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Effect of Cement Dust Pollution on Morphology and Photosynthetic Pigments of Some Legume Plants Grown in Ariyalur District, Tamil Nadu

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Abstract

Keywords

Cement klin dust pollution; Chl a/b ratio; chl/carotenoid ratio, proline, phenols. Air pollution has assumed a mammoth proportion in recent times because of causation of health hazards and loss of economic productivity of crop plants. The present study was carried out to assess the impact of cement klin dust pollution on some selected legume crops viz. Arachis hypogaea, Vigna mungo, V. radiate, Dolichos lablab and Cajanus cajan which are grown around the cement industry. Effect of cement dust on morphology, photosynthetic pigments, proline and total phenol content was studied in the plants collected near the factory site (Polluted) and 15 km away from the factory site (Control). Cement crust formation on leaf surfaces was evident in plants growing in polluted site. The Chlorophyll pigments were reduced in dust-exposed plant species compared with control, wherein a reduction up to 88 % was noted. Of the chlorophyll greater reduction was observed in chl b. The chl a/b ratio, chl/carotenoid ratio was also reduced. Further there was increase in accumulation of the stress indicators proline and phenols. In general, pollution by the cement dust has caused adverse effects on the photosynthetic pigments. Of the selected plants A. hypogaeae was more resistant to dust pollution followed by C. cajan and V. mungo. The decrease in the chlorophyll/carotenoid ratio in stressed plants suggested that these relationships could be used as an indicator of tolerance and physiological status of the plants under cement dust stress conditions.

Introduction

Cement dust pollution although a localized one, has become a serious environmental problem in recent times because of mushroom growth of cement industries in different localities of every state in India. Fallout levels of kiln exhaust dust as well as physical and chemical properties of the exhaust are determined by various factors such as the nature of raw materials, cement manufacturing process and more importantly on the type of equipment employed for the control of particulate matter emissions (Sarala Thambavani and Saravanakumar, 2011). The cement plants erected in several states of India have been reported to affect the

growth and economic productivity of a number of plants and animals. The Tamil Nadu Cements Corporation Limited (TANCEM) at Ariyalur, Tamil Nadu (India), which has been adopted in the present study, is one of the largest cement plants in India. TANCEM is a dry processing unit with an average capacity of 2000 MT/day. The exhaust pollutants like dust and gases are emitted from three stacks. These stacks, being closely located constitute a point source of pollution. The cement dust emitted through smoke stacks are carried over to a radius of 8-10 km from the area of the cement plant, depending on the prevailing wind direction and its

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velocity. The larger particles usually settle near the source, but the smaller ones are blown away to varying distances depending on the wind current. In due course, the smaller particles probably get agglomerated and coaled and these finally settle down on soil surfaces, vegetation and water bodies nearby. The cement dust containing oxides of calcium, potassium and sodium is a common air pollutant affecting plants in various ways. For instance, cement dust and cement crust on leaves plug stomata, interrupt absorption of light as well as diffusion of gases. The dust is also well known to reduce starch formation and thereby a severe reduction in fruit setting. Premature leaf fall has also been reported (Armbrust, 1986).

Another alarming situation is that cement dust has been reported to alter the physico-chemical properties of the soil which generally make the soil quite unsuitable for crop plant growth (Sarala Thambavani and Saravanakumar, 2011; Sadhana et al., 2013). So from the reports cited it is evident that cement kiln exhausts affect not only plant growth and productivity but also the physico-chemical properties of the soil. It may be of greater relevance at this juncture to mention that in developing countries like that of ours springing forth of cement factories cannot be prevented because of greater demand for cement than ever before. Therefore, it becomes imperative that either resistant crop varieties should be evolved or fast growing tree species to shield the dust from falling on annual crop plants should be recommended to reduce severity of pollution. With this view in mind, the present study was carried out to assess the effect of cement dust pollution on growth and biochemical properties of selected legume crops viz. Arachis hypogaea L., Vigna mungo L., V. radiata L. and Cajanus cajan L. with a view of elucidating the changes that are induced in these plants and identify the tolerant species which can be cultivated in cement klin dust polluted sites.

Materials and Methods

Location of study area

The Tamil Nadu Cements Corporation situated at about 70 kms from Trichirapalli city was selected for the above study. The soil is sandy clay in nature and for control studies a similar type of soil situated beyond 15km from the cement factory was chosen. The cement factory adopting a dry process was commissioned during 1979 with a capacity of 2000 MT/day with a stack emission capacity of 1,65, 000 m/hr.

