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Estimation of amino acid, protein, proline and sugars in *Vigna mungo* L. exposed to cement dust pollution

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Abstract

Estimation of amino acid, protein, proline and sugars in *Vigna mungo* L. exposed to cement dust. Field experiment was conducted with blackgram var. Vamban 3. The different levels (5, 10, 15 and 20 g m^{-2} day^{-1}) of the cement dust were dusted by foliar application. pollution.These parameters decreased with increasing level of cement dust. But, the content of amino acid and proline increased with the increasing levels of cement dust. Cement dust as significant change on the biochemical content.

Keywords: *Vigna mungo* L, Cement Dust Pollution, Proline, Amino Acid and Urbanization.

Introduction

Soil is nature's gift to nurture the plants, which intern nourishes the biotic community thus ecosystem. Now a day's this soil as become pool for various, toxins, syntactic non degradable chemicals, heavy metals etc. Soil has been polluted in all possible means, polluted water being reservoirs of chemicals heavy metals let this chemical percolate into the soil. Polluted air caring all the toxin dust depositing on the soil, Human population explosion, rapid industrialization, increased deforestation, unplanned urbanization, scientific and technological advancement etc. have further hyped all kinds of pollution (Narendra Kuppan, 2012).

Now a day, cement industry caused environmental pollution problems; the pollutants of the cement industry produced the adverse impact on Air, Water and Land. Cement industry is the one of the 17 most polluting industries listed by Central Pollution Control Board. During the last decades, the emission of dust from cement factories has been increased alarmingly due to expansion of more cement plants to meet the requirement of cement materials for construction of building. In comparison with gaseous air pollutants, many of which are readily recognized as being the cause of injure to various types of vegetation. Relatively little known and limited studies have been carried out on the effect of cement dust pollution on the growth of plants.

Cement contains 3-8% aluminium oxide, 0.5-0.6% iron-oxide, 60-70% calcium oxide, 17-25% silicon oxide, 0.1-4% magnesium oxide and 1-3% sulphur trioxide (Ade-Ademilua and Umebese, 2007). The pH of the cement-polluted soils was alkaline but that of the polluted soil was more alkaline. Similar studies on cement dust pollution show elevated levels of soil pH (Mandre et al., 1998). Cement dust is a mixture of Ca, K, Si and Na which often include heavy metals like As, Al, Cd, Pb, Zn, Fe, and Cr. Majority of these elements in excess amounts are potentially harmful to the biotic and abiotic components of the environment (Gbadebe and Bankole, 2007). Likewise, increased concentration of cement dust pollutants causes invisible injuries like progressive decline in the physiological process such as photosynthetic ability and respiration rate of leaves. Similarly, visible injuries such as closure leaf stomata, a marked reduction in growth and productivity were observed due to cement dust. Farmer (1993) reported that cement dust pollutants block the stomata, reduction in number of annual crops. (D. Raajasubramanian *et al 2011*)also reported that due to cement dust decreased the productivity and concentration of chlorophyll in a number of crops. Air is one of the five basic natural ingredients of life system and air quality affects our way of life. Substances introduced into the air by the activity of mankind in such concentrations sufficient to cause serious effects on health, vegetation, property or interference with the enjoyment of his property is defined as air pollution (Senthilnathan and Raju, 2003).

Materials and Methods

Seed material

The seeds of black gram (*Vigna mungo* L. Hepper var. Vamban 3) were procured from National Pulse Research Station, Regional Research Station of Tamil Nadu Agricultural University located at Vamban, Pudukkottai district, Tamil Nadu, India. The healthy seeds were chosen and used for both laboratory and field experiments.

Estimation of amino acid (Moore and Stein, 1948)

Extraction

Five hundred mg of plant materials were weighed and macerated with a pestle and mortar with 10 ml of 80 per cent ethanol. The homogenate was centrifuged for 10 minutes at 800 rpm. The supernatant was saved. The extract was used for the estimation of amino acids.

Estimation

One ml of the extract was pipetted out into a test tube.A drop of methyl red indicator was added. The sample was neutralized with 1 ml of 0.1 N sodium hydroxide. To this, 1 ml of ninhydrin reagent was added and mixed thoroughly. The content of the test tube was heated for 20 minutes in a boiling water bath. Five ml of the diluent solution was added and heated in water bath for 10 minutes. The tubes were cooled under the running water and the contents were mixed thoroughly. Blank was prepared without extract. The absorbance was read at 570 nm in a UV-Spectrophotometer (Hitachi U-2900). The amino acid contents are expressed in mg g^{-1}frwt basis.

Ninhydrin reagent

Eight hundred mg of hydrated stannous chloride was dissolved in 500 ml of citrate buffer at pH 5.0 and 20 g of recrystallized ninhydrin was dissolved in 500 ml of methyl cellosolve. Then these two solutions were mixed.

Estimation of protein (Lowry *et al.*, 1951)

Extraction

Five hundred mg of plant materials were weighed and macerated in a pestle and mortar with 10 ml of 20 per cent trichloroacetic acid. The homogenate was centrifuged for 15 min at 600 rpm. The supernatant was discarded. To the pellet, 5 ml of 0.1 N NaOH was added and centrifuged for 5 min. The supernatant was saved and made upto 10 ml with 0.1 N NaOH. This extract was used for the estimation of protein.

Estimation

One ml of the extract was taken in a 10 ml test tube and 5 ml of reagent 'C' was added. The solution was mixed and kept in darkness for 10 min. Later, 0.5 ml of folin-phenol reagent was added and the mixture was kept in dark for 30 minutes. The sample was read at 660 nm in the UV-Spectrophotometer (Hitachi U-2900). The protein contents are expressed in mg g^{-1}frwt basis.

Preparation of reagents

Reagent A: 0.4 g of sodium hydroxide was dissolved in 100 ml of distilled water. To this solution, 2 g of sodium carbonate was added.

Reagent B: One per cent of copper sulphate was mixed with equal volume of 2 per cent sodium potassium tartarate.

Reagent C: Fifty ml of reagent A and 1 ml of reagent B were taken and mixed and it was prepared freshly at the time of experiment.

Folin-phenol reagent: One ml of folin-phenol reagent was diluted with 2 ml of distilled water.

Proline (Bates *et al*., 1973)

Extraction

Five hundred mg of plant material was taken in a pestle and mortar and homogenized with 10 ml of 3 per cent aqueous sulfosalicylic acid. Then, the homogenate was filtered through whatman No. 2 filter paper. The residue was re-extracted two times with 3 per cent sulfosalicylic acid and pooled. The filtrates were made upto 20 ml with 3 per cent sulfosalicylic acid and used for the estimation of proline.

