RESEARCH PAPER:

Effect of cement dust on photosynthetic pigments of selected plant species

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SUMMARY

The study aimed to appraise the effect of cement dust pollution on photosynthetic pigments of selected plant species. Changes in the concentration of different photosynthetic pigments like chlorophyll 'a', chlorophyll 'b', total chlorophyll and total carotenoids were determined in the leaves of selected plant species exposed to cement dust pollution. The plant species selected for the study were *Azhadirachta indica*, *Polyalthia longifolia*, *Ficus religiosa*, *Pongamia pinnata* and *Delonex regia*. Reduction in chlorophyll 'a', chlorophyll 'b', total chlorophyll and total carotenoids were recorded in the leaf samples of all selected cement dusted plant species and compared with non-dusted plant species. The data obtained were further analyzed by using two-way ANOVA and also obtained significant changes in all the parameters from the polluted plant species compared with control. There was maximum reduction (43.32%) of chlorophyll 'a' in the leaves of *Ficus religiosa* and minimum (22.92%) reduction in *Azhadirachta indica* while maximum reduction (41.85%) of chlorophyll 'b' was depleted in *Delonex regia* and minimum reduction (25.39%) in *Azhadirachta indica*. The highest reduction (51.81%) in total chlorophyll was observed in *Delonex regia* whereas the lowest reduction (24.67%) was recorded in *Azhadirachta indica*. Similarly, in case of carotenoid contents, highest reduction (65.55%) was observed in *Pongamia pinnata* and lowest in *Ficus religiosa* (29.01%).

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Key Words :

Cement dust, Bio-indicators, Photosynthetic pigments, Chlorophyll, Carotenoid, Assimilating pigments

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Air pollution is a major problem arising mainly from industrialization (Odilara, *et al.*, 2006). Air pollution can directly affect plants via leaves or indirectly via soil acidification (Steubing, *et al.*, 1989). It has also been reported that when exposed to air pollutants, most plant experience physiological changes before exhibiting visual damage to leaves (Dohmen, *et al.*, 1990). Air pollutants, responsible for vegetation injury and crop yield losses, are causing increased concern (Fuji, 1973). Air pollution has become a major threat to the survival of plants in the industrial areas (Gupta and Mishra,1994). Rapid industrialization and addition of the toxic substances to the environment are responsible for altering the ecosystem (Mudd and Kozlowski, 1975: Niragau and Davidson, 1986; Clayton and 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.

The pollutants when absorbed by the leaves that can cause a reduction in the concentration of photosynthetic pigments *viz.,* chlorophyll and carotenoids, which directly affect to the plant productivity. Chlorophyll is the principal photoreceptor in photosynthesis, the light-driven process in which carbon dioxide is "fixed" to yield carbohydrates and oxygen. 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). Furthermore, leaf chlorophyll content is closely related to plant stress and senescence (Hendry *et al.,* 1987, Merziyak and Gitelson 1995, *Peñuelas and Filella* 1998, *Merziyak 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 *Azhadirachta indica*, *Polyalthia longifolia*, *Ficus religiosa*, *Pongamia pinnata* and *Delonex regia*.

EXPERIMENTAL METHODOLOGY

Study area:

Madurai city has 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' north latitude and $78^0$7' east longitude and the city is covering 51.96 sq.kms that comprises of a total population of 25,78,201 persons (Census 2011), whereas the Madurai Urban agglomeration comprising the city and surrounding settlements accommodates a population of 12,03,095 persons. The climate of Madurai town is hot and dry and the temperature ranged between a maximum and minimum of 42^0C and 21^0C, respectively. April and May are the hottest months and rainfall is irregular and intermittent, with an average of approximately 85 cm per annum. The wind blows from northeast direction during January to February and from southwest direction during May to July. The phenomenal growth in population coupled with the growth of vehicles and increasing transport demand have created numerous transportation problems in the city, particularly deterioration of environmental quality, resulting in an increased air pollution and traffic noise (Sivacoumar *et al.,* 2000).

Experimental procedure:

The experiment was conducted in pots under natural conditions. Mature ripe seeds of five plant species like *Azhadirachta indica*, *Polyalthia longifolia*, *Ficus religiosa*, *Pongamia pinnata* and *Delonex regia* 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 colour 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 colour 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 centre of 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 centres 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 per cent acetone. Initially, added 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. Added 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. Poued the extract into a 15ml centrifuge tube and centrifuged in the bench top centrifuged for 3 to 5 min. Removed the extract to a 10ml graduated cylinder using a Pasteur pipette. Transferred an aliquot of the clear leaf extract (supernatant) with a pipette to a 1-cm-pathlength cuvette and took 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 (ìg/ml extract solution).Once the baseline has been run from 700-400nm using acetone in the cuvette, ran 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. Cursor can be used 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 *Azhadirachta indica*, *Polyalthia longifolia*, *Ficus religiosa*, *Pongamia pinnata* and *Delonex regia* 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 A661.6 – 2.04 A644.8
Chl-b (µg/ml) = 20.13 A644.8 – 4.19 A661.6
Carotenoids = (1000 A470 – 1.90 Ca - 63.14 Cb)/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).

