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Effects of Cement Dust on the Physiological Activities of *Arabidopsis thaliana*

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Abstract: Air pollution exerts detrimental effects on plant ecosystems and restricts agricultural productivity. Cement dust is considered to be one of the most harmful air pollutants of industrial origin and is a limiting factor for plant growth and productivity. This study aims to uncover the impacts of cement dust on photosynthetic pigments, protein content, hydrogen peroxide (H_2O_2) accumulation, lipid per oxidation and antioxidant enzyme activities of *Arabidopsis thaliana*. In this study, Arabidopsis plants were dusted with cement at a rate of 1.5 g per 1-m^2 area and measurements were undertaken at 10 days after cement dust application. Treatment with cement dust resulted insignificant reductions in chlorophyll content and total soluble protein accumulation. Neither carotenoid nor starch content of Arabidopsis plants was affected by exposure to cement dust, whereas protease activity was significantly enhanced in cement dust-treated plants. Furthermore, exposure to cement dust significantly enhanced the production of H_2O_2, a product of oxidative stress, in the leaves of Arabidopsis plants. Moreover, Malondialdehyde (MDA) content, a product of lipid per oxidation, significantly increased after exposure to cement dust. In response to cement dust, activities of scavenging enzymes such as Ascorbate Peroxides (APX), Superoxide Dismutase (SOD) and Guaiacol Peroxidase (GPX) increased, whereas the activity of Catalase (CAT) activity decreased. The present results suggest that cement dust induced oxidative stress in Arabidopsis plants through the generation of Reactive Oxygen Species (ROS), induction of lipid peroxidation and up regulation of antioxidant enzyme activities.

Keywords: Antioxidant Enzymes, *Arabidopsis thaliana*, Cement Dust, Hydrogen Peroxide, Lipid Peroxidation, Photosynthetic Pigments

Introduction

Plants are frequently exposed to different biotic and abiotic environmental stress factors, which occur separately or in combination and exert detrimental effects on plant ecosystems and restricting agricultural productivity (Mittler, 2002; Abu-Romman and Suwwan, 2012). Abiotic stresses are commonly caused by water deficit, salinity, alkalinity, heat shock, freezing, ultraviolet irradiation, deficient or excess nutrients, anoxia and pollutants (Mittler, 2006; Wang *et al*., 2003). Air pollution is a major problem of modern urbanization and industrialization, resulting in undesirable effects on humans, animals and plants (Taylor *et al*., 1986; Wilson *et al*., 2004).

Dust is a major particulate air pollutant, particularly in dry climates (Naidoo and Chirkoot, 2004). Dust pollution is important near roads, quarries, cement works and other industrial areas. Cement dust is considered to be one of the most hazardous dust of industrial origin because it not only forms crusts but also reacts with the atmospheric moisture; thus, it is chemically active (Kabir and Madugu, 2010).

Almost every stage of cement manufacture, including extraction of raw materials, crushing, packing and dispatching of cement from the facility, involves the emission of considerable amounts of dust

(Mutlu *et al*., 2013). In addition to dust, the combustion of fuels during cement manufacturing releases injurious gases such as sulfur oxides, nitrogen oxides and carbon dioxide (Kampa and Castanas, 2008). Cement dust contains heavy metal pollutants, including chromium, nickel, cobalt, lead and mercury (Baby *et al*., 2008). Moreover, cement contains other substances, such as crystalline silica, lime and gypsum, which can be hazardous (Isikli *et al*., 2006; Schuhmacher *et al*., 2002).

Exposure to cement dustcan cause serious health problems through contact with skin, eyes, or breathing passages and might result in hospitalization (Anderson *et al*., 2003; Ballester *et al*., 2001). Exposure to cement dust is associated with inflammatory and pathological changes in the nasal and pharyngeal epithelia, leading to areduction in ventilatorycapacity (Al-Neaimi *et al*., 2001).

