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The monthly changes of chloroplast pigments content in selected plant species exposed to cement dust pollution

Authors:
Sarala Thambavani D[1],
Saravana Kumar R[2]

Institution:
1. Department of Chemistry, Sri Meenakshi Govt. Arts College for women, Madurai - 625 002. Tamil Nadu, India.
2. Department of Chemistry, N.P.R. College of Engineering and Technology, Natham, Dindigul 624401. TamilNadu, India.

Corresponding author:
Saravana Kumar R

ABSTRACT:

Chloroplast pigments were shown to be very sensitive to various environmental influences. Changes in chlorophyll and carotenoid content were investigated in selected plant species exposed to alkaline dust emitted by the cement industry. Pigments were extracted consequently for 30, 60, 90, 120, 150 and 180 days interval and quantified spectrophotometrically. In comparison with control site all measured pigments were reduced in dust-exposed plant species. This is due to deceleration of the biosynthetic processes rather than degradation of pigments. Chlorophyll b content appeared to be more sensitive than chlorophyll a in polluted plants. Total carotenoids needed a longer period of time to reach nearly the same level as in controls. The progression of pigment decline in polluted sites appeared not to be dramatically accelerated. It might thus be concluded that polluted sites had sufficient biosynthetic capacity to prevent irreversible damage by cement dust.

Keywords:
Cement dust, Bio-indicators, Photosynthetic pigments, Chlorophyll, Carotenoid, assimilating pigments and acetone.

Email:
sarala_dr@yahoo.in
gsivasaravanan@gmail.com

Phone No:
99423-68797

Web Address:
http://jresearchbiology.com/Documents/RA0165.pdf.

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INTRODUCTION

The impact of the cement industry on the surrounding vegetation has been widely investigated (Farmer, 1993) although research on the effects of dust pollution on plants has never received the same level of attention as that given to phytotoxic pollutants such as SO_2, NO_2 and O_3. Results from research that has been undertaken, together with repeated observations of dust deposits on vegetation, suggest that the effects of dust may be important and are worthy of greater investigative attention. Rapid industrialization and addition of the toxic substances to the environment are responsible for altering the ecosystem (Mudd & Kozlowski, 1975: Nriagu & Davidson, 1986; Clayton & Clayton, 1982).

The Cement industry plays a vital role in the imbalances of the environment and produces air pollution hazards (Stern, 1976). In comparison with gaseous air pollutants, many of which are readily recognized as being the cause of injury to various types of vegetation, relatively little is known and limited studies have been carried out on the effects of cement dust pollution on the growth of plants. Carotenoids are a class of natural fat-soluble pigments found principally in plants, algae and photosynthetic bacteria, where they play a critical role in the photosynthetic process (Ong and Tee, 1992; Britton, 1995) and also protect chlorophyll from photo oxidative destruction (Siefermann-Harms, 1987). When plants are exposed to the environmental pollution above the normal physiologically acceptable range, photosynthesis gets inactivated (Miszalski and Mydiarz, 1990). The amount of solar radiation absorbed by a leaf is a function of the photosynthetic pigment content; thus, chlorophyll content can directly determine photosynthetic potential and primary production (Curran *et al.,* 1990, Filella *et al.,* 1995). Furthermore, leaf chlorophyll content is closely related to plant stress and senescence (Hendry 1987, Merzlyak and Gitelson 1995, Penuelas and Filella 1998, Merzlyak *et al.,* 1999). A periodical study was carried out to study the effect of cement dust pollution on the growth of selected plant species such as *Azadirachta indica(L), Polyalthia longifolia (L), Ficus religiosa(L), Pongamia pinnata(L) and Delonix regia(L)*.

MATERIALS AND METHODS

Study Area Description

Madurai city is grown on both sides of river Vaigai and its terrain is mostly flat. The ground rises from the city, towards outward, on all sides except the south, which is a gradually sloping terrain. It is surrounded on the outskirts by small and prominent hills. The city is about 100 meters above mean sea level and it is situated on $9^0$55' NL and $78^0$7' EL and the city is covering 51.96 Sq.kms. The climate of Madurai town is hot and dry and the temperature range between a maximum and minimum of 42^0C and 21^0C respectively.

