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The spectral determination of chlorophylls A, B and total carotenoids using various solvents for tree species growing near sugar mill

■ D. SARALA THAMBAVANI AND M.A. SABITHA

Author for Correspondence -
D. SARALA THAMBAVANI
Department of Chemistry, Sri Meenakshi Government Arts College for Women (Autonomous), MADURAI (T.N.) INDIA
sarala_dr@yahoo.in

See end of the article for authors affiliation

ABSTRACT - 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, 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. In the present study, the extraction of chlorophyll a, chlorophyll b and carotenoid pigments were studied in the five tree species such as *Ficus benghalensis, Delonix regia, Ficus religiosa, Azadirachta indica* and *Pongamia pinnata*. Extraction was made by using solvents such as acetone (80% and 100%), ethanol and ethyl acetate. The spectrophotometric determination of chlorophyll a, chlorophyll b and carotenoid pigments were studied. The study area was polluted with organic pollutants which results in reduced pigment levels in the leaves. It was observed that Acetone (100%) showed higher significance during the extraction process when compared to ethanol, ethyl acetate and acetone (80%).

Key words - Air pollutants, Bagasse, Chlorophyll a and b, Carotenoid, Organic pollutants, Extraction process, Solvents

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Industrialization is an important tool for the development of any nation. Consequently, the industrial activity has expanded so much all over the world. Today, it has become a matter of major concern in the deterioration of the environment (Tiwari *et al*, 1993). With the rapid growth of industries (sugar, paper, tannery, textile, sago, dye industries) in the country, pollution of natural water by industrial waste water has increased tremendously (Amathussalam *et al.*, 2002). Among them, sugar industry plays a major role in producing a higher amount of water pollution and soil pollution because they contain large quantities of chemical elements. They contain higher amounts of total hardness, total dissolved solids, biological oxygen demand and chemical oxygen. The effluent not only affects the plant growth but also deteriorate the soil properties when used for irrigation (Maliwal *et al.*, 2004). In addition to that, some traceable amount of heavy metals such as zinc, copper and lead were also present in the effluent (Borale *et al.*, 2004). These effluents not only increase the nutrient level but also excess tolerance limits and cause toxicity (Mishra *et al.*, 1999).

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 (Bevan, 1971, Hendrickson *et al.,* 1971).

Gaseous emissions such as CO_x, SO_x and NO_x were reported both from process and fired equipment from sugar industry (Khwaja and Quraishi, 2003). Air pollutants can directly affect plants *via* leaves or indirectly *via* soil acidification (Steubing *et al.*, 1989). When exposed to airborne pollutants, most plants experienced physiological changes before exhibiting visible damage to leaves (Dohmen *et al.*, 1990). Plants provide an enormous leaf area for impingement, absorption and accumulation of air pollutants to reduce the pollutant level in the air environment (Escobedo *et al.*, 2008), with a various extent for different species (Hove *et al.*, 1999). 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 (Sarala *et al.*, 2011). Some can be absorbed by the leaf surface itself, although most remain on the plant surface (Joshi *et al.*, 2008). Previous studies also showed the impact of air pollution on ascorbic acid content (Hoque *et al.*, 2007), chlorophyll content (Flowers *et al.,* 2007; Sarala *et al.*, 2009a), leaf extract pH (Klumpp *et al.*, 2000; Sarala *et al.*, 2009b) and relative water content (Rao, 1979).

Chlorophyll itself is not a single molecule but a family of related molecules, designated as chlorophyll a, b, c and d. Chlorophyll pigments are major supplement source in the production of nutraceutical products (Higdon, 2004). Chlorophyll a is most abundant in nature; the main function of chlorophyll b is to gather the light energy, working together with chlorophyll a and carotenoids. The most important one in the carotenoids; they play vital role in the photosynthetic pathway and are called as accessory pigments because, they don't dissolve in water, and must attach within the pathway, but must pass their absorbed energy to chlorophyll.

