it was suggested to grow these species to reduce the load of pollution near sugar mills.

Key words: Air pollutants, visible damage, organic pollutants, chlorophyll, degradation, pheophytin a and b.

Introduction

Sugar industry is one of the most important agro based industries in India and is highly responsible for creating significant impact on rural economy in particular and countries economy in general. Sugar industries rank second amongst mavar agro based industries in India. Sugar industry is seasonal in nature and operates only for 120 to 200 days in a year (early November to April). A significant large amount of waste is generated during the manufacture of sugar and contains a high amount of production load particularly in items of suspended solids, organic matters, press-mud, and bagasses and air pollution[1,2,3].

Gaseous emissions such as CO_x, SO_x and NO_x were reported both from process and fired equipment from sugar industry[4]. Air pollutants can directly affect plants via leaves or indirectly via soil acidification[5]. When exposed to airborne pollutants, most plants experienced physiological changes before exhibiting visible damage to leaves[6]. Plants provide an enormous leaf area for impingement, absorption and accumulation of air pollutants to reduce the pollutant level in the air environment[7], with a various extent for different species[8]. Trees provide a large leaf surface onto which particles are deposited and gases are removed. Pollution is removed by nearly all parts of a tree; the soil, roots and vegetative portions of the tree species.

Trees respirate and exchange gases through stomata or holes, on their leaves; these gases include those necessary for the tree's functioning as well as other gaseous air pollutants. Once inside the leaf, gases diffuse into the spaces between the cells of the leaf to be absorbed by water films or chemically altered by plant tissues. Trees also reduce air pollution by intercepting airborne particles and retaining them on the leaf surface, called dry deposition[9]. Some can be absorbed by the leaf surface itself, although most remain on the plant surface[10]. Previous studies also showed the impact of air pollution on ascorbic acid content[11], chlorophyll content[12,13], leaf extract pH[14,15] and relative water content[16].

There are many pigments in the higher plants, such as chlorophyll (chl), carotenoid (car), phytochrome, flavinoid, anthocyanin, tannin and so on. Chlorophyll contains two different chemical structures, a and b, while the former contains CH_3 at the position 7 of ring B, the latter contains CHO at the same position. The major absorbance of both chlorophyll a and b is in the blue and red visible light. All carotenoids absorb blue light. Phytochrome transforms its structure between red and far red light. Flavonoids, including anthocyanins and tannins, absorb green and ultra-violet light. These pigments offer an all-oriented network of interception, reflection and protection for plant body, allowing plant to use sunlight and cut off its photodamage. Plant pigments are degraded into small molecules recycling into the nature after completing their mission. Eight steps are involved in the degradation pathway of chlorophyll to pheophorbide[17] (figure 1).

With acid gases or vapors, the initial action on the chlorophyll pigments is conversion to the corresponding pheophytins. The extent of decomposition of chlorophyll or conversion to pheophytin is a measure of the degree of air pollution damage to leaves of plants, and this can be determined readily by spectrochemical methods.

Cholorotic injury is the result of a breakdown of the chlorophyll in the leaf and may be either a temporary or permanent condition. The leaf tissue may still exercise its functions, but at a reduced rate. In the transient form, the chlorosis consists of a slight paling of the normal green color which is usually restored within a few days. Where the chlorosis is of the permanent type, the loss in chlorophyll content is evident in the yellow or yellowish'green appearance of the leaves. The gross symptoms of permanent chlorosis are similar in appearance to senescence and may result from the continued absorption of gas and its accumulation as sulfate in the leaf beyond the tolerance limit. When the leaf chlorophyll and other green pigments are decomposed, the leaf assumes a bleached, reddish'brown, brown, or ivory appearance, depending upon the nature of the species.

In order to determine the effect of air pollution exposure and damage on the chlorophyll and pheophytin content of living plants, five tree species growing near sugar mill and residential area were selected as the most suitable species for study purposes.