Experimental procedure

The experiment was conducted in pots under natural conditions. Mature ripe seeds of five plant species like *Azadirachta indica (L), Polyalthia longifolia(L), Ficus religiosa (L), Pongamia pinnata (L) and Delonix regia (L)* were collected. The seeds of all the three species were sown in medium sized clay pots with three parts fine sand and one part of natural manure. When the seedlings reached a suitable height, they were transferred to pots, 23.0 cm in diameter and 21.0 cm in depth and the five plants were planted in three replicates. One gram of cement dust was sprinkled regularly on the aerial parts of each plant twice a week, except the control and all the plants were watered daily with tap water.

Photosynthetic pigments Analysis

Chlorophyll' a' and' b' impart the green color that one associate with plant leaves. Carotenoids, which are yellow pigments, are also present in leaves but are usually masked by the chlorophylls. It is only in the fall when the chlorophylls are degraded faster than the carotenoid that the yellow color becomes visible to us. The chlorophyll and carotenoid contents of plants can vary markedly with its age or depend on environmental factors such as light intensity or quality during growth. Carotenoids and chlorophylls are found in the chloroplasts and are associated with the thylakoids, the internal membrane network of these organelles. It is now established that all chlorophylls are organized as discrete chlorophyll-protein complexes within the lipid matrix of the photosynthetic membrane. The majority of chlorophyll 'a' molecules (and all chlorophyll 'b' and carotenoid molecules) functions as antenna pigments. In combination with proteins, they form the light-harvesting complexes, which absorb and funnel light energy to the reaction center chlorophylls, thereby allowing the plant to utilize a broad spectrum of wavelengths for photosynthesis. Some of the chlorophyll 'a' molecules serve specialized functions in the reaction centers of photo systems I and II, where the light energy is used to drive the reduction of components of the electron transport chain.

Extraction of Photosynthetic pigments

Extract photosynthetic pigments by grinding 1g of leaves, torn into small pieces, in a mortar with a pinch of clean sand and a total of 10 ml of 100% acetone. Initially, add only a small amount of acetone to begin the grinding process. It is much easier to grind the leaves if the extract is a pasty consistency. Add more solvent in small increments while continuing to grind the leaves. For some species may need to add more than the suggested 10ml of acetone. Pour the extract into a 15ml centrifuge tube and centrifuge in the bench top centrifuge for 3 to 5 min. Remove the extract to a 10ml graduated cylinder using a Pasteur pipette. Transfer an aliquot of the clear leaf extract (supernatant) with a pipette to a 1-cm-pathlength cuvette and take absorbance readings against a solvent blank in a UV-VIS spectrophotometer at four different wavelengths.

750 nm ($A750 = 0$ for clear extract)
662 nm (chlorophyll *a* maximum using 100% acetone)
645 nm (chlorophyll *b* maximum using 100% acetone)
470 nm (carotenoids).

Apply measured absorbance values to equations given by Lichtenthaler (1987) for acetone to determine pigment content (µg/ml extract solution).Once the baseline has been run from 700-400nm using acetone in the cuvette, run an absorption spectrum for each pigment, rinsing the sample cuvette with acetone between readings. The peaks and valleys will be adjusted automatically by the Lamda-35 UV-VIS spectrophotometer, by changing the range of percentage absorbance on the y-axis. We can use the cursor to obtain the wavelengths of the spectral peaks, or estimate them from the printed spectra. These peak wavelengths will be useful for determining the identities of the pigments associated with the spectra. The studies were conducted on *Azadirachta indica(L), Polyalthia longifolia(L), Ficus religiosa(L), Pongamia pinnata(L) and Delonix regia(L)* plants growing under natural conditions. The plant samples were analyzed at every 30-day of intervals. The concentrations of photosynthetic pigments like chlorophyll-a, chlorophyll-b and carotenoids (mg/g fresh weight) we obtained using the following formula given by Lichtenthaler 1987.