Besides health problems in humans, environmental damage associated with cement production and use includes water and soil pollution. Cement dust can severely reduce water quality and subsequently have damaging effects on animals and vegetation. Cement particles can enter into soil as dry, humid, or occult deposits (Al-Khashman and Shawabkeh, 2006) and can undermine its physicochemical properties, leading to a reduction in soil fertility (Sivakumar and Britto, 1995) and subsequently destroying the vegetation cover in the affected area (Schuhmacher *et al*., 2002).

Successful crop production is highly dependent on environmental conditions, with air quality playing a major role (Agrawal *et al*., 2006). The impact of the cement useandindustry 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 sulfur dioxide, nitrogen dioxide and ozone. Results from these types of studies on dust pollution that have 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. Cement dust causes adverse effects on plants grown near construction sites and cement manufacturing. Early investigations (Pierce, 1909) dealt with the impact of cement dust on the stomata of *Vitisvinifera* and *Quercuslobata*. Subsequent investigations, mostly concerning commercial species, pointed out the influence of cement dust on growth (Iqbal and Shafig, 2001; Zargari and Shoar, 2008), transpiration (Singh and Rao, 1981), photosynthesis (Armbrust, 1986), crop productivity (Saralabai and Vivekanandan, 1997), cation levels (Ade-Ademilua and Umebese, 2007), enzyme activities (Borka, 1980), starch production (Kloseiko, 2005) and leaf necrosis (Lepedus *et al*., 2003).

Understanding the physiological and metabolic responses of the stressed plant to such pollutants will facilitate the management of crop cultivation in affected areas. Therefore, the aim of the present study is to evaluate the impact of cement dust on photosynthetic pigments, protein content, H_2O_2 production, lipid peroxidation and antioxidant enzymes of *Arabidopsis thaliana*.

Materials and Methods

Plant Growth and Treatment

Seeds of *A. thaliana* of the Columbia-0 ecotype were soaked for 5 days at 4°C in 0.1% agarose to break dormancy. Seeds were then sown in pots filled with peatmoss. The cultures were maintained in the greenhouse and plants were watered daily.

Only healthy seedlings were chosen for the treatment. One-month-old Arabidopsis seedlings were exposed to cement dust under greenhouse conditions at the rate of 1.5 g of Portland cement per m^2 area. Dusting was fixed for all seedlings at approximately 30 cm above the surface of the plant. Conditions for control plants were identical to those for treated plants, except in case of cement dusting. The treatment was continued for 10 days. After 10 days of cement dust application, leaf tissues were collected from both control and treated plants, frozen in liquid nitrogen and kept at −60°C until use.

Determination of Photosynthetic Pigments

Plant contents of the photosynthetic pigments chlorophyll *a* (Chl*a*), chlorophyll *b* (Chl*b*) and total carotenoids were determined from control and cement dust-treated plants. Leaf samples of approximately 80 mg were extracted with chilled 80% acetone. Homogenates were centrifuged at 4°C in the dark at 8000 *g* for 10 min, after which the absorbance of acetone extracts was measured at 663, 645 and 470 nm. Specific absorption coefficients of Chl*a*, Chl*b* and carotenoids reported by Lichtenthaler (1987) were used. The total chlorophyll content was obtained by the addition of Chl*a* and Chl*b* values. All values were normalized to the Fresh Weight (FW) of each sample.

Determination of Total Protein Content, Protease Activity and Starch Content

Approximately 80 mg of leaf material was ground into a fine powder in liquid nitrogen. Then, 300 µL of protein extraction buffer for total protein was added to the powder in a microfuge tube and the mixture was homogenized. The cell debris was pelleted in a centrifuge at 8000 *g* and at 4°C for 10 min. Approximately 200 µL of the supernatant was transferred into a new microfuge tube and stored at −20°C until use.

Protein concentration was estimated by a colorimetric assay according to Bradford (1976). To measure the concentration of proteins in leaf extract, 800 µL of

protein extracts at an appropriate dilution were mixed with 200 µL of Bradford reagent (Abcam, UK) and incubated for 15 min at room temperature. The protein absorbance was measured at 595 nm; protein concentration was determined by plotting the OD_{595} against a standard curve for bovine serum albumin.

Protease activity was assayed using a Protease Activity Assay Kit (Abcam, UK) according to the manufacturer's protocol.