Determination of chloroplast pigments

Morphological observation of the leaves of selected legume plants collected from control and cement dust polluted areas were collected in clean polythene bags, brought to the laboratory and processed suitably for Scanning Electron Microscopy studies (Pathan *et al.*, 2008). Peelings of leaves collected from control and cement dust polluted sites were taken and the changes in the epidermal and stomata were noted by observing under a Cos Lab Photo microscope fitted with Nikon digital camera.

Determination of chloroplast pigments

Healthy leaves with no symptoms of disease were collected randomly from 40 days old selected legume crop plants and washed free of dust particles. The photo synthetic pigment were extracted from leaves in 80% acetone and centrifuged at 3000 rpm for 15 minutes to remove the debris. The volume of clear extract was made up to 100 ml by the addition of 80% acetone and its absorbance at 645 and 663 nm measured with a spectrophotometer. Concentration of chlorophyll a and b were determined using the formulae given by Arnon, (1949).

mg chlorophyll a/g tissue = 12.7 (A663) – 2.69 (A645) \times 1000 \times W / V

mg chlorophyll b/g tissue = 22.9 (A645) – 4.68 (A663) \times 1000 \times W / V

and

mg chlorophyll/g tissue = 20.2 (A645) + 8.02 (A663) $\times 1000 \times W / V$

Where

A = Absorbance at specific wavelengths,

V = Final volume of chlorophyll extract in 80 % acetone, and

W = Fresh weight of tissue extracted

Total carotenoids were determined in the same 80% aqueous acetone extract following the extinction coefficient proposed by Goodwin (1954).

Determination of Free proline

Total free proline was determined in the plant extracts prepared using aqueous sulphosalicylic acid (2%) which was subsequently extracted with the toluene bearing the chrompohore. The methodology followed for determination of free praline was by Bates *et al.* (1973).

Determination of total phenol

Leaf tissue was extracted with 10ml of (80%) ethanol. 1ml of extract was added to1ml of (20%) sodium carbonate and 0.5ml of Folin - phenol reagent and it was kept in a boiling water bath at 100° C for 10 minutes. Total volume was made up to 10ml by adding distilled water and absorbance was read at 650nm.The amount of phenols present in the sample was calculated from a standard curve prepared from catechol (Sadasivam and Manikam, 1992).

Results and Discussion

Morphological changes on leaves

The cement kiln exhaust dust were found to be carried over to a distance of 10 km from the location of the factory. The direction of flow of dust was dependent on the direction of the wind. Larger particles were found to settle within a radius of 3 to 4 km. The larger particles were about 15 micron in size whereas the smaller particles were less than 15 micron. The plants that were growing in the vicinity of the cement plant were found to be smeared with ashy cement kiln dust. Once the dust started accumulating on the plants, in due course it resulted in the formation of a thick crust covering the entire body of the plant including leaves, twigs, flowers and fruits (Fig. 1). The deposition of cement particles were found to occur mainly on the upper surface and were found to clog the stomata (Figs. 2, 3).

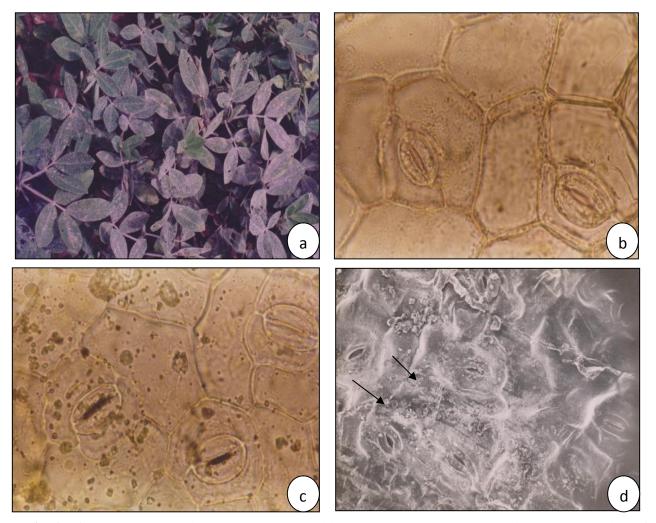


Fig. 1. (a) Photograph showing cement dust deposition on groundnut leaves grown in the polluted site; (b).Microphotograph of peeling of ground nut taken from the control site x 150; (c) Microphotograph of peeling of ground nut taken from the polluted site x 150; (d) SEM photograph showing the crust formation and clogging of stomata (arrow) of groundnut plants collected from the polluted site

Changes in Photosynthetic pigments

The changes in the photosynthetic pigments in plants grown in control and cement dust polluted soils is shown in Table -1. When compared with plants growing far from the factory (control site), dust accumulation altered the chlorophyll and carotenoid contents in all plants in the polluted location (near the factory). Greater decrease in chlorophyll a content was clearly observed in Vigna radiata and Dolichos lablab. (88..20% and 76.62% respectively). With regard to Chl b greater reduction was observed in Cajanus cajan followed by Arachis hypogeae. Among the different chlorophyll, greater reduction was observed in Chl b, wherein a reduction of up to 44% was observed in V. radiata and this followed by D. lablab (53%) and V. mungo (58%). There was not much reduction in the Chl b content of C. cajan.