Quantification of pigments (For 100% Acetone)

Chl-a (µg/ml) = $11.24\ A_{661.6} - 2.04\ A_{644.8}$
Chl-b (µg/ml) = $20.13\ A_{644.8} - 4.19\ A_{661.6}$
Carotenoids = $(1000\ A_{470} - 1.90\ C_a - 63.14\ C_b)/214$

Statistical Analysis

Data from the two selected sites for the plant materials were subjected to the two way analysis of variance (ANOVA). Using ANOVA the comparison made between control plant species and polluted plant species. Significance difference was calculated at 0.05%, 0.01% and 0.001% level as per standard method of Gomez and Gomez (1984).

The present investigation has been undertaken to study the effect of cement dust pollutant on total chlorophyll, carotenoids, chlorophyll ‘a’ and chlorophyll ‘b’ of selected plant species. In the present study, the cement pollution effects on the performance of selected plant species was observed and the total chlorophyll content decreased significantly in response to cement dust pollutants in polluted plant leaves compared with control of *Azadirachta indica(L), Polyalthia longifolia(L), Ficus religiosa(L), Pongamia pinnata(L) and Delonix regia*(L).3.

RESULTS AND DISCUSSION

The quantitative analysis of chloroplast pigments revealed difference between the selected plant species exposed to cement dust pollution and those from control. The mean values of all measured parameters are shown in **Fig. 1** to 4. Chlorophyll a content was higher in control site compared with polluted site. The amount of chlorophyll a varied from 1.18 to 1.78 mg g^{-1}(FW) in control site and from 0.61 to 1.33 mg g^{-1}(FW) in the polluted site **(Table 1).**

The chlorophyll ‘b’ content was lowered in polluted site compared with control site in all the plant species. The values varied from 0.81 to 1.43 mg g^{-1}(FW) and 0.54 to 1.02 mg g^{-1}(FW) in control and polluted sites respectively (**Table 2**, **fig. 2**). The amount of chlorophyll reduced in all the polluted

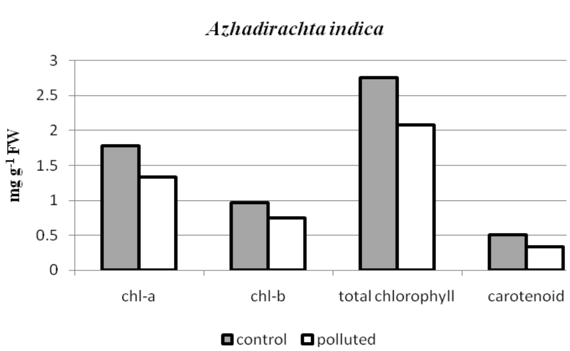

Figure 1. The mean value of chloroplast pigments in Azhadirachta indica

Table 1. The monthly mean values of chlorophyll a (mg g^{-1} FW; ± SD) in control and polluted leaves of selecteplant species. P(t)- percent of similarity; NS- not significant difference.

Plants	control	Polluted	P(t)
Azhadiracha indica(L)	1.78 ±0.42	1.33* ±0.31	<0.05%
Polyalthia longifolia(L)	1.40 ±0.34	1.02** ±0.29	<0.01%
Ficus religiosa (L)	1.18±0.22	0.64** ±0.14	<0.01%
Pongamia pinnata(L)	1.54 ± 0.42	0.94* ±0.35	<0.05%
Delonex regia(L)	1.49±0.47	0.61*±0.21	<0.05%

plants than control plants due to alkaline dust pollution. The highest value of total chlorophyll is 2.08 mg g^{-1}(fw) in A.indica and lowest value 1.19 mg g^{-1}(fw) in F.religiosa at polluted site compared with control (**Table 3, fig. 3**).