Starch was extracted from leaf samples with 1 mL of 10 N NaOH in a boiling water bath for 5 min. Following extraction, supernatants were neutralized with 1 mL of 10 M H_3PO_4; each sample was centrifuged at 8000*g* for 2 min. Subsequently, starch in the supernatant was assayed using a Starch Assay Kit (Abcam, UK) according to the manufacturer's protocol.

Determination of Hydrogen Peroxide Content and Lipid Peroxidation

Hydrogen peroxide (H_2O_2) content was assayed in control and treated plants. Leaf samples (80 mg) were ground in liquid nitrogen. Samples were suspended in 20m sodium phosphate buffer and assayed using a Hydrogen Peroxide Assay Kit (Abcam, UK) following the protocols of Shin and Schachtman (2004) and expressed as $\mu mol \cdot g^{-1} FW$

The Thiobarbituric Acid (TBA) test, which identifies Malondialdehyde (MDA) as an endproduct of lipid peroxidation, was used to analyze lipid peroxidation (Loreto and Velikova, 2001; Sunkar *et al*., 2003). Leaf tissues (80 mg) were incubated in 1 ml of 0.25% (w/v) TBA prepared in 10% (w/v) trichloroacetic acid. The mixture was kept in a boiling water bath for 20 min and then cooled at room temperature. After centrifugation at 8000 *g*for 15 min, the absorbance at 535 nm was measured and corrected for nonspecific absorbance at 600 nm. The MDA concentration was calculated using its extinction coefficient 155 $mM^{-1} \cdot cm^{-1}$ (Heath and Packer, 1968). Results are expressed as nmol MDA g^{-1}FW.

Determination of Antioxidant Enzyme Activities

Ascorbate peroxidase (APX; EC 1.11.1.11) activity was assayed in an APX assay buffer (50 mM potassium phosphate buffer, pH 7.0, 0.5 mM ascorbic acid, 0.2 mM H_2O_2 and 0.1 mM EDTA). The resulting homogenates were filtered and centrifuged at 3000*g* for 25 min. The APX activities were measured by monitoring the decrease in absorbance at 290 nm and the molar extinction coefficient of ascorbic acid (2.8 $mM^{-1} \cdot cm^{-1}$) was used for calculation (Amako *et al*., 1994).

To determine the activity of Superoxide Dismutase (SOD; EC 1.15.1.1), tissues were homogenized in ice-cold 0.1 M Tris/HCl at pH 7.4 containing 0.5% Triton X-100, 5 mMβ-mercaptoethanol and 0.1 mg mL^{-1} PMSF. The tissue homogenate was centrifuged at 8500 *g* rpm for 5 min at 4°C. The activity of SOD in the supernatant was assayed using a Superoxide Dismutase Activity Assay Kit (Abcam, UK) according to the manufacturer's protocol.

Regarding Catalase (CAT; EC 1.11.1.6) activity, sample preparation and measurements were performed with a Catalase Assay Kit (Abcam, UK) according to the manufacturer's protocol. Guaiacol Peroxidase (GPX; EC 1.11.1.7) activity was assayed after homogenizing leaf tissues in 50 mMTris/HCl, pH 7.5, 5 mM EDTA and 1 mM DTT. The resulting supernatant was used to assess the GPX activity using a Glutathione Peroxidase Assay Kit (Abcam, UK) according to the manufacturer's protocol.

Statistical Analysis

A two-tailed Student's *t*-test ($P = 0.05$) was applied to evaluate the significant differences between control and treated plants. All experimental data reported are average means of three independent assays with five replicates ($n = 15$).

Results

Chlorophyll *a* (Chl*a*), Chlorophyll *b* (Chl*b*) and total chlorophyll contents were investigated in Arabidopsis plants exposed to cement dust (Table 1). Treating Arabidopsis plants with cement dust significantly reduced Chl*a* and Chl*b* contents by 29% and 9%, respectively, when compared with control plants. Compared with control plants, exposure to cement dust significantly reduced total chlorophyll content of treated plants by 25.83%. Carotenoid accumulation was also investigated in control and cement dust-treated plants (Table 1). Carotenoid content was not significantly altered in response to the treatment.