Experimental Findings and Discussion

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 *Azhadirachta indica*, *Polyalthia longifolia*, *Ficus religiosa*, *Pongamia pinnata* and *Delonex regia* which is shown in Table 1(Fig. 1).

Interpretation of chlorophyll and carotenoid content:

The concentration of Chlorophyll 'a' and 'b' in plant material can be quantified with different reference systems. Reference systems currently in use include mg Chl a+b/m^2 leaf area (or µg/cm^2 leaf area), µg Chl a+b/g dry weight and mg Chl a+b/g fresh weight (less suitable than dry weight). The weight ratio of Chl 'a' and Chl 'b' (Chl a/b ratio) is an indicator of the functional pigment equipment and light adaptation of photosynthetic apparatus (Lichtenthaler *et al.,* 1981). Chl 'b' is found exclusively in the pigment antenna system, whereas Chl 'a' is present in the reaction centres of photo systems I and II and in the pigment antenna, whereas the light-harvesting pigment protein LHC-I of the photosynthetic pigment system PS I has an a/b ratio of 3, that of LHC-II of PS II exhibits a/b ratio of 1.1 to 1.3. The level of LHC-II of PS II is variable and shows a light adaptation response. Thus, a decrease in the Chl a/b ratio may be interpreted as an enlargement of the antenna system of PS II.

The weight ratio of Chls 'a' and 'b' to total carotenoids (a+b)/(x+c) is an indicator of the greenness of plants. The ratio (a+b)/(x+c) normally lies between 4.2 and 5 in sun leaves and sun-exposed plants, and between 5.5 and 7 in shade leaves and shade-exposed plants. Lower values of the ratio (a+b)/(x+c) are an indicator of senescence, stress, and damage to the plant and photosynthetic apparatus, which is expressed by a faster breakdown of chlorophylls than carotenoids. Leaves become more yellowish-green and exhibit values for (a+b)/(x+c) of 3.5, or even as low as 2.5 to 3.0 as senescence progresses. Also during chromoplost development in ripening fruits or fruit scales, which turn from green to yellow or orange or red, the ratio (a+b)/(x+c) decreases continuously and reaches values below 1.0.

The results obtained with cement-dusted and non-dusted *Azhadirachta indica*, *Polyalthia longifolia*, *Ficus religiosa*, *Pongamia pinnata* and *Delonex regia* were compared (Table 1). In general, plants showed a decrease in photosynthetic pigments due to cement dust treatment. *Azhadirachta indica*, *Polyalthia longifolia, Ficus religiosa*, *Pongamia pinnata* and *Delonex regia* showed the significant ($p<0.01$ and $p<0.05$) reduction in pigment content in the study period of the selected plant species. The relatively reduction in Chl 'a', Chl 'b', total chlorophyll and total carotenoids for the selected species such as *Azhadirachta indica*, *Polyalthia longifolia*

Ficus religiosa, *Pongamia pinnata* and *Delonex regia* were related to cement dust pollution. The following observations were made.

Fig.1 indicates that *Azhadirachta indica* showed 22.92 per cent reduction in the chlorophyll 'a' when exposed to the cement dust pollution. There was 25.39 per cent reduction in chlorophyll 'b' when compared with control and cement dusted plant species. *Azhadirachta indica* showed 42.06 per cent reduction in the total carotenoids. There was 24.6 per cent reduction in the total chlorophyll. Cement dust reduced the photosynthetic pigments such as chlorophyll 'a', chlorophyll 'b', total chlorophyll and total carotenoids. *Polyalthia longifolia* exhibited 27.34 per cent, 26.61 per cent, 61.15 per cent and 28.2 per cent reduction in chlorophyll 'a', chlorophyll 'b', total carotenoids and total chlorophyll, respectively. There was 43.32 per cent reduction in chlorophyll 'a', 32.39 per cent reduction in chlorophyll 'b', 29.01 per cent reduction in total carotenoids and 40.1 per cent reduction in total chlorophyll in the selected species of *Ficus religiosa*, *Pongamia pinnata* exhibited the decreasing trend in the photosynthetic pigments such as 38.78 per cent, 37.65 per cent, 65.55 per cent and 41.2 per cent reduction in chlorophyll 'a', chlorophyll 'b', total carotenoids and total chlorophyll, respectively. *Delonex regia* exhibited the maximum reduction in the photosynthetic pigments such as 37.65 per cent, 41.85 per cent, 38.37 per cent and 51.8 per cent 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* and minimum (22.92%) reduction in *Azhadirachta indica* while maximum reduction (41.85%) of chlorophyll 'b' was depleted in *Delonex regia* and minimum reduction (25.39%) in *Azhadirachta indica*. The highest reduction (51.81%) in total chlorophyll was observed in *Delonex regia* whereas the lowest reduction (24.67%) was recorded in *Azhadirachta indica*. Similarly, in case of carotenoid contents, highest reduction (65.55%) was observed in *Pongamia pinnata* and lowest in *Ficus religiosa* (29.01%). *Polyalthia longifolia* and *Ficus religiosa* showed significant reduction in chlorophyll 'a' ($p<0.01$) and other three species showed the significance level ($p<0.05$%) (Fig. 1).