The carotene pigments are the most important photosynthetic pigments, and they prevent the chlorophyll and thylakoid membrane from the damage of absorbed energy by photooxidation (Vechetel *et al.*, 1992). It was also determined that the various limiting factors such high light, lack of nitrogen and limited nutrient, influence the change in pigment level. A solvent is a liquid or gas that dissolves a solid, liquid, or gaseous solute, resulting in a solution. Solvents play a major role in the process of extracting the pigments. The spectrophotometric absorbance properties of pigments facilitate the qualitative and quantitative analysis of them using different solvents (Nusch, 1980 and Sartory, 1982) and the contribution of these solvents to the extraction in various species were comparatively studied. Furthermore, the determination of suitable methods and solvents that could be used in studies on pigments was aimed.

In the present study, chlorophyll a, chlorophyll b and carotenoids of five tree species growing near sugar mill were determined using various solvents for extracting various pigments and were examined comparatively.

EXPERIMENTAL METHODOLOGY

Selection of sampling area and sampling details:

Table A : Formula used in the calculation of pigments

Sr. No.	Solvents	Formulae
1.	80% Acetone	Chlorophyll a = 12.21 A_{663} - 281 A_{646}
		Chlorophyll b = 20.13 A_{646} - 5.03 A_{663}
		Carotenoids = $\frac{1000\ A_{470} - 3.27[\text{Chl a}] - 104\ [\text{Chl b}]}{227}$
		Total pigments = chlorophyll a + chlorophyll b + carotenoids
2.	100% Acetone	Chlorophyll a = 11.75 A662 – 2.350 A645
		Chlorophyll b = 18.61 A645 – 3.960 A662
		Carotenoids = 1000 A470 – 2.270 [Chl a] – 81.4 [Chl b]/230
		Total pigments = chlorophyll a + chlorophyll b + carotenoids
3.	Ethanol	Chlorophyll a = 15.65 A666 – 7.340 A653
		Chlorophyll b = 27.05 A653 – 11.21 A666
		Carotenoids = 1000 A470 – 2.860 [Chl a] – 85.9 [Chl b]/245
		Total pigments = chlorophyll a + chlorophyll b + carotenoids
4.	Acetone	Chlorophyll a = 11.75 A_{662} – 2.350 A_{645}
		Chlorophyll b = 18.61 A_{645} – 3.960 A_{662}
		Carotenoids = 1000 A_{470} – 2.270 [Chl a] – 81.4 [Chl b]/230
		Total pigments = chlorophyll a + chlorophyll b + carotenoids

The research work was mainly confined near sugar factory. Leaves of five tree species such as *Ficusbenghalensis, Delonix regia, Ficus religiosa, Azadirachta indica* and *Pongamia pinnata* were collected from polluted 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 (1999 - 2000). 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 (22°C) under suitable condition for further biochemical analysis.

Extraction process:

The preweighed samples of five tree species were put separately in acetone (80% and 100%), ethanol and ethyl acetate (20ml per each gram) were 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 400 – 700 nm on UV spectrophotometer. Maximum absorbance of chlorophyll a, is at 662 nm, chlorophyll b, is at 646 nm and for total carotenoid, 470 nm. The experiment was repeated thrice for statistical analysis. The amounts of pigments present in them were calculated according to the formula of Lichtentaler and Wellburn (1985) and tabulated in Table A.

EXPERIMENTAL FINDINGS AND ANALYSIS

The pigment content of five tree species such as *Ficus benghalensis, Delonix regia, Ficus religiosa, Azadirachta indica* and *Pongamia pinnata* were extracted using three different solvents. The extraction was complete because when compared to other compound chlorophyll and carotene pigments are non-polar organic substances. Chlorophyll and carotenes did not dissolve in water, but they dissolve in organic solvents. In this study, chlorophyll a was higher in *Azadirachta indica* when extracted with solvent Acetone (80%), although chlorophyll a in other four tree species showed some significant variation between each other. The calculated pigment values were tabulated in Table 1.