Our data also indicates that the exposure of plants to dust can alter several physiological and biochemical parameters which are triggered to assist the plant to overcome the stress and induce tolerance. The most apparent effect of stress induced by dust, described in numerous species, is leaf damage (Naidoo and Chirkoot, 2004). Nanos and Ilias. (2007) reported that leaf injury is due to diverse alterations at the sub cellular level. Various studies have shown that the main detrimental effect of dust at the sub cellular level is photo system damage (Santosh and Tripathi, 2008). The results of the present study indicated that dust altered several biochemical aspects, such as photosynthetic pigment in leaves of plants growing near the vicinity of the cement factory.

The reports on the effect of cement dust on chlorophyll pigments are contrary. While many studies have reported a reduction in all the photosynthetic pigments, few reports especially on tree species show an increasing trend. For instance the Chlorophyll a, b, total chlorophyll and chl a/b ratio were significantly increased in *Ficus religiosa* (L) together with increasing dust accumulation in plant leaves near to the factory (Saravana Kuma and Sarala Thambavani, 2012),

Changes in chla/b ratio

A significant increase in chl a/b ratio was observed in *V. radiata* followed by *D. lablab* and *V. mungo*,

which was mainly caused by an decrease in chlorophyll *a* content associated with drastic decrease in chlorophyll *b* content. Our results are consistent with that of Nanos and Ilias, (2007) who reported that cement dust decreased the leaf total chlorophyll content and chlorophyll a/chlorophyll b ratio. As a result of which the photosynthetic rate and quantum yield decreased. The changes in chlorophyll a and b are possibly due to shading and/or photo system damage due to dust accumulation between the petioles or other effects on stomata. Dust from a cement factory seems to cause substantial changes to leaf physiology, possibly leading to reduced plant productivity. The results of the present study indicates the presence of protection mechanism of the chloroplast towards the dust pollution. Lichtenthaler et al., (2000) stated that the increase in the ratio Chl a/bis always associated with a change in pigment composition of the photosynthetic apparatus towards a more sun-type like chloroplast which possesses less light harvesting chlorophyll proteins (LHCPs).

Changes in Carotenoid content

With regard to the carotenoids content, there was significant reduction in the carotenoids content in 3 legumes studied (viz. A. hypogeae, V. mungo and V. radiata) where there was an increase in the carotenoids content in C. cajan and D. lablab (Table -1). Our results also confirms earlier reports that chloroplast of plants growing in polluted site are known to contain higher carotenoid content than their counterparts in non polluted sites, as indicated by the lower values for chlorophylls (a+b) to carotenoids ratios (Table - 1). In addition, the increased carotenoids, in the dust-stressed plants, not only play a role as accessory light harvesting pigments but they also protect the photosynthetic systems against reactive oxygen species (Young, 1991; Asada et al., 1998; Loggini et al., 1999). Moreover, Young, (1991) reported that this response is induced, not only by changes in the irradiation under which the plants are grown, but also by diverse chemicals including cement dust that are often associated with long term stress. Thus the response of studied plants seems to have characteristics in common to dust pollution and are in agreement with the suggestions made for other species by Shinozaki and Shinozaki, (1996) and Tabaeizadeh (1998).