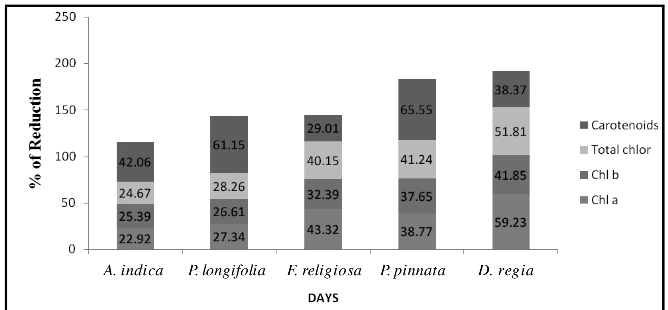

Fig. 1 : Significant reduction of all the photosynthetic pigments in the selected plant species

Total chlorophyll (a+b) was reduced maximum (32.28%) after 90-days but the total carotenoid was reduced to the maximum (67.62%) after 30-days itself. It is inferred that *Azhadirachta indica* showed the maximum reduction of carotenoid pigment after 30-days itself. *Polyalthia longifolia* showed the greater extent of reduction (164.41%) in total carotenoid and 32.61% reduction in total chlorophyll for 60-days, respectively. The highest reduction of total chlorophyll (45.44%) was observed after 90-days in *Ficus religiosa* but the maximum reduction was recorded (59.09%) after 120-days itself. The greater extend of total chlorophyll reduction (50.5%) was after 60-days in *Pongamia pinnata*. Maximum reduction was observed (289.31%) in the total carotenoid after 30-days itself. Likewise, the highest decreasing trend of total chlorophyll was recorded (71.12%) in *Delonex regia* after 30-days, but the total carotenoid was observed highest reduction(177.24%) after 60-days itself.

Table 1 : Variation of different photosynthetic pigments of five selected plant species

Plant species	Chlorophyll A		Chlorophyll B		Total carotenoid		Total chlorophyll	
	Control	Polluted	Control	Polluted	Control	polluted	Control	Polluted
Azhadiracha indica	1.78 ±0.42	1.33* ±0.31	0.97 ±0.20	0.75** ±.017	0.51 ±0.14	0.33* ±0.11	2.76 ±0.62	2.08** ±0.47
Polyalthia longifolia	1.40 ±0.34	1.02** ±0.29	1.43 ±0.16	1.02** ±0.11	0.33 ±0.12	0.21 ns ±0.10	2.83 ±0.49	2.04** ±0.38
Ficus religiosa	1.18 ±0.22	0.64** ±0.14	0.81 ±0.12	0.54** ±0.08	0.32 ±0.09	0.19 ns ±0.05	1.99 ±0.32	1.19** ±0.22
Pongamia pinnata	1.54 ± 0.42	0.94* ±0.35	1.13 ±0.24	0.70* ±0.14	0.28 ±0.11	0.18 ns ±0.08	2.67 ±0.65	1.64* ±0.46
Delonex regia	1.13 ±0.24	0.70* ±0.14	1.25 ±0.29	0.74* ±0.21	0.25 ±0.17	0.15 ns ±0.11	2.74 ±0.64	1.35** ±0.41

* and ** indicate significance of values at $p_{=}$ 0.05 and 0.01, respectively ns $_{=}$ non- significatnt

Total average amount of assimilating pigments (Table 2 and Fig. 2):

Azhadiracta indica:

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 *Azhadirachta indica* compared to the control species of *Azhadirachta indica*. Similarly, the weight ratio of Chl 'a' and Chl 'b' to total carotenoids indicates the stress experienced by the plant species. The ratio said to be decreasing from 30-days to 180-days drastically from 20.96 to 5.24.