Out of the five species chlorophyll a (3.39 µg/ml) and chlorophyll b (2.70 µg/ml) were very low in *Ficus religiosa* in 80per cent Acetone. Chlorophyll b was found to be high in *Pongamia pinnata* (9.79 µg/ml). 9.36 µg/ml of carotenoid was encountered for *Delonix regia* and minimum value of 2.76 µg/ml was obtained for *Ficus religiosa*. In 100 per cent Acetone, chlorophyll a and chlorophyll b were found to be maximum for *Ficus religiosa* (28.82 µg/ml and 13.29 µg/ml, respectively) and it was 10.08 µg/ml for *Azadirachta indica*. *Pongamia pinnata* showed minimum value of 4.87 µg/ml for chlorophyll b. Carotenoid content was maximum in *Delonix regia* (10.22 µg/ml) and minimum in *Azadirachta indica* (2.18 µg/ml).

15.88 µg/ml of chlorophyll a was extracted in ethanol where as it was 2.24 µg/ml for *Ficus benghalensis*. The extraction of chlorophyll b was very low in ethanol for *Ficus benghalensis* (1.19 µg/ml) and it was found to be high for *Pongamia pinnata* (8.35 µg/ml). Carotenoid showed lower values for *Ficus benghalensis* and *Azadirachta indica*. The extraction of chlorophyll a and chlorophyll b in ethyl acetate was high for *Delonix regia* (18.35 µg/ml and 15.66 µg/ml, respectively). It was found to be very low for *Ficus benghalensis* (0.81 µg/ml and 0.42 µg/ml, respectively). Carotenoid extraction was maximum for *Delonix regia* (6.01 µg/ml) and minimum for *Ficus benghalensis* (0.34 µg/ml).

The total pigments were high for trees extracted using acetone (80% and 100%). 18.01 µg/ml, 30.05 µg/ml, 8.85 µg/ml, 32.83 µg/ml and 29.19 µg/ml were calculated for *Ficus benghalensis, Delonix regia, Ficus religiosa, Azadirachta indica* and *Pongamia pinnata*, respectively using 80 per cent acetone whereas 18.96 µg/ml, 40.07 µg/ml, 49.91 µg/ml, 17.34 µg/ml and 21.58 µg/ml were calculated in extraction using 100

Table 1 : Effect of solvents on pigments

Species	80% Acetone				100% Acetone				Ethanol				Ethyl acetate			
	Chl a	Chl b	Car	TP	Chl a	Chl b	Car	TP	Chl a	Chl b	Car	TP	Chl a	Chl b	Car	TP
Ficus benghalesni	7.09	7.67	3.25	18.01	10.63	5.45	2.88	18.96	2.24	1.19	0.54	3.97	0.81	0.42	0.34	1.57
Delonix regia	13.21	7.93	9.36	30.50	20.72	9.13	10.22	40.07	9.26	4.94	2.50	16.7	18.35	15.66	6.01	40.02
Ficus religiosa	3.39	2.70	2.76	8.85	28.82	13.29	7.80	49.91	15.88	8.24	3.69	27.81	14.72	5.58	5.03	25.33
Azadirachta indica	20.91	7.66	4.26	32.83	10.08	5.08	2.18	17.34	5.51	3.86	0.72	10.09	6.37	3.98	2.06	12.41
Pongamia pinnata	16.08	9.79	3.32	29.19	13.25	4.87	3.46	21.58	14.42	8.35	2.25	25.02	7.56	2.03	3.01	12.60

per cent acetone.

Most importantly, a solvent plays a major role in the extraction process of chlorophylls and carotenes. The pigments were calculated and formulated based on the formulas of Lichtentaler and Wellburn (1983). It was determined that the solvent used were important in the determination of pigments. In the present study, Acetone (80% and 100%) was considered as the best solvent for the extraction of pigments because acetone is easily miscible and the extraction was complete when compared to ethanol and ethyl acetate.