Treatment of Arabidopsis plants with cement dust resulted in a significant decrease in total protein content (Table 2). Total protein content was decreased by 34.56% compared with control plants. In the present study, protease activity was significantly enhanced in cement dust-treated plants (Table 2). The level of protease activity was enhanced by 41.07% in stressed plants as compared with control plants. This result is in accordance with the observed decrease in total protein content. The present study indicated that cement dust has no significant impact on starch accumulation in Arabidopsis plants (Table 2).

Figure 1a shows H_2O_2content in control and cement dust-treated plants. H_2O_2 level in cement dust-treated plants increased by 50.23% as compared with control plants. The effect of cement dust on lipid peroxidation was determined by evaluating MDA level (Fig. 1b). MDA content was significantly increased in treated plants to reach a level of 23.23 $ng \cdot g^{-1}$ FW.

Table 1. Effect of cement dust on photosynthetic pigments of *A. thaliana* plants. Values are presented as mean ± Standard Deviation (SD) of 15 biological replicates

	Control	Treatment	*t*value[a]
Chlorophyll *a*(μg·g^{-1} FW)	40.40 (0.54)	28.67 (0.48)	21.78
Chlorophyll *b* (μg·g^{-1} FW)	7.68 (0.178)	6.99 (0.184)	2.95
Total chlorophyll (μg·g^{-1} FW)	48.08 (0.55)	35.66 (0.53)	22.02
Carotenoids (μg·g^{-1} FW)	15.47 (0.26)	14.73 (0.30)	1.78

[a]The difference between control and treatment was considered significant if the calculated value exceeded the tabular *t* value. Tabular *t* value = 2.14 (α′= 0.05 and *n* = 15)

Table 2. Effect of cement dust on total protein content, protease activity and starch content of *A. thaliana* plants Values are presented as mean ± Standard Deviation (SD) of 15 biological replicates

	Control	Treatment	*t*value[a]
Protein (mg·g^{-1} FW)	20.02 (0.297)	13.1 (0.227)	24.8
Protease (U·mg^{-1} protein)	0.627 (0.011)	1.064 (0.026)	−10.28
Starch (mg·g^{-1} FW)	52.44 (1.12)	53.3 (0.81)	−0.712

[a]The difference between control and treatment was considered significant if the calculated *t* value exceeded the tabular *t* value. Tabular *t* value = 2.14 (α′= 0.05 and *n* = 15)

Table 3. Effect of cement dust on the activities of antioxidant enzymes in *A. thaliana* plants Values are presented as mean ± Standard Deviation (SD) of 15 biological replicates

	Control	Treatment	*t*value[a]
APX (μmol·mg^{-1} protein)	535.4 (9.42)	1652.1 (19.58)	−81.88
SOD (U·mg^{-1} protein)	23.86 (0.52)	32.33 (0.94)	−7.53
CAT (U·mg^{-1} protein)	41.27 (0.87)	15.23 (0.35)	30.45
GPX (μmol·mg^{-1} protein)	0.215(0.005)	0.350 (0.01)	−11.35

[a]The difference between control and treatment was considered to be significant if the calculated *t* value exceeded the tabular t value. Tabular t value = 2.14 (α′= 0.05 and n = 15)

Changes in activities of major antioxidant enzymes in Arabidopsis plants were measured after cement dust application (Table 3). Activity of APX increased significantly in response to cement dust to reach 1652.1 μmol·mg^{-1}of protein, whereas, in control plants, a value of only 535.4 μmol·mg^{-1}of protein was obtained. Activity of SOD was significantly enhanced in plants treated with cement dust. An increase in SOD activity of approximately 26.5% in cement dust-treated plants was obtained when compared with control plants (Table 3). As shown in Table 3, cement dust application to Arabidopsis plants resulted in a significant decrease in CAT activity (approximately 63.1%). Compared with the control plants, cement dust treatment significantly increased the activity of GPX (Table 3). The level of GPX activity increased up to 38.5% in response to the treatment.