Estimation

Two ml of extract was taken in a test tube and 2 ml of acid ninhydrin reagent and 2 ml of glacial acetic acid were added to it. The mixture was incubated for an hour at 100`C in a water bath. The tubes were transferred to an ice bath to terminate the reaction. Then, to each test tube, 4 ml of toluene was added and mixed vigorously using a test tube and stirred for 10-20 seconds. The toluene containing the chromophore was separated from the aqueous phase with the help of separating funnel and the absorbance was measured at 520 nm in a UV- Spectrophotometer (Hitachi U-2900) using an appropriate blank. The proline content was determined from a standard curve prepared with proline and the results are expressed in mg g^{-1}frwt basis.

Estimation of sugars (Nelson, 1944)

Extraction

Five hundred mg of plant materials were weighed and macerated in a pestle and mortar with 10 ml of 80 per cent ethanol. The homogenate was centrifuged for 10 minutes at 800 rpm. The supernatant was saved. Then, the ethanol was evaporated in a water bath at 50 °C. The net content was made upto 20 ml with distilled water and the extract was used for the estimation of reducing sugar.

Estimation

One ml of extract was taken in a 25 ml marked test tube. 1 ml of reagent 'C' was added. Then, the mixture was heated for 20 min at 100 °C in a boiling water bath, cooled and 1 ml of arsenomolybdate reagent was added. The solution was thoroughly mixed and diluted to 25 ml with distilled water. The sample was read in a UV-Spectrophotometer (Hitachi U-2900)at 520 nm. The sugar contents are expressed in mg g^{-1}frwt basis.

Total sugar

Preparation of reagents

Reagent A: Twenty five grams of anhydrous sodium carbonate, 25 g of sodium potassium tartarate, 20 g of sodium bicarbonate and 200 g of anhydrous sodium sulphate were dissolved in 800 ml of distilled water and made upto 1000 ml. Then, it was filtered and stored in a glass stoppered brown bottle.

Reagent B: Fifteen per cent copper sulphate containing 1 or 2 drops of concentrated sulphuric acid.

Reagent C: Fifty ml of reagent A and one ml of reagent B were mixed well and it was prepared freshly at the time of experiment.

Arsenomolybdate reagent: To 450 ml of distilled water, 25 g of ammonium molybdate, 21 ml of concentrated sulphuric acid were added and 3 g of sodium arsenate was dissolved in 25 ml of distilled water. The mixture was kept in a water bath at 37 °C for 24 to 48 hrs. The reagent was stored in a glass stoppered brown bottle.

Non-reducing sugars (Nelson, 1944)

Non-reducing sugars present in the ethanol extracts (extraction as in reducing sugars) were hydrolysed with sulphuric acid to reducing sugars. Reducing sugars present in the hydrolysates were estimated following Nelson's method. The differences between the total sugars and the reducing sugars correspond to the non-reducing sugars.

Hydrolysis

One ml of extract was taken in a test tube and evaporated to dryness in a water bath for 15 minutes. To the residue, 1 ml of distilled water and 1 ml of 0.1 N sulphuricacid were added. The mixture was hydrolysed by incubating at 49 °C for 30 min in a thermostat. The solution was neutralized with 0.1 N NaOH (5 ml) and the methyl red as indicator. To this, 1 ml of reagent C was added and heated for 20 minutes, cooled and 1 ml of arsenomolybdate reagent was added. The content was made upto 25 ml and the absorbance was read at 495 nm in a UV-Spectrophotometer (Hitachi U-2900). The reducing sugar contents are expressed in mg g^{-1}frwt basis. Blank was prepared with 1 ml of distilled water.

Field preparation

The field was thoroughly ploughed three times before sowing. The entire field was irrigated with bore well water for two days before sowing. Blackgram seeds were sown with a spacing of 20 × 20 cm. Field management were employed under normal agronomical practices.

Cement dust treatment

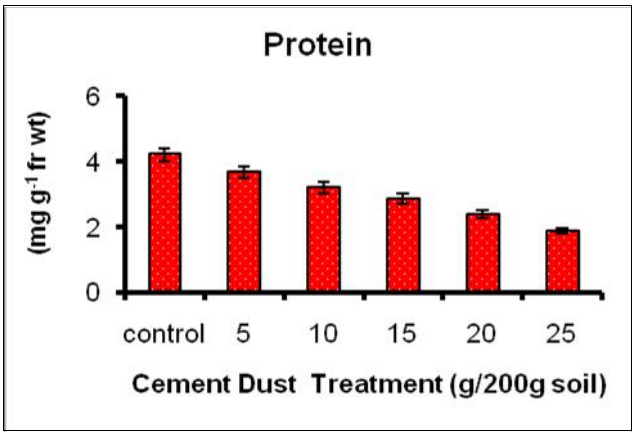
Different amounts (5, 10, 15, and 20 g m^{-2} day^{-1}) of cement dust application were done daily on the aerial parts of the experimental crops. The crops grown without cement dust were treated as control.

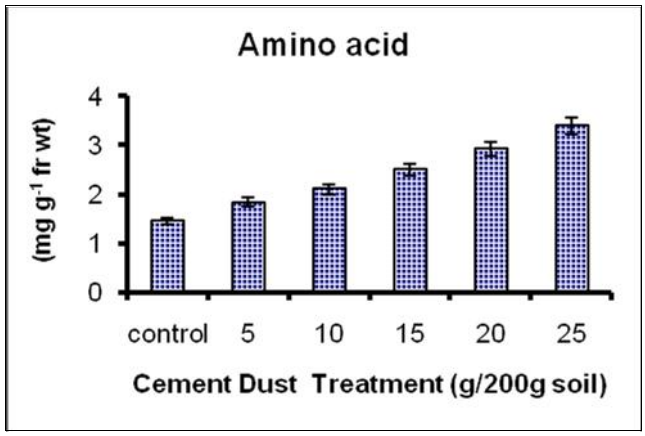
Protein, proline and sugars in both root and shoot were estimated and recorded at 15, 30, 45 and 60 DAS. The methods of extraction and estimation were mentioned earlier in the studies.