Earlier data have suggested that methanol and ethanol were used in the extraction of chlorophyll. These pigments were better opted than acetone for the extraction process. According to the recommendation of Elder, till 1995 the evaluation of the chlorophyll content was performed by using extraction method with acetone after that ethanol was suggested for the extraction process. In this study the use of acetone in the extraction process of chlorophyll and carotene have showed significant good results when compared to the ethanol and ethyl acetate.

Considerable loss in chlorophyll in the leaves of plants exposed to air pollution stress supports the argument that the chloroplast is the primary site of attack by air pollutants such as SO_2 and NO_X (Tripathi and Gautam, 2007; Sarala *et al*., 2008). Air pollutants make their entrance into the tissues through the stomata and cause partial denaturation of the chloroplast and decrease pigment contents in the cells of polluted leaves. High amount of gaseous SO_2 causes destruction of chlorophyll and that might be due to the replacement of Mg^{2+} by two hydrogen atoms and degradation of chlorophyll molecules to phaeophytin (Rao and Leblanc, 1966). Hence, it becomes important to study the chlorophyll content of tree species near sugar mill to assess the air pollution effect.

Conclusion:

In fact acetone, chloroform, diethyl ether, ethyl acetate, Dimethyl formamide and methanol were used in the studies of chlorophyll extraction even from higher plants (Wellburn, 1994). The extraction varies and specifically depends on the extraction rate of every solvent. Here in this method acetone used showed better results for extraction of pigments from the tree species.

Authors Affiliation :

M.A. SABITHA, Department of Chemistry, J.A. College for Women (Autonomous), PERIYAKULAM (T.N.) INDIA
Email : sabiarsh@yahoo.in

REFERENCES

Amathussalam, A., Abusbacker, M.N. and Jayabal, N.J. (2002). Indian Pollution Control, pp: 118-119.

Bevan (1971). The disposal of sugar mills effluents in Queensland, 40^{th} proceeding of the T. S. S. C.T. Louisinia October-November, 1971; pp. 150, H–1516.

Borale, D.D. and Patil, P.R. (2004). Pollution Resident, pp. 23-83.

Dohmen, GP., Koppers, A. and Langebartels, C. (1990). Biochemical response of Norway Spruce [*Picea abies* (L.) Karst.] towards 14-month exposure to ozone and acid mist: effects on amino acid, glutathione and polyamine titers, *Environ. Pollution*, **64** : 375-383.

Elder, L. (1979). Recommendations on methods for marine biological studies in the Baltic sea. Phytoplankton and chlorophyll. *Baltic Marine Biol*., **5**:38.

Escobedo, FJ., Wagner, JE. and Nowak, DJ. (2008). Analyzing the cost effectiveness of Santiago, Chile's policy of using urban forests to improve air quality, *J. Environ. Management*, **86** : 148-157.

Flowers, MD., Fiscus, EL. and Burkey, K.O. (2007). Photosynthesis, chlorophyll fluorescence and yield of snap bean (*Phaseolus vulgaris* L) genotype differing in sensitivity to ozone. *Environ. Exp. Bot*., **61**: 190-198.

Hendrickson (1971). New sugar factory waste and their control 40^{th} proceeding of the T. S. S. C. T. October-November, 1971; pp. 1552–1559.

Higdon, J. (2004). Chlorophyll and Chlorophyllin. http://lpi.oregonstate.edu/infocentre/ phytochemicals/chlorophylls.

Hoque, MA., Banu, MNA. and Okuma, E. (2007). Exogenous proline and glycinebetaine increase NaCl-induced ascorbate –glutathione cycle enzymes activities, and praline improves salt tolerance more than glycinebetaine in tobacco bright yellow -2 suspension – cultured cells. *J. Plant Physiol*., **164**: 1457 – 1468.

Hove, LWA., Bossen, ME. and Bok, FAM. (1999). The uptake of O_3 by poplar leaves: The impact of a long-term exposure to low O_3-concentrations, *Atmospheric Environ.*, **33** : 907-917.