Polyalthia longifolia:

The total photosynthetic pigments (Chl 'a'+ Chl 'b'+ carotenoid) were found to be minimum compared to the control plant species. The total photosynthetic pigments were dropped after 120-days extensively compared to the remaining days. The reduction in total pigments is mostly caused by Chl 'a' and Chl 'b' which have significant value and also the low carotenoid pigments. The ratio of Chl 'a' to Chl 'b' from the polluted plant species was very less compared to the control area species. The ratio was said to be between 0.5915 (mg/g fw) to 1.8065 (mg/g fw). The low ratio value indicates the plant species subjected to the dust pollution. The ratio of Chl 'a' and Chl 'b' to carotenoid showed the variation between 30-days to 180 days. Cement dust had a significant negative effect on the photosynthetic pigments. This finding coincides with the results of the earlier studies made (Rauk, 1995). The rather large dust load and its long-term effect have brought about alkalization of the growth environment. This complicates mineral nutrition and misbalances the content of micro- and macro elements in the organisms. Changes in the primary metabolism and mineral nutrition of trees are accompanied by changes in secondary metabolism and growth processes (Mandre, 1995c).

Ficus religiosa:

The ratio of Chl'a' to Chl'b' was found to be minimum compared to the control species. After 120-days, the ratio of Chl'a' and Chl'b' was found to be below 0.95 (mg/gfw). The ratio of Chl'a' and Chl'b' to carotenoid was found to be 7.47(mg/g), 9.44(mg/g), 6.91(mg/g), 5.31(mg/g), 6.38(mg/g) and 5.17(mg/g) compared to the control species 10.23(mg/g) 12.60(mg/g), 11.86(mg/g), 4.91(mg/g), 5.65(mg/g) and 4.67(mg/g), respectively for 30, 60, 90, 120, 150 and 180 days, respectively. The observed variation in photosynthetic pigment was contributed due to the air pollutant and sensitivity of the plant.

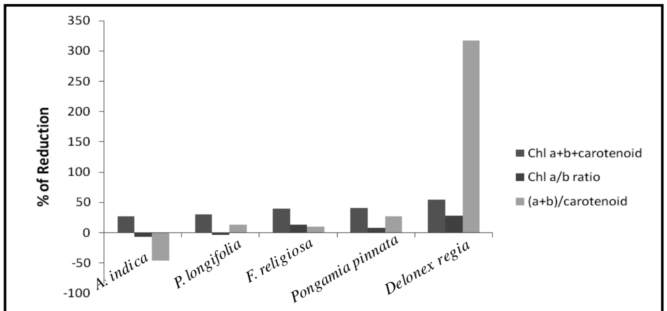

Fig. 2 : Significant reduction of assimilating pigments of selected plant species

Delonex regia:

Total average amount of assimilating pigments (a+b+c) in the control plant leaf was minimum (0.9624 mg/g) in 30-days and maximum (6.8869 mg/g) in 180-days. In the polluted plant leaf, this amount droped significantly from 30-days to 180 days (88.44% to 41.02%) except at 150-days. The total chlorophyll (a+b), carotenoidic pigments, (a+b)/c ratio from the polluted leaves generally has lower values compared to the control.

Pongamia pinnata

Total average amount of assimilating pigments (a+b+c) from the polluted leafs was lower than the control, its minimum reading being 1.2436 mg/g in 30-days. Polluted leaves reduced significantly from 30-days to 150-days (54.43% to 24.94%) except at 180 days. The Chl'a' and Chl'b', a/b ratio from the polluted leaves has values lower than the control leaves except at 30-days. The Chl

Table 2: Variation of assimilating pigments of selected plant species

Plant species	(Chl-a + Chl-b + Carotenoid) %of reduction	Chlorophyll a/b ratio % of reduction	Chl (a + b)/carotenoid % of reduction
Azhadiracta indica	27.18	-7.11	-45.68
Polyalthia longifolia	30.15	-3.97	13.01
Ficus religiosa	39.50	13.41	10.28
Pongamia pinnata	41.25	8.01	27.08
Delonex regia	54.41	27.77	316.97

a+b, carotenodic pigments, (a+b)/c ratio has extremely low values compared to the control plant species. It indicates the plant species were 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 stomata (Ahmed and Qadir, 1975) reveal 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 (Table 1 and Fig. 1). The results presented in the paper showed that cement dust pollution significantly reduced the photosynthetic pigments of *Delonex regia* compared to other four species. It is also clear that *Delonex regia* was very sensitive species compared to the other four species.

It is concluded that the presence of toxic pollutants 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 and 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.

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