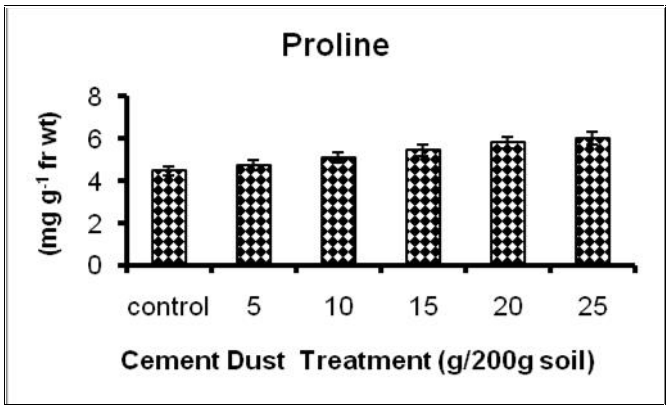
Results

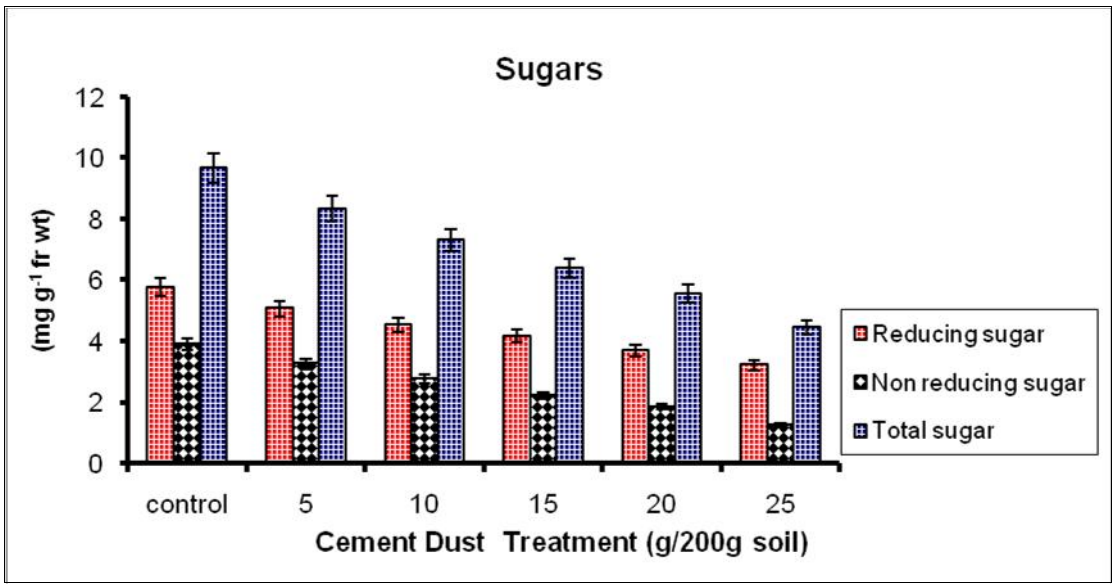
The impact of cement dust on biochemical contents of black gram seedling is presented in Figures1, and are expressed in mg g^{-1} frwt basis. The highest protein (4.211 mg g^{-1} frwt), amino acid (1.454 mg g^{-1} frwt), reducing sugar (5.773 mg g^{-1} frwt), non- reducing sugar (3.916 mg g^{-1} frwt) and total sugar (9.689 mg g^{-1} frwt). The lowest content of proline (4.464 mg g^{-1} frwt) was recorded in control plants. The lowest amino acid (3.403 mg g^{-1} frwt), reducing sugar (3.225 mg g^{-1} frwt), non- reducing sugar (1.245 mg g^{-1} frwt) and total sugar (4.47 mg g^{-1} frwt) were recorded in the seedlings grown in 25 g of cement dust mixed with 200 g of soil. The highest content of proline (5.998 mg g^{-1} frwt) was also recorded in the seedling grown in cement dust mixed soil.

Fig. 1: Effect of cement dust on biochemical content (protein, amino acid, proline, reducing sugar, non-reducing sugar and total sugar (mg g^{-1} frwt) of black gram seedlings.