Material and Methods

Selection of sampling area and sampling details: The research work was mainly confined near sugar factory. Leaves of five tree species such as *Ficus benghalensis, Delonix regia, Ficus religiosa, Azadirachta indica* and *Pongamia pinnata* were collected from polluted area and residential area. The screening and selection of the tree species was partly based on literature survey of similar work and guidelines of Central Pollution Control Board. The five leaf samples were collected at the lower most position of canopy at a height of 6-7ft from the ground surface. Samples were cleaned with distilled water and then refrigerated (22ºC) under suitable condition for further biochemical analysis.

Extraction process: Chlorophyll a, b and Carotenoid: The preweighed samples of five tree species were dissolved in 80% acetone (20ml per each gram) and grained using mortar and pestle and then homogenized using a homogenizer at 1000 rpm for about 5 minutes. Then the samples were filtered using cheese cloth. The extracts obtained were centrifuged at 5000 rpm for about 10 minutes. The supernatants were separated and absorbances were read at 200 – 700 nm on UV spectrophotometer. The experiment was repeated thrice for statistical analysis. The content of chlorophyll a, b and carotenoid were calculated using the equations of Porra et al[20] and Holm[21] respectively.

Chl a = $12.25\ A_{663.6} - 2.55\ A_{646.6}$ (µg/ml), Chl b = $20.31\ A_{646.6} - 4.91\ A_{663.6}$ (µg/ml), Car = $4.69\ A_{440.5}$ -0.267 Chl_{a+b} (µg/ml)

Porphyrins: The above acetone extract was mixed with equal amount of hexane and kept undisturbed till interface appears. The upper phase contains less polar compounds dissolved in hexane and the lower phase contains more polar compounds dissolved in acetone. The upper and lower fractions were separated and the lower fraction was used to measure the absorbance at 575, 590 and 628 nm. The equation of Khan et al[22] was hired to determine the content of protoporphyrin IX (PPIX), magnesium-protoporphyrin IX (MGPP) and protochlorophyllide (Pchlide).

PPIX = $196.25\ A_{575} - 46.6\ A_{590} - 58.68\ A_{628}$ (nmole), MGPP = $61.81\ A_{590} - 23.77\ A_{575} - 3.55\ A_{628}$ (nmole), Pchlide = $42.59\ A_{628} - 34.22\ A_{575} - 7.25\ A_{590}$ (nmole)

Chlorophyllide (Chlide) a and b: The lower acetone fraction was used to measure the absorbance at 667 and 650 nm. The Beer-Lambert law and method of McFeeters[23] was used to calculate the content of chlorophyllide a and b. Chlide a = A_{667}/ 74.9 (mM), Chlide b = A_{650}/ 47.2 (mM)

Pheophytin (Phe) a and b: The upper hexane fraction was dried with nitrogen and dissolved in 80% acetone. The absorbance were measured at 665.4 and 663.4 nm, which were major absorption peaks of pheophytin a and b respectively. The content of pheophytin a and b were calculated using the equation of Lichtenthaler[24]. Phe a = $22.42\ A_{665.4} - 6.81\ A_{653.4}$ (µg/ml), Phe b = $40.17\ A_{653.4} - 18.58\ A_{665.4}$ (µg/ml)

Less polar caroteoid (LP Car): The acetone fraction dissolved in HCl was used and the absorbance was measured at 470 nm. The content of less polar carotenoid was calculated using Lichtenthaler equation. LP Car = $(1000\ A_{470} - 4.28\ A_{665.4} - 4.78\ A_{653.4})/ 164$ (µg/ml)

Results and Discussion

The chlorophyll and its derivatives calculated for the five tree species collected from residential area and sugar mill were tabulated in table 1 and 2. All the five tree species showed reduction in chlorophyll a, b and carotenoids at the polluted site. Chlorophyll a, b and carotenoid of *Ficus benghalensis* was declined by 22.84%, 57.12% and 55.03% respectively near sugar mill. The percentage reduction of other species was also very high. The total chlorophyll and carotenoid of *Delonix regia* was decreased by 62.74% and 70.57% respectively. Higher percentage reductions were seen in *Azadirachta indica* (81.07% and 85.92%) *and Pongamia pinnata* (70.13% and 49.07%) for total chlorophyll and carotenoid. *Ficus religiosa* showed 45.73% decline in carotenoid pigment compared to the unpolluted site.