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Name of the	Treatment	Total	Chlorophyll	Chlorophyll	Chl a/b	Carotenoids	Chl/ Cart
Plant	11 catiliciti	Chlorophyll	а	b	ratio	Carotenoius	ratio
Arachis	Control	2.26 ± 0.58	1.34 ± 0.32	0.92 ± 0.28	1.46	0.77 ± 0.15	2.94
hyogaea	Polluted	1.58 ± 0.48	0.98 ± 0.28	0.6 ± 0.18	1.63	0.46 ± 0.13	3.43
Cajanus	Control	4.45 ± 1.24	$2.86\ \pm 0.86$	1.59 ± 0.34	1.80	0.83 ± 0.10	5.36
cajan	Polluted	3.34 ± 1.26	1.74 ± 0.48	1.6 ± 0.35	1.09	1.02 ± 0.58	3.27
Vigna	Control	4.82 ± 1.18	2.12 ± 0.64	2.7 ± 0.56	0.79	0.98 ± 0.23	4.92
radiata	Polluted	3.06 ± 0.98	1.87 ± 0.43	1.19 ± 0.46	1.57	0.78 ± 0.19	3.92
Dolichos	Control	2.87 ± 0.72	1.54 ± 0.60	1.33 ± 0.35	1.16	0.87 ± 0.24	2.56
lablab	Polluted	1.89 ± 0.52	1.18 ± 0.39	0.71 ± 0.18	1.66	1.12 ± 0.36	1.43
Vigna mungo	Control	3.67 ± 0.76	1.86 ± 0.47	1.81 ± 0.42	1.03	1.32 ± 0.53	5.40
	Polluted	2.50 ± 0.88	1.45 ± 0.68	1.05 ± 0.44	1.38	0.68 ± 0.21	2.94

Table – 1. Changes in the photosynthetic pigments in certain legume plants grown in control and cement klin dust
polluted sites. (Mean \pm SD; n=10)

Changes in Proline content

In most of the crop legumes the content of free proline increased under severity of pollution as shown in Figure 2. The increased free proline content under any type of stress including salt, drought and air pollution has been very well documented in literature. *Eichhornia crassipes* exposed to low concentrations of metals for a short period had lower proline contents in their leaves compared to those treated with higher concentrations and a longer period. The differences between the means of the foliar proline contents were statistically significant. For each metal, the proline content increased rapidly with increase in concentration and treatment duration. There was also a significant difference between means of foliar proline when different metals were compared at the same concentration (Odjegba and Fasidi, 2006). Increased foliar proline levels likely acts as an antioxidant in metal and other stressed cells. Proline reduces metalinduced free radical damage and maintains a more reducing environment (higher glutothione levels) in the cell (Siripornadulsil, 2002).

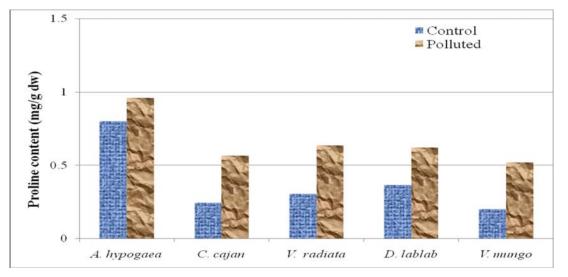


Fig. 2. Effect of Cement dust pollution on Proline content in selected legume plants

Changes in Phenol Content

Increased phenol concentration was observed in plant that were collected from the cement klin dust polluted sites. The increase was more prominent in *A. hypogeae* and *D. lablab* and lesser in *V. mungo* (Fig. 3). The increase in phenol content and its role in providing resistance to pathogen and oxidative stress are well documented (Maletsika *et al.*, 2015). The increase in phenol content may be due to the increase in the oxidative enzymes like polyphenol oxidae in plants that turn more active when plant is subjected to any stress. In this case it is the stress developed by the presence of various kinds of pollutants in the ambient air (Uma *et al.*, 2003).

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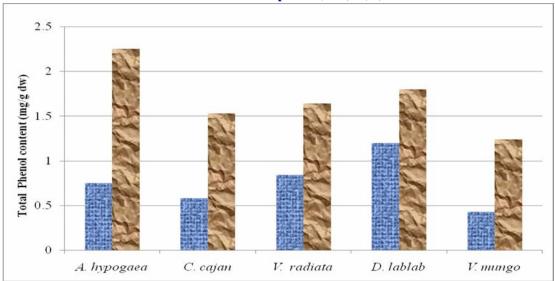


Fig. 3. Effect of Cement dust pollution on Total phenol content in selected legume plants

Conclusion

This study indicates that the total chlorophyll in control plants was always higher than that of the plants grown in cement klin exhaust dust polluted soil. It also revealed, that the exposure to particulate deposition may alter plant growth and their production even though there may or may not be any marked physical damage to the plant. The reduction in yield may be attributed to the reduction in the photosynthetic pigments especially chlorophyll a and the deposition of cement dust which leads to the clogging of stomata which also interferes with gaseous exchange. Of the five legume plants tested in the present study A. hypogaeae was more resistant to dust pollution followed by C. cajan and V. mungo. The decrease in the chlorophyll/carotenoid ratio in stressed plants suggested that these relationships could be used as an indicator of tolerance and physiological status of the plants under cement dust stress conditions.

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