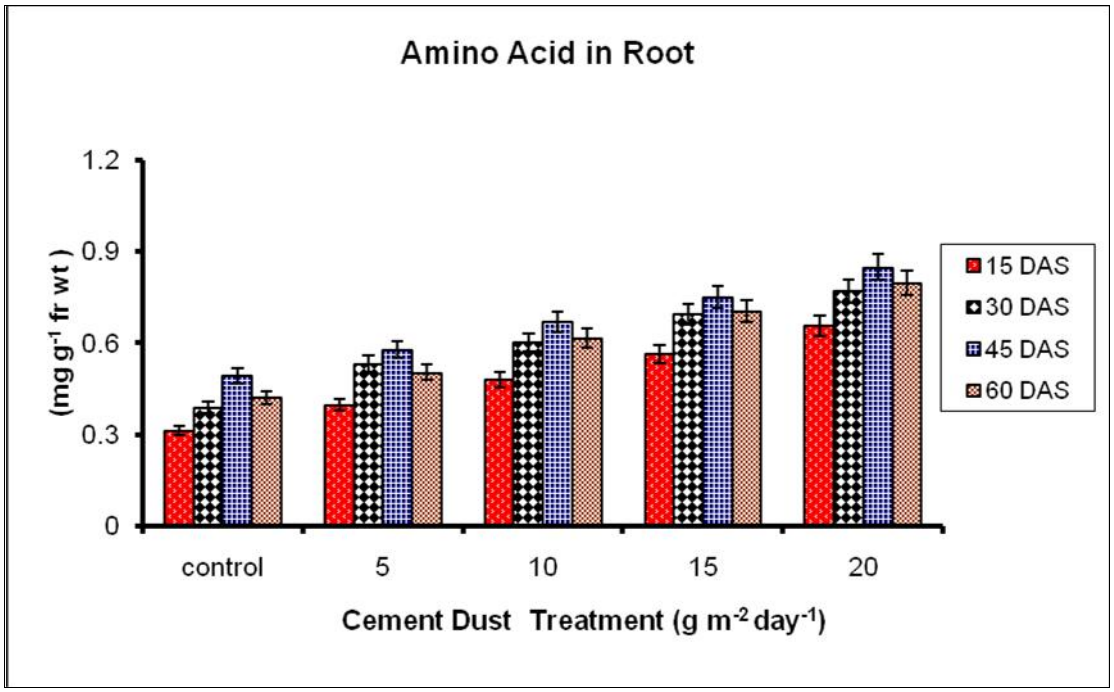


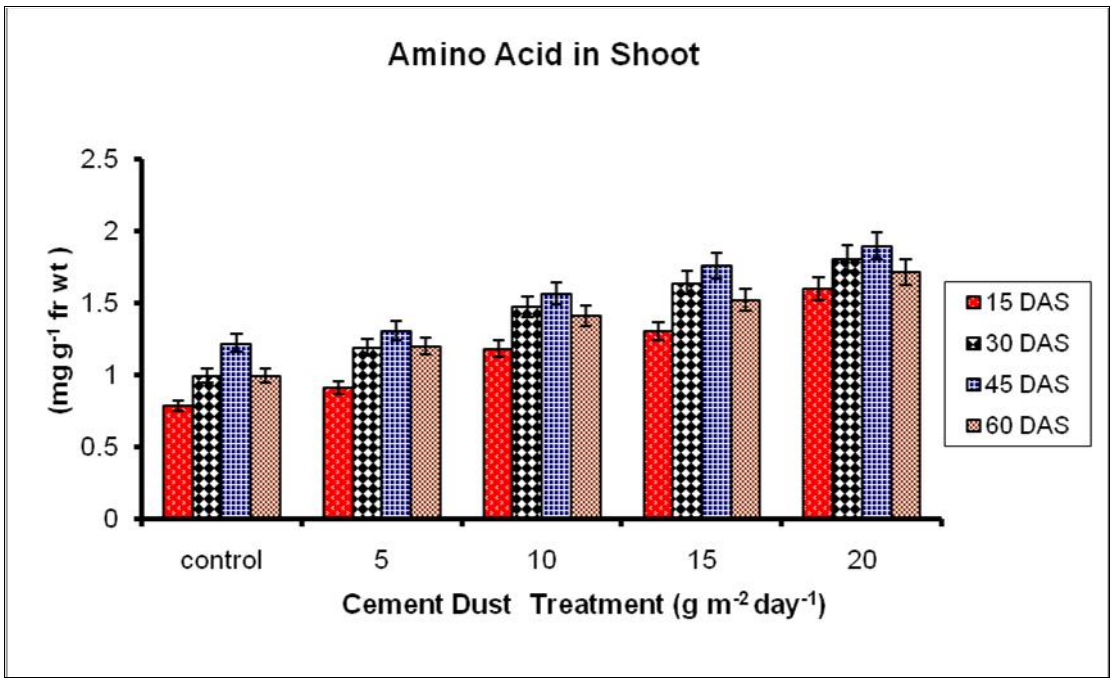


Amino acid

The effect of various amounts of cement dust on amino acid contents (mg g^{-1}frwt) of black gram root and shoot was recorded at 15, 30, 45 and 60 DAS of its growth and it was shown in Figure 2. The highest amount of amino acid content of root (0.657, 0.771, 0.848 and 0.798 mg g^{-1}frwt) and shoot (1.597, 1.808, 1.892 and 1.712 mg g^{-1} frwt) was recorded in 20 g m^{-2} day^{-1} cement dusted plant at 15, 30, 45 and 60 DAS respectively. The lowest amino acid content of root (0.313, 0.389, 0.492 and 0.421 mg g^{-1}frwt) and shoot (0.784, 0.991, 1.219 and 0.994 mg g^{-1}frwt) was recorded in control plants at 15, 30, 45 and 60 DAS respectively.

Fig. 2: Effect of cement dust on amino acid content (mg g^{-1}frwt) of black gram at various stages of its growth.

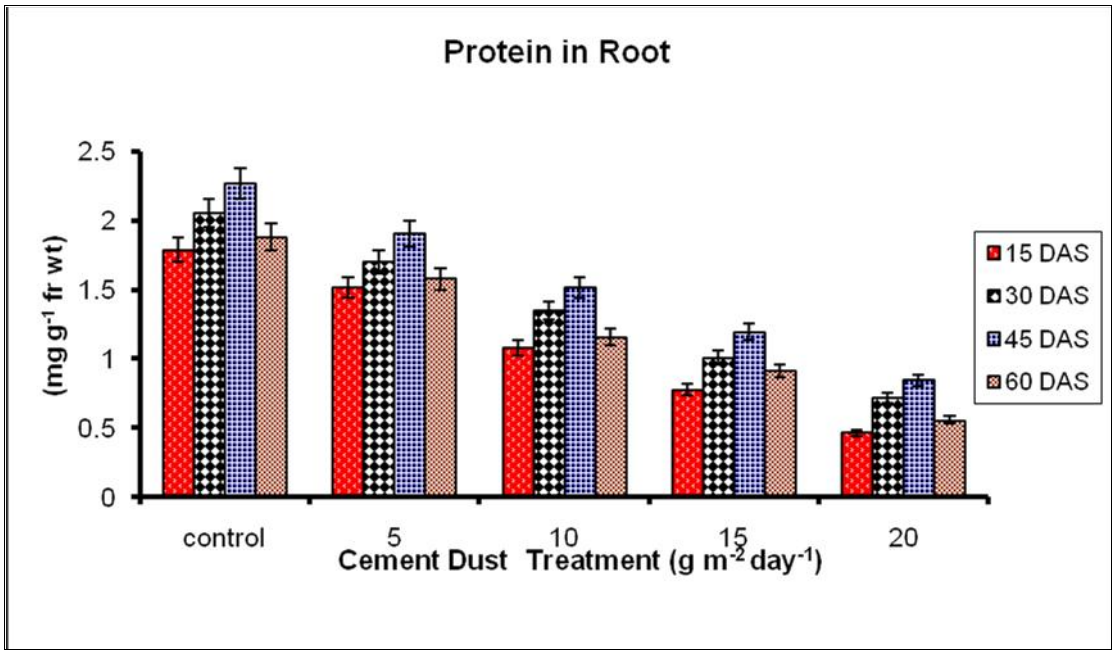


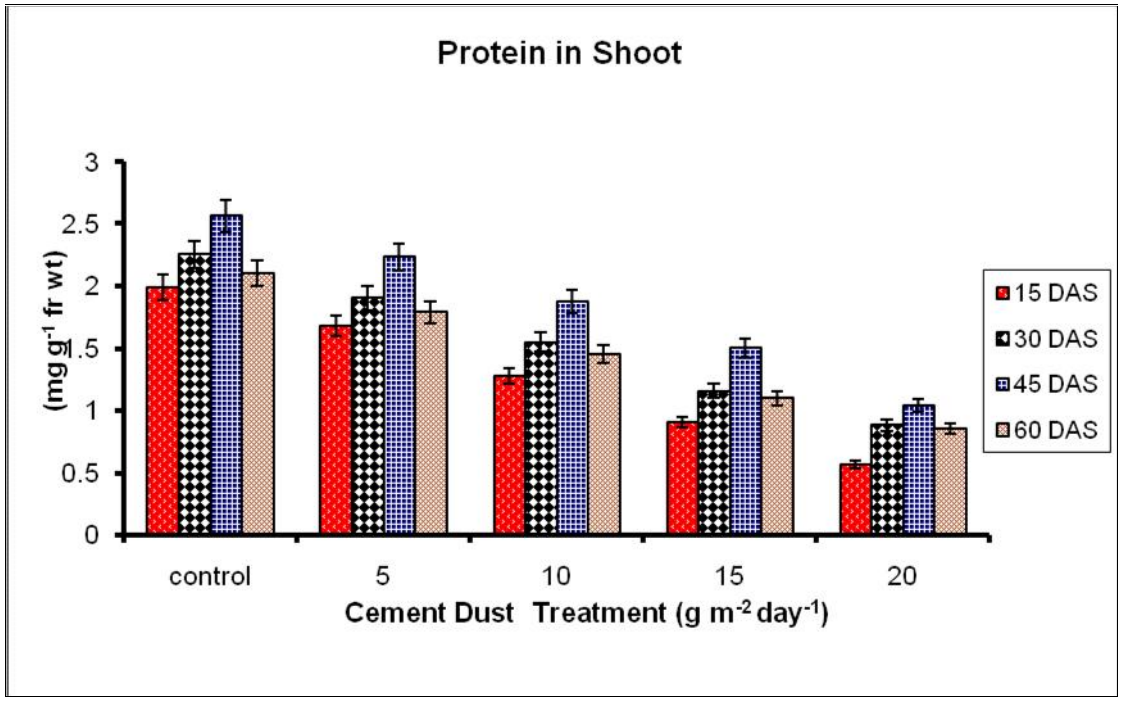


Protein

The influence of various amounts of cement dust on protein content (mg g^{-1}frwt) of black gram root and shoot was shown Figure 3. The highest protein content of root (1.789, 2.053, 2.267 and 1.881 mg g^{-1}frwt) and shoot (1.989, 2.253, 2.567 and 2.101 mg g^{-1}frwt) of black gram was recorded in 15, 30, 45 and 60 DAS of control plants respectively. The lowest amount of protein content of root (0.465, 0.719, 0.843 and 0.554 mg g^{-1}frwt) and shoot (0.565, 0.879, 1.043 and 0.854 mg g^{-1}frwt) were recorded in 20 g m^{-2} day^{-1} cement dusted plants at 15, 30, 45 and 60 DAS respectively.