The amount of total carotenoid in control and polluted sites varied from 0.25 to0.51 mg g^{-1} and 0.15 to 0.33 mg g^{-1} respectively (**Table 4, fig. 4**). All the selected plant species showed the significant ($p<0.01$ and $p<0.05$) reduction in pigment content during the study period. In general, plants showed a decrease in photosynthetic pigments due to cement dust treatment. The relatively reduction in Chl 'a', Chl 'b', total chlorophyll and total carotenoids for the selected species such as *Azadirachta indica(L), Polyalthia longifolia(L) Ficus religiosa(L), Pongamia pinnata (L) and Delonix regia(L)* were related to cement dust pollution. The following observations were made.

Azadirachta indica (L) showed 22.92% reduction in the Chlorophyll 'a' when exposed to the cement dust pollution. There were 25.39% reduction in Chlorophyll 'b' when compared with control and cement dusted plant species. *Azadirachta indica* (L) showed 42.06% reduction in the total carotenoids. There were 24.6% reductions in the total chlorophyll. Cement dust reduced the photosynthetic pigments such as chlorophyll 'a', chlorophyll 'b', total chlorophyll and total carotenoids. *Polyalthia longifolia* (L) exhibited 27.34%, 26.61%, 61.15% and 28.2% reduction in chlorophyll 'a', chlorophyll 'b', total carotenoids and total chlorophyll respectively. There were 43.32% reduction in chlorophyll 'a', 32.39% reduction in chlorophyll 'b', 29.01% reduction in total carotenoids and 40.1% reduction in total chlorophyll in the selected species of *Ficus religiosa* (L). *Pongamia pinnata* (L) exhibited the decreasing trend in the photosynthetic pigments such as 38.78%, 37.65%, 65.55% and 41.2% reduction in chlorophyll 'a', chlorophyll 'b', total carotenoids and total chlorophyll respectively. *Delonix regia* (L) exhibited the maximum reduction in the photosynthetic pigments such as 37.65%,41.85%, 38.37% and 51.8% in chlorophyll 'a', chlorophyll 'b', total carotenoid and total chlorophyll respectively.

There was maximum reduction (43.32%) of Chlorophyll 'a' in the leaves of *Ficus religiosa* (L) and minimum (22.92%) reduction in *Azadirachta indica* (L) while maximum reduction (41.85%) of Chlorophyll 'b' was depleted in *Delonix regia* (L) and minimum reduction (25.39%) in *Azadirachta*

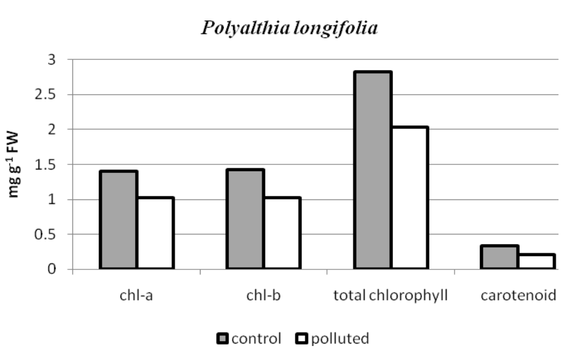

Figure 2. The mean value of chloroplast pigments in *Polyalthia longifolia*

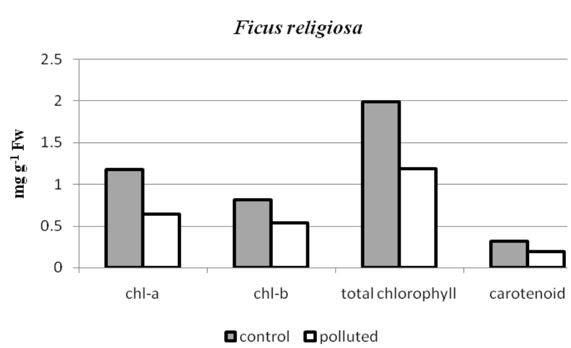

Figure 3. The mean value of chloroplast pigments in *Ficus religiosa*

Table 2. The monthly mean values of chlorophyll b (mg g^{-1} FW; ± SD) in control and polluted leaves of selected plant species. P(t)- percent of similarity; NS- not significant difference.