The decomposed products of chlorophyll (protoporphyrin and magnesium-protoporphyrin) were formed more than hundred percent in *Ficus benghalensis* and *Azadirachta indica* near sugar mill compared to residential area. In *Delonix regia*, the percentage decompositions were 35.56% and 76.47% respectively. *Pongamia pinnata* showed 54.38% formation of protoporphyrin and greater than hundred percent formation of magnesium-protoporphyrin.

Another decomposition product protochlorophyllide (PChlide) was also formed in higher percentage for *Ficus benghalensis*, *Delonix regia* and *Ficus religiosa* where as less decomposed product than the residential area was reported in *Azadirachta indica* and *Pongamia pinnata*. The formation of chlide a and b were very low in both the polluted and unpolluted sites.

The classical investigations of Willstatter and Stoll[25] showed that when chlorophyll is treated with dilute acids there is formed a derivative, pheophytin, by quantitative replacement of the magnesium atom of the chlorophyll nucleus with hydrogen. Molisch[26] found pheophytin to be present in gas damaged leaves[27]. Subsequently, Roben and Dorries[28] in 1932 described experiments on the effect of sulfurous acid on Elodea canadensis in which the formation of pheophytin from chlorophyll was noted in shorter exposure periods, followed by bleaching of the green pigment and subsequent disappearance of the pheophytin as well, with time or at higher acid concentrations. It was also noted that sulfuric acid was far less damaging to the plant than sulfurous acid at equivalent hydrogen ion concentration.

The pheophytin a and b were formed greater than hundred percent in *Delonix regia Azadirachta indica* and *Pongamia pinnata*. For *Ficus benghalensis*, pheophytin a was found to be 27.17% and pheophytin b was more than hundred percent. In *Ficus religiosa*, pheophytin a was formed by 41.09% where as pheophytin b was produced less than the residential area. Less polar carotenoids was reported to be formed more than hundred percent in *Ficus benghalensis* and *Delonix regia* where as it was only 41.22% in *Ficus religiosa*. These results indicate that the decomposition of chlorophyll was higher near sugar mill because of the emission of gases[26]. The minimum decomposition of chlorophyll at the residential area was due to the natural autumnal yellowing or senescence of plants by organic acids released in the leaves in contact with the chlorophyll, as a result of the disintegration of plasma proteins[29].

Table 1
The chlorophyll and its derivatives for the five tree species at residential area

Pigments	Tree species				
	Ficus benghalensis	***Delonix regia***	***Ficus religiosa***	***Azadirachta indica***	***Pongamia pinnata***
Chl a	7.2624	13.4413	3.4549	21.2096	16.3653
Chl b	7.8518	8.2105	2.7814	8.0479	10.1246
Chl a+ b	15.1141	21.6518	6.2363	29.2575	26.4898
Car	4.8005	10.4651	4.5257	6.0144	4.7742
PPIX	16.8961	22.1329	18.8731	7.7797	29.4871
MGPP	7.3455	7.8210	5.9539	3.7771	9.6977
Pchlide	1.4307	0.0959	0.0707	0.2956	0.5897
Chlide a	0.0072	0.0062	0.0033	0.0045	0.0056
Chlide b	0.0069	0.0064	0.0044	0.0045	0.0064
Phe a	9.5859	8.0775	4.0579	6.1425	7.0022
Phe b	4.3818	4.5270	4.0257	2.3414	5.0729
LP Car	4.0427	4.4298	3.9569	1.1300	4.8460

Table 2
The chlorophyll and its derivatives for the five tree species near sugar mill

Pigments	Tree species				
	Ficus benghalensis	***Delonix regia***	***Ficus religiosa***	***Azadirachta indica***	***Pongamia pinnata***
Chl a	5.6035	4.7087	2.4545	3.8346	4.0656
Chl b	3.3668	3.3590	2.8738	1.7034	3.8482
Chl a+ b	8.9703	8.0677	5.3283	5.5380	7.9138
Car	2.1589	3.0799	2.4559	0.9367	2.4316
PPIX	64.0508	30.0043	12.0914	22.3197	45.5225
MGPP	19.0808	13.8019	4.8366	15.9559	19.5078
Pchlide	3.4422	3.8909	1.8781	9.4298	3.2889
Chlide a	0.0099	0.0167	0.0044	0.0261	0.0196
Chlide b	0.0125	0.0169	0.0049	0.0217	0.0207
Phe a	12.1901	22.4446	5.7253	36.0295	26.7548
Phe b	10.9589	11.8519	3.8524	11.4006	15.1852
LP Car	9.4571	17.7582	5.5878	11.0845	11.0446