Fig.3: Effect of cement dust on protein content (mg g^{-1} frwt) of black gram at various stages of its growth.

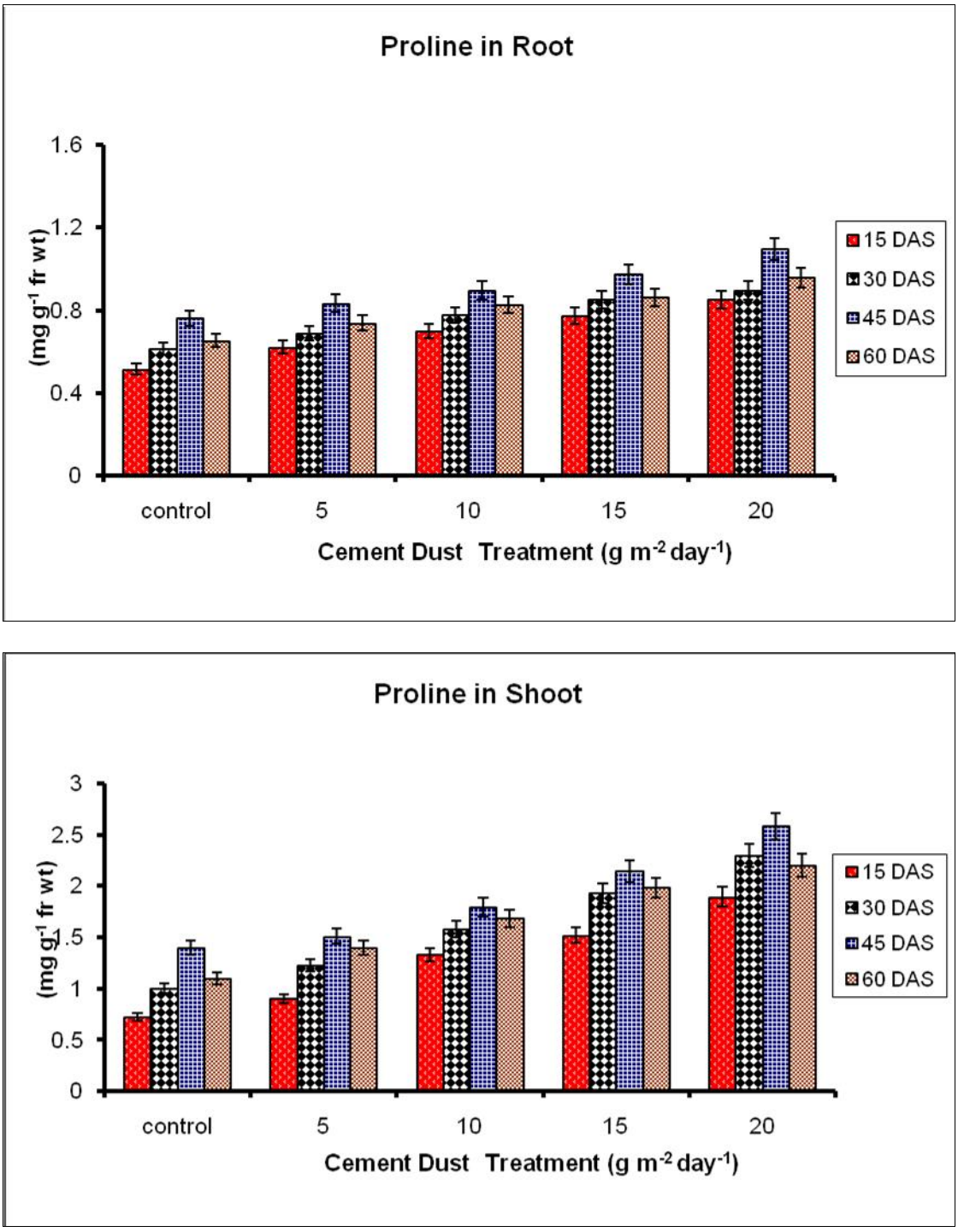




Proline

The effect of different amounts of cement dust on proline content (mg g^{-1}frwt) of blackgram root and shoot was given in Figure 4. The lowest amount of proline content of root (0.513, 0.613, 0.759 and 0.651 mg g^{-1}frwt) and shoot (0.723, 0.997, 1.399 and 1.097 mg g^{-1}frwt) was recorded in control plants of 15, 30, 45 and 60 DAS in control plants respectively. The highest proline content of root (0.851, 0.892, 1.093 and 0. 0.956 mg g^{-1}frwt) and shoot (1.892, 2.297, 2.582 and 2.199 mg g^{-1}frwt) were recorded in 20 g m^{-2} day^{-1} concentration of cement dusted plants at 15, 30, 45 and 60 DAS respectively.