Joshi, P.C. and Chauhan, A. (2008). Performance of locally grown rice plants (*Oryza sativa* L.) exposed to air pollutants in rapidly growing industrial area of district Haridwar, Uttarakhand, India. *Life Sci. J.*, **5**(3): 57 – 61.

Khwaja, Mahmood A. and Quraishi, Masood H. (2003). Self-Monitoring and Reporting for Industry Islamabad: Sustainable Development Policy Institute (SDPI).

Klump, G., Furlan, C.M. and Domingos, M. (2000). Response of stress indicators and growth parameters of tibouchina pulchra logn. Exposed to air and soil pollution near the industrial complex of cubatao, *Brazil Sci. Total Environ*., **246** : 79-91.

Lichtenthaler, H.K. and Wellburn, A.R. (1985). Determination of total carotenoids and chlorophylls A and B or leaf in dissolved solvents. *Biol. Soc. Trans*., **11**: 591-592.

Maliwal, G.L., Patel, K.P., Patel, K.C. and Petel, M.N. (2004). Pollution Resident, pp. 231-169.

Mishra, P.C. and Sahoo, S. (1997). Production and energetics of earthworm population (*Lampito mauritii*, Kinberg) and metabolism in soil under paper mill waste water irrigation, *Ecol. & Environ. Conservation,* **3**(1) : 49-61.

Nusch, E.A. (1980). Comparision of methods for chlorophyll and phaeopigment determination. *Arch. Hydrobiol. Limnol.*, **14** : 14-36.

Rao, D.N. and Leblanc, F. (1966). Effect of SO_2 on the lichen, algae with special reference to chlorophyll. *Biologist.*, **69** : 69-95.

Rao, DN. (1979). Plant leaf as pollution monitoring Device. *Fertilizer News*, 25-28.

Sarala Thambavani, D. and Sabitha, M.A. (2009a). Tolerance of plants to air pollution near leather tanneries, *J. Ecotoxicol. & Environ. Monitoring*, **1**(6): 609-612.

Sarala Thambavani, D. and Syed Ali Fatima Latif (2008). Biochemical changes in certain plants species under automobile exhaust pollution, *Eco. Chronicle*, 257-264.

Sarala Thambavani, D., Sabitha, M.A. and Selvasundari, R. (2009b). Air pollution Tolerance index of tree species growing in traffic area of Madurai, Tamil Nadu, *Asian J. Experimental Chem.*, **4**(1&2): 126-132.

Sarala Thambavani, D. and Sabitha, M.A. (2011). Variation in air pollution tolerance index and anticipated performance index of plants near a sugar factory: implications for landscape-plant species selection for industrial areas, *J. Res. Biol.*, **1**(6): 001-009.

Sartory, D.P. (1982). Extraction of chlorophyll A from fresh water phytoplankton for spectrophotometric analysis. M.Sc., Thesis. Univ. Orange Free State. Bioemfontein. Republic of S. Africa.

Steubing, L., Fangmeier, A. and Both, R. (1989). Effects of SO_2, NO_2, and O_3 on population development and morphological and physiological parameters of native herb layer species in a beech forest, *Environ. Pollu.*, **58** : 281-302.

Tiwari, P. (1993). Elecy and C.B. Indu Bhahia, Geobiosphere, pp: 20-27.

Tripathi, AK. and Gautam, M. (2007). Biochemical parameters of plant as indicators of air pollution, *J. Environ. Biol.*, **28**(1):127-132.

Vechetel, B.W. and Ruppel, H.G. (1992). Lipid bodies in Eremophaera viridis de bary (Chlorophyceae). *Plant & Cells Phys.*, **31**:41-48.

Wellburn, A.R. (1994). The spectral determination of chlorophylls A and B, as well as Total caroteinds, using various solvents with Spectrophotometers of different resolution. *J. Plant. Phys.*, **144** : 307-313.

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