Discussion

Measurement of photosynthetic pigments is an important parameter frequently used to evaluate the effects of pollutants on plants because photosynthetic pigments play critical roles in plant metabolism (Wagh *et al*., 2006). Several investigators have reported a reduction in chlorophyll content in plants subjected to cement dust (Mandre and Tuulments, 1997; Nanos and Ilias, 2007).

The shading of leaf surfaces caused by cement dust particles reportedly reduces photosynthesis, thereby reducing chlorophyll content (Darley, 1966). Moreover, the incorporation of cement kiln dust into leaf tissues was reported to enhance chloroplast degradation and, therefore, reduce the chlorophyll content (Singh and Rao, 1981). The observed reduction in chlorophyll content (Table 1) might be because of both the shading effects of cement particles and membrane damage, indicating enhanced lipid peroxidation (Fig. 1). Carotenoid content of Arabidopsis plants was not affected by cement dust (Table 2).

Reduction in protein content in plants because of air pollution has been reported by many investigators (Guo *et al*., 2007; Rajput and Agrawal, 2005). Protein degradation and increased proteolytic activities have been suggested as an index of oxidative stress in plants (Romero-Puertas *et al*., 2002; Xiong *et al*., 2007). In the present study, exposure of Arabidopsis plants to cement dust caused reduction in total protein content and a considerable increase in protease activity (Table 2). This observation could be attributed to cement dust-induced oxidation of proteins, enhanced H_2O_2 accumulation and increased protease activities.

Metabolism of carbohydrates in plants is known to be strongly affected by dust pollution. Mandre *et al*. (2000) showed that starch content in the leaves of some conifer species was decreased in response to cement dust, an effect that can apparently be explained by the shading effect of the dust layer, which would negatively influence carbon metabolism and photosynthesis. The present study showed no change in the starch content of Arabidopsis plants in response to exposure to cement dust (Table 2). This result might suggest that cement dust does not induce changes in starch/sugar conversion and remobilization under our experimental conditions.

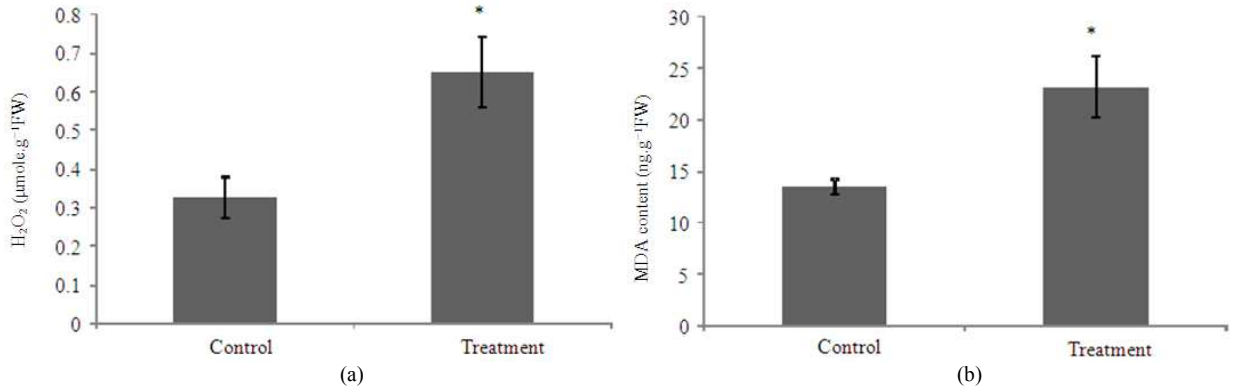

Fig. 1. Effect of cement dust on H_2O_2 (a) and MDA (b) contents of *A. thaliana* plants. Data are presented as the mean of 15 biological replicates and bars represent Standard Errors (SE). *: significant difference from control at a *P* value of≤0.05 by applying Student's *t*-test

Environmental stresses, including pollutants, mediate their effects through oxidative stress caused by enhanced accumulation of ROS (Mittler, 2002; Scandalios, 2005). ROS are highly reactive and toxic molecules that can cause lipid peroxidation and oxidative damage to photosynthetic pigments, DNA and proteins (Møller *et al*., 2007; Scandalios, 2002).