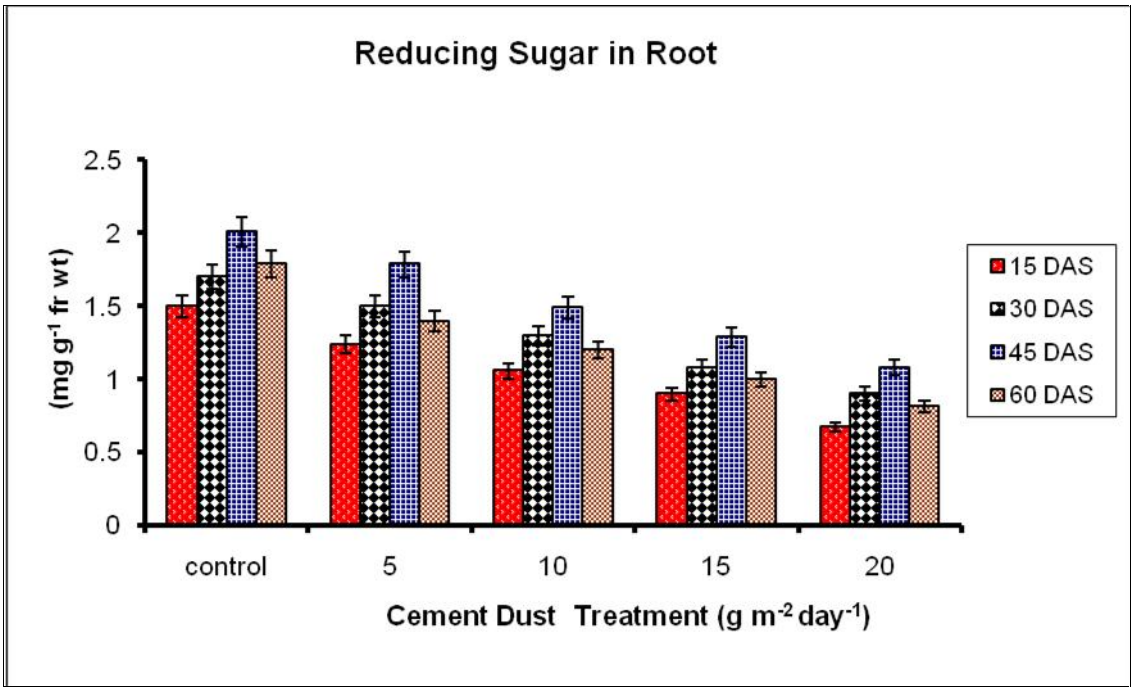
Fig. 4: Effect of cement dust on proline content (mg g^{-1}frwt) of black gram at various stages of its growth.

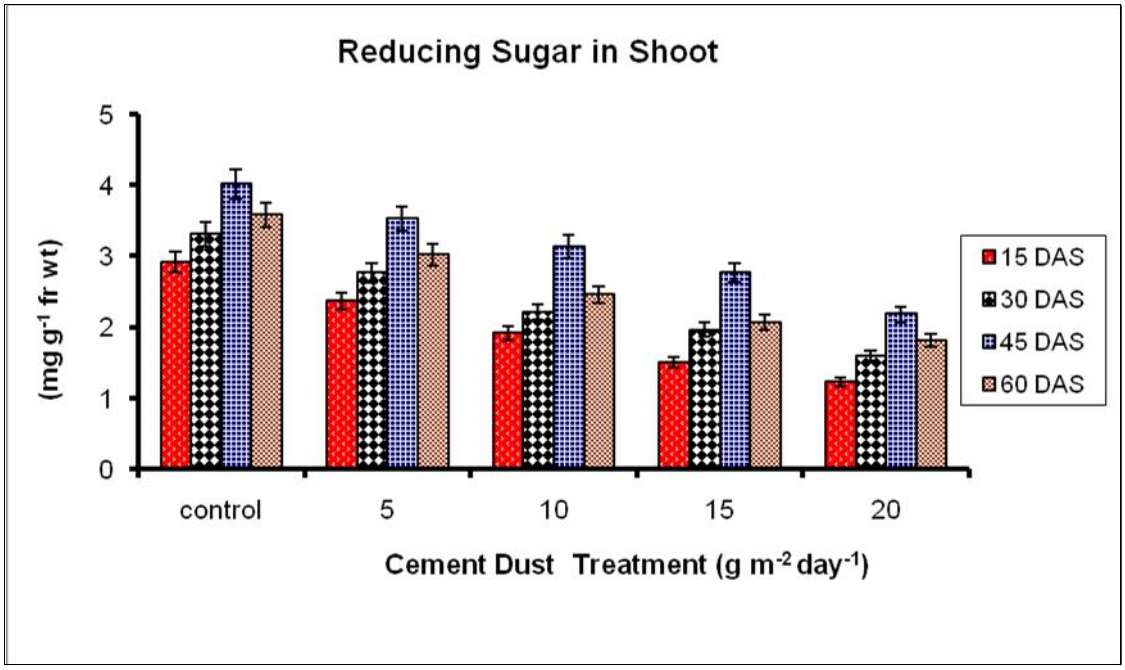


Reducing sugar

The impact of different amounts of cement dust on reducing sugar contents (mg g^{-1}frwt) in root and shoot of black gram at 15, 30, 45 and 60 DAS was given in Figure 5. The highest reducing sugar content of root (1.497, 1.702, 2.006 and 1.788 mg g^{-1}frwt) and shoot (2.913, 3.313, 4.017 and 3.581mg g^{-1}frwt) was recorded in control plants at 15, 30, 45 and 60 DAS respectively. The lowest reducing sugar content of root (0.671, 0.901, 1.081and 0.813 mg g^{-1}frwt) and shoot (1.232, 1.599, 2.185 and 1.811 mg g^{-1}frwt) was observed in 20 g m^{-2} day^{-1} of cement dusted plants at 15, 30, 45 and 60 DAS respectively.

Fig. 5: Effect of cement dust on reducing sugar content (mg g^{-1}frwt) of black gram at various stages of its growth.