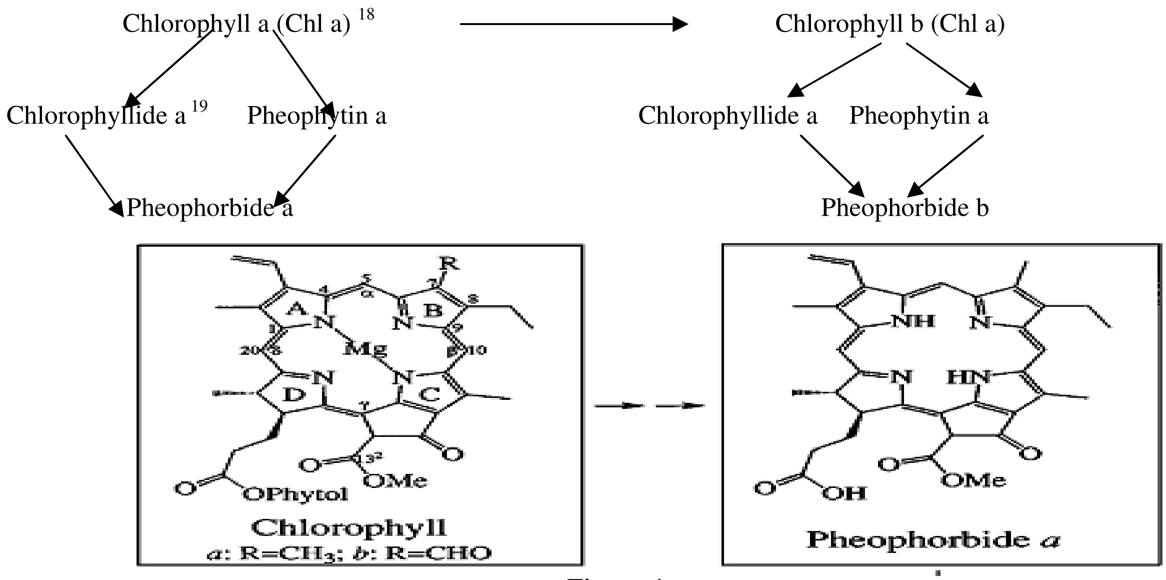

Figure-1
Degradation pathway of chlorophyll

Conclusion

Air pollution damage to vegetation produces a variety of gross symptoms on leaves which may have similar features as to color changes, location of lesions, and boundary characteristics[30, 31]. The order of susceptibility of various species to air pollution damage is a useful guide as to the probable causal agent. Chemical analysis of the leaf tissue provides additional help in diagnosis[32, 33].

The evidence in the present study on chlorophyll and pheophytin conversion in leaves of five tree species indicates that acid gases, such as sulfur dioxide in sufficiently high concentration, convert the chlorophyll pigments into the corresponding pheophytins. This process does not take place to an appreciable extent, however, unless the threshold value for sulfite in the living cells is exceeded. The effect may be produced by either relatively short exposures to high concentrations of gas or more prolonged exposures to lower concentrations.

Transformation of an appreciable proportion of chlorophyll to pheophytin is accompanied by visible symptoms in the tissue, such as plasmolysis, shrinkage of cells, and fading of the normal green color. The destruction of the chlorophyll, pheophytin and other pigments in the chloroplasts results merely in the appearance of bleached or ivory colored areas. Sulfur dioxide absorption by leaves in subtoxic amounts produces no deleterious influence on photosynthesis and rate of growth[34, 35]. The gas is stored in the tissue largely as sulfate, and this may reach a value of 3 to 5 times the quantity normally present before it becomes toxic[36].

Among the five tree species growing near sugar mill, *Ficus religiosa* showed minimum decomposition of chlorophyll and it was suggested to grow these species to reduce the load of pollution near sugar mills.

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