In the present study, cement dust application resulted in a marked increase in H_2O_2 level in Arabidopsis plants (Fig. 1a). Moreover, the level of MDA, an indicator of lipid peroxidation and membrane damage, increased in Arabidopsis leaves upon exposure to cement dust (Fig. 1b). The results of the present study are similar to those of the previous studies that have shown that cement dust enhances lipid peroxidation in affected plants (Dziri and Hosni, 2012). In the present study, increased level of H_2O_2 along with enhanced lipid peroxidation indicate that cement dust disrupts cellular integrity and induces oxidative stress. H_2O_2 was proposed to enhance the Haber-Weiss reaction, resulting in hydroxyl radical production and, thus, leading to lipid peroxidation (Mittler, 2002).

The plant cell can be protected from the damaging effects of the overproduction of ROS and the associated oxidative damages by operating efficient antioxidant systems (Alscher *et al*., 1997). These systems employ ROS-scavenging enzymes, such as SOD, CAT, APX and GPX, as well as no enzymatic antioxidants such as glutathione and ascorbate (Apel and Hirt, 2004; Foyer and Noctor, 2011; Halliwell, 2006).

SOD and CAT are considered to be the first lines of defense in the antioxidant system against peroxidation reactions. SODs are metalloenzymes containing Cu/Zn, Fe, or Mn in their prosthetic groups. SOD catalyzes the dismutationof superoxide anion into H_2O_2 and oxygen (Alscher *et al*., 2002). Our results showed increased activity of SOD in Arabidopsis plants exposed to cement dust. Induction of SOD activity has been reported under various stressful conditions, including cementdust pollution (Abu-Romman and Shatnawi, 2011; Dziri and Hosni, 2012; Kasai *et al*., 2006).

Catalase (CAT) is a potent antioxidant enzyme that catalyzes the dismutation of H_2O_2 to oxygen and water (Mhamdi *et al*., 2010). In plants, CATs comprise a multigene family and are differentially regulated in various tissues during development by a variety of environmental stimuli (Du *et al*., 2008; Frugoli *et al*., 1996; Purev *et al*., 2010). The present study showed a decline in CAT activity in response to cement dust; this result might explain the enhanced accumulation of H_2O_2 observed in this studyand suggested that CAT activity is negatively affected by oxidative stress. Decreased CAT activity was frequently reported in response to abiotic stresses in different plant species (Neto *et al*., 2006; Hameed *et al*., 2012).

APX is a potent scavenger of H_2O_2 produced in chloroplasts and in the cytosol. This enzyme catalyzes the reduction of H_2O_2 using ascorbate as the specific electron donor (Asada, 1992; Teixeira *et al*., 2004). In our study, treatment with cement dust enhanced APX activity in Arabidopsis plants. GPXs are enzymes involved in the detoxification of H_2O_2 (Blokhina *et al*., 2003). In the present study, the observed increased activity of GPX in response to cement dust might be because of increased H_2O_2 level in the cytosol and cell wall. The enhanced activities of APX and GPX observed in this study were unable to compensate for the decline in CAT activity because H_2O_2 level significantly increased in plant leaves.

Conclusion

In conclusion, the results of the present study showed that cement dust decreased chlorophyll and total protein contents and enhanced protease activity. Moreover, the data obtained from this study showed that cement dust induced oxidative stress on Arabidopsis plants

through the enhanced generation of H_2O_2, increased peroxidation of lipid and activation of antioxidant enzymes. The increased levels of antioxidant enzymes (except CAT) indicated the activation of a secondary defense mechanism in response to cement dust; however, they might not be efficient for protecting Arabidopsis plants from oxidative stress induced by cement dust and were not sufficient to block H_2O_2 accumulation and lipid peroxidation.

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Author's Contributions

Saeid Abu-Romman: Contributed to conception and design of the experiment, analyzed and interpreted data and wrote the manuscript.

Jarrah Alzubi: Analyzed and interpreted data and wrote the manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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