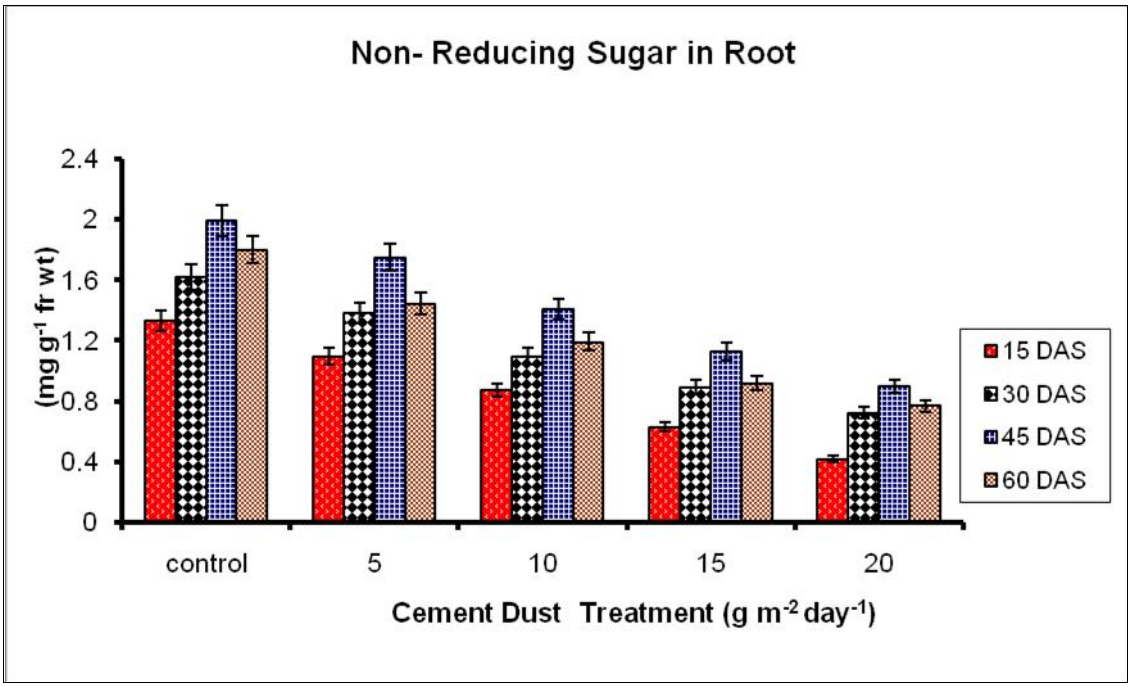


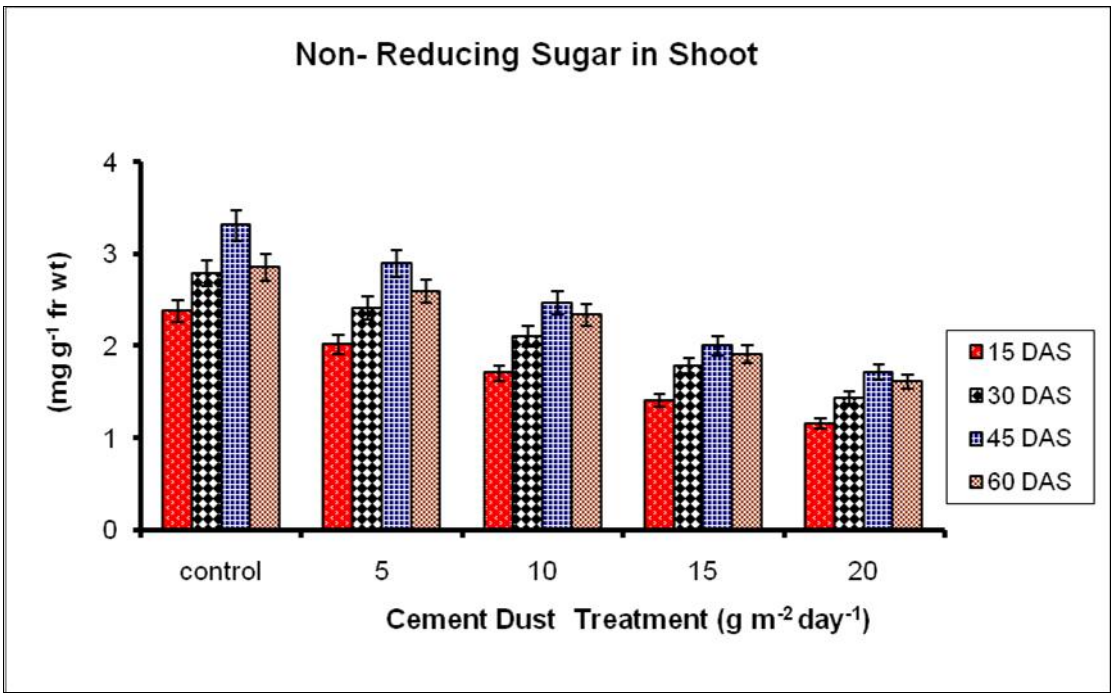


Non-reducing sugar

The influence of various amounts of cement dust on non-reducing sugar contents (mg g^{-1}frwt) of black gram root and shoot in 15, 30, 45 and 60 DAS was given in Figure 6.The highest non - reducing sugar content in root (1.331, 1.619, 1.991 and 1.798 mg g^{-1}frwt) and shoot (2.376, 2.789, 3.311 and 2.854 mg g^{-1}frwt) was recorded in control plants at 15, 30, 45 and 60 DAS of growth respectively. The lowest amount of non-reducing sugar contents in root (0.421, 0.725, 0.897 and 0.769 mg g^{-1}frwt) and shoot (1.155, 1.437, 1.711and 1.609 mg g^{-1}frwt) was observed in 20 g m^{-2} day^{-1} cement dusted plants at 15, 30, 45 and 60 DAS respectively.

Fig. 6: Effect of cement dust on non- reducing sugar content (mg g^{-1}frwt) of black gram at various stages of its growth.