Plants	control	Polluted	P(t)
Azhadiracha indica(L)	0.97 ±0.20	0.75**±0.17	<0.01%
Polyalthia ongifolia(L)	1.43±0.16	1.02**±0.11	<0.01%
Ficus religiosa (L)	0.81±0.12	0.54**±0.08	<0.01%
Pongamia pinnata(L)	1.13±0.24	0.70*±0.14	<0.05%
Delonex regia(L)	1.25±0.29	0.74*±0.21	<0.05%

Table 3. The monthly mean values of total chlorophyll (mg g^{-1} FW; ± SD) in control and polluted leaves of selected plant species. P(t)- percent of similarity; NS- not significant difference.

Plants	control	Polluted	P(t)
Azhadiracha indica(L)	2.76 ±0.62	2.08**±0.47	<0.01%
Polyalthia longifolia(L)	2.83±0.49	2.04**±0.38	<0.01%
Ficus religiosa (L)	1.99±0.32	1.19**±0.22	<0.01%
Pongamia pinnata(L)	2.67±0.65	1.64*±0.46	<0.05%
Delonex regia(L)	2.74±0.64	1.35**±0.41	<0.01%

indica (L). The highest reduction (51.81%) in total chlorophyll was observed in *Delonix regia* (L) whereas the lowest reduction (24.67%) was recorded in *Azadirachta indica*(L). Similarly, in case of carotenoid contents, highest reduction (65.55%) was observed in *Pongamia pinnata*(L) and lowest in *Ficus religiosa*(L) (29.01%). *Polyalthia longifolia*(L) and *Ficus religiosa*(L) showed significant reduction in chlorophyll 'a' ($p<0.01$) and other three species showed the significance level ($p<0.05$%).

Mandre and Tuulmets (1997) reported a decrease of chlorophyll content in Norway spruce needles caused by cement dust.

Total average amount of assimilating pigments:

The weight ratio of Chl 'a' and Chl 'b' indicates the functional characters of photosynthetic pigments. The ratio of Chl 'a' and Chl 'b' was found to be higher for the polluted environment exposed to the *Azadirachta indica* (L) compared to the control. Similarly, the weight ratio of Chl 'a' and Chl 'b' to total carotenoids indicates the stress experienced by the plant species. The average amount of chlorophyll a to chlorophyll b (a/b) is minimum in *pongamia pinnata*(L) (0.76 mg g^{-1}fw) and maximum in *Azadirachta indica*(L) (1.80 mg g^{-1}fw) (**Table 5**). The total photosynthetic pigments (Chl 'a'+ Chl 'b'+ carotenoid) were found to minimum in polluted sites compared to the control. The average amount of total photosynthetic pigments in polluted plants was less in *Ficus religiosa* (L) (1.37 mg g^{-1}fw) and more in *Azadirachta indica* (L) (2.40 mg g^{-1}fw) compared to all the control plant species. The reduction in total pigments is mostly caused by Chl 'a' and Chl 'b' which have significant value and also the low carotenoidic pigments. The low ratio value indicates the plant species subjected to the dust pollution.

The observed variation in photosynthetic pigment was contributed due to the air pollutant and sensitivity of the plant. The ratio of Chl a+b to carotenodic pigments, (a+b)/c) has extremely low values compared to the control plant species. It