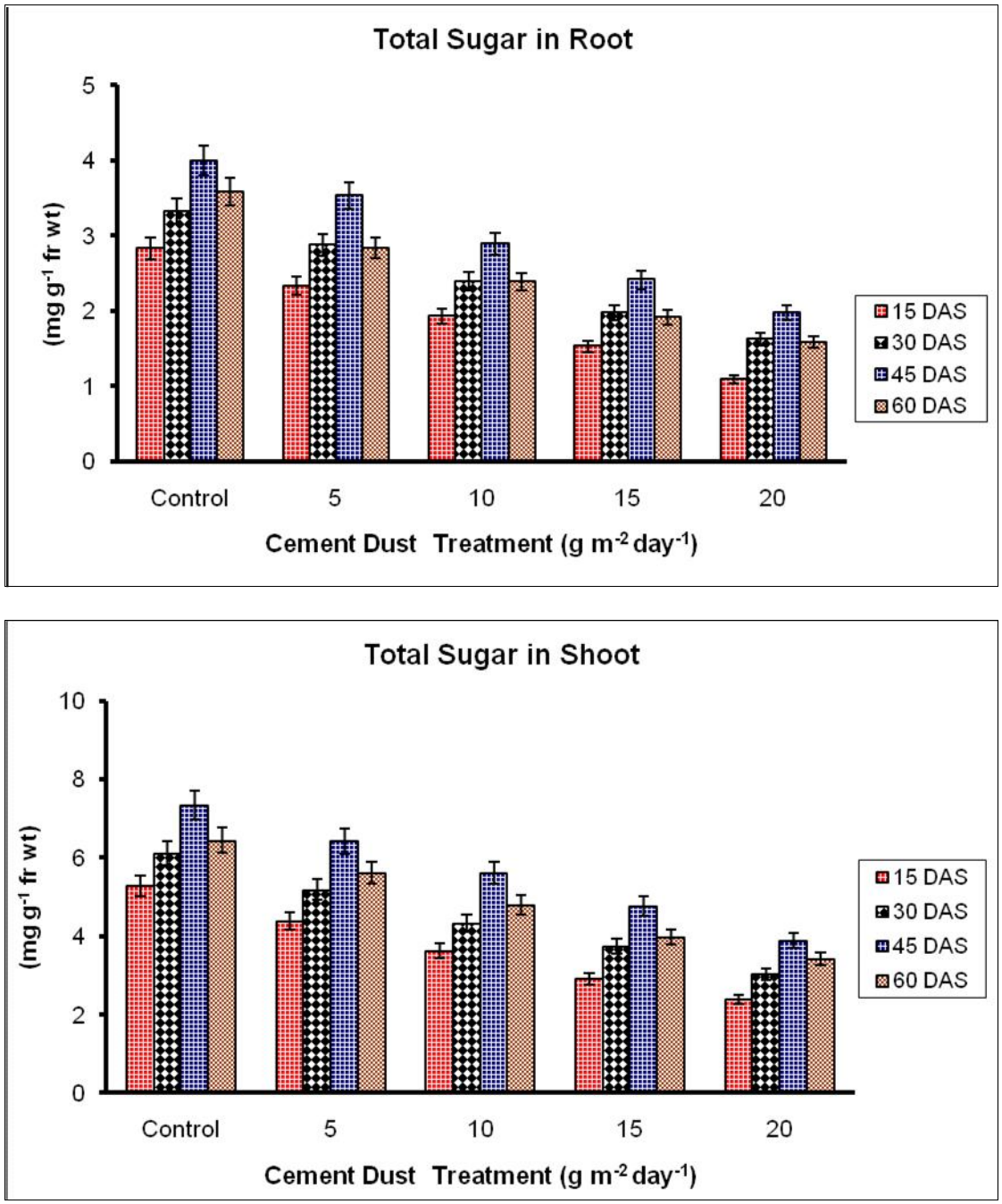




Total sugar

The effect of different amounts of cement dust on total sugar content (mg g^{-1}frwt) of black gram root and shoot was given in Figure 7. The highest total sugar content in root (2.828, 3.321, 3.997 and 3.586 mg g^{-1}frwt) and shoot (5.289, 6.102, 7.328 and 6.435 mg g^{-1}frwt) was noted in control plants of 15, 30, 45 and 60 DAS respectively. The lowest total sugar content of root (1.092, 1.626, 1.978 and 1.582 mg g^{-1}frwt) and shoot (2.387, 3.036, 3.896 and 3.42 mg g^{-1}frwt) was observed in 20 g m^{-2} day^{-1} cement dusted plants at 15, 30, 45 and 60 DAS respectively.

Fig. 7: Effect of cement dust on total sugar content (mg g^{-1}frwt) of black gram at various stages of its growth.



Discussion

Nature is wonderful engineer and its system works flawlessly until and unless we indulge we use, utilize nature in recent times we have been exploiting nature rather than using it. Air pollution has become a serious environmental problem in recent years due to rapid growth of cement factories, thermal power stations, and steel and coal industries. It is physical, chemical and biological agents that modify the natural characteristics of the atmosphere. Air pollution is responsible for vegetation injury, crop yield loss and has become a major threat to the survival of plants in industrial area (Raajasubramanian *et al*. (2015).

Amino acid

Amino acid is the monomer of protein, the common reserve food material manufactured by plant system. Increases in amino acid content of black gram crop with increasing amount of cement dust were recorded. Similar trend of increase in amino acid content was already reported in *Psidium guajava* (Lal and Ambasat, 1982), *Brassica juncea* and *Crotalaria juncea* (Uma and Rao, 1993 and Uma *et al*., 1994) and *Hibiscus cannabinus* (Uma and Rao, 1996). Prasad *et al*. (1991) reported that the increasing level of amino acid in *Cajanus cajan* due to cement dust. The increasing trend of amino acid was also reported in some legumes (Saralabai and Vivekanandan, 1995) and some tree species such as *Mangifera indica, Cassia fistula* and *Eucalyptus* (Tripathi and Mukesh Gautam, 2007) exposed to air pollution. Similar trend of amino acid content in groundnut was reported by Raajasubramanian *et al*. (2011) due to cement dust pollution. The increase in amino acid content could be attributed to the production of sulphur containing amino acids like cysteine and methionine or hydrolysis of proteins as a response to the pollutant exposure (Krishnamurthy *et al*., 1994). The changes in the amino acid concentration could be due to the readjustment of metabolic potentialities of the cell to achieve a mechanism of stabilization of the cellular pH, through an increase in organic bases- alkaline amino acids, polyamines (Pierre and Queoroz, 1981).

Proline

Plants accumulate several kinds of osmolytes such as proline, glycine betaine and soluble sugars under stress condition. Proline has been implicated as anti-stress organic molecule, in some higher plants (Greenway and Munns, 1980) and it is known to accumulate in response to environmental stress (Aspinall and Paleg, 1981). In our study, proline content of black gram grown in cement dust polluted condition was estimated and they were found to be increased with the increasing concentrations of cement dust. It is quite interesting to note that the crop plants grown in cement dust polluted environment showed a gradual increase in the level of free proline. It has also been reported that the plants grown under stress condition exhibit a remarkable increase in proline content in some legumes (Saralabai and Vivekanandan, 1995). Proline accumulation was normally observed during stress condition A considerable increases in proline content in halophytic species was also recorded under direct exposure of cement dust.

Increased accumulation of proline is to maintain intercellular osmoticum during stress condition. The higher magnitude of proline accumulation may help plants to tolerate the degradation by maintaining cell turgidity as recorded earlier by Sivakumar *et al*. (1998) and may protect plants against induced damage. The accumulation of proline content under stress may be due to increased synthesis of protein bound proline (Krishnamurthy *et al*., 1994).

Protein

Protein is one of the reserved food materials that is utilized for the growth of seedlings and further growth of plants. In the present study, the protein content was found to be decreased in both germination studies and field experiments. Similar results of reduction in protein content were recorded in *Phaseolus aureus* (Nandi *et al*., 1987) groundnut and black gram (Prasad and Inamdar, 1990, 1991) and *Hibiscus cannabinus* (Uma and Rao, 1996). Reduction in protein content might be due to the enhanced rate of protein denaturation (Prasad and Inamdar, 1990 and Tripathi and Gautham, 2007). The enhanced protein denaturation and breakdown of existing protein to amino acid is the main cause of reduction in protein content (Constantinidou and Kozlowski, 1979).

The reduction in protein content of the dusted plants may be attributed to breakdown of soluble proteins or increase in activity of protease or catabolic enzymes that were stimulated. A deficiency of one element such as iron usually produces correlative changes in the rate of protein biosynthesis (Suzuki *et al*. 1981).

Sugar

Sugar is an important constituent and source of energy for all living organisms. Plants manufacture this organic substance during photosynthesis and breakdown during respiration. The concentration of soluble sugar is indicative of the physiological activity of a plant and it determines the sensitivity of plants to air pollution. In the present study, the sugar content of black gram was found to be reduced with the increase in the amount of cement dust applied. The reduction in total sugar content of *Hibiscus cannabinus* was recorded at all stages of its growth (Uma and Rao, 1996). A similar result of reduction in sugar content was already reported in *Vigna mungo*, *Cajanus cajan* (Prasad and Inamdar, 1990, Prasad *et al*., 1991), groundnut (Raajasubramanian *et al*., 2011) and *Mangifera indica*, *Cassia fistula* and *Eucalyptus* exposed to different air pollution (Tripathi and Mukesh Gautam, 2007). Reduction in soluble sugar content in polluted stations can be attributed to increased respiration and decreased CO_2 fixation because of chlorophyll deterioration (Tripathi and Mukesh Gautam, 2007).Phosphorus deficiency decrease reducing sugar accumulation in the shoot and root (Foyer, 1988).

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