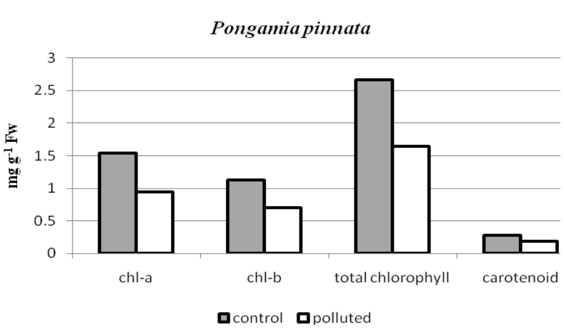

Figure 4. The mean value of chloroplast pigments in Pongamia pinnata

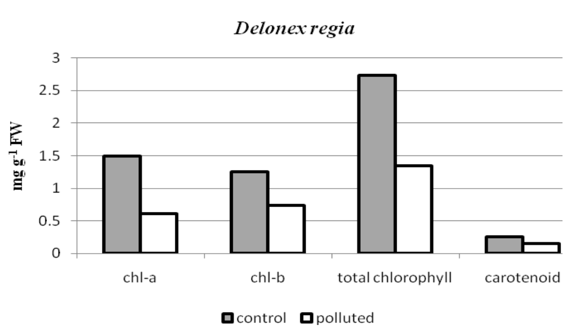

Figure 5. The mean value of chloroplast pigments in Delonex regia

Table 4. The monthly mean values of total carotenoid (mg g^{-1} FW; ± SD) in control and polluted leaves of selected plant species. P(t)- percent of similarity; NS- not significant difference.

Plants	control	Polluted	P(t)
Azhadiracha indica (L)	0.51±0.14	0.33*±0.11	<0.05%
Polyalthia longifolia (L)	0.33±0.12	0.21±0.10	NS
Ficus religiosa (L)	0.32±0.09	0.19±0.05	NS
Pongamia pinnata (L)	0.28±0.11	0.18±0.08	NS
Delonex regia (L)	0.25±0.17	0.15±0.11	NS

in cement dust might be responsible for the reduction in plant species pigments. Traces of toxic metals such as Chromium and Copper are common in some varieties of Portland cement and are harmful to human beings and other living systems (Omar & Jasim, 1990). Cement dust pollution imparts more stress on the plant species. Bio-monitoring of the plants is an important tool to evaluate the impact of cement dust pollutants on pollution.

Table 5. The mean values of assimilating pigments (mg g^{-1}fw) of selected Plant Species

Plant Species	(Chl-a + Chl-b + Ca-rotenoid)		Chlorophyll a/b ratio		Chl (a + b)/Carotenoid	
	Control	Polluted	Control	polluted	Control	polluted
A.indica(L)	3.26	2.40	1.75	1.80	5.98	9.16
Polyalthia longifolia(L)	3.15	2.24	0.92	0.95	11.70	8.88
Ficus religiosa (L)	2.30	1.37	1.43	1.12	8.31	6.78
Pongamia pinnata(L)	2.98	1.50	1.14	0.76	10.68	-27.70
Delonex regia(L)	2.94	1.83	1.28	1.25	17.73	5.31

indicates that the plant species are under stress and also had damage due to cement dust pollution.

CONCLUSION

The Cement dust had a significant effect on the photosynthetic pigments such as Chl'a', Chl'b' and total carotenoid. Plant response varies between plant species of a given genus and between varieties within a given species. Plants do not necessarily showed similar susceptibility to different pollutants. Major variations in response to different species to air pollutants have been documented by Jacobson and Hill (1970). Studies of biochemical changes and pollution effects on the plant metabolism, that is, reduction in chlorophyll and completely clogged stomates (Ahmed & Qadir, 1975) reveals that these parameters are important in regulating the productivity and also the number of flowers and seeds produced. Although all the species showed significant reduction in the photosynthetic pigments, the extent up to which the plant species were affected varied from species to species and days to days. Almost all the species showed maximum variation in photosynthetic pigments (table1 and fig1). The results presented in the paper shows that cement dust pollution significantly reduced the photosynthetic pigments of *Delonix regia* (L) compared to other four species. It is also clear that *Delonix regia* (L) is very sensitive species compared to the other four species.

It is concluded that the presence of toxic pollutants

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URL: http//www.madurai maps of India.