0273-2289/95/5001—0001$06.00

Does Application of Cement Kiln Exhausts Affect Root Nodule Biochemistry and Soil N_2-Fixing Microbes?

V. C. SARALABAI AND M. VIVEKANANDAN*

Department of Biotechnology, School of Life Sciences, Bharathidasan University, Tiruchirapalli-620 024, Tamilnadu, India

Received September 28, 1993; Accepted March 1, 1994

ABSTRACT

The research work carried out on cement exhausts centers mostly around vegetation and crop productivity (*1-3*) with little or no work on root nodulation. Soil plus foliar application of exhaust dusts did not affect soil/nodule rhizobial population, nodule initiation, and possible N_2-fixing capacity in *Cajanus cajan, Vigna radiata, Vigna mungo, Vigna catjung,* and *Glycine max*. The nodular biochemistry was investigated in detail. The heme protein leghemoglobin was higher compared to the control. The levels of intermediary N compounds like total ureides of the nodules, which may serve as indirect evidence of symbiotic N_2 fixation, were higher in the treated plants. There were also increments in free proline, free amino acids, soluble proteins, soluble starch, soluble sugars, total nitrogen, and phenols in the treated plants. The levels of total nitrates, soluble sucrose, and soluble SH compounds of the nodule of the control and treated plants did not show a significant difference. The activities of ascorbate peroxidase, catalase, glutathione reductase, and superoxide dismutase were significantly higher, possibly indicating their role in alleviation of H_2O_2 and O_2^- damage by the exhausts. Enzymes like nitrate reductase, nitrite reductase, and glutamine synthetase, and also the activities of acid and alkaline phosphatases were not affected. The presence of

*Author to whom all correspondence and reprint requests should be addressed.

beneficial soil microbes like *Azotobacter, Azospirillum,* and mycorrhizae was not affected at all.

Index Entries: Beneficial soil microbes; cement kiln exhausts; heme protein; nodule biochemistry; *Rhizobia;* symbiotic N_2 fixation.

INTRODUCTION

Air pollution is considered to be a serious problem in recent times (*1*). In the present study, cement exhaust dust on root nodule biochemistry has been taken up for a detailed investigation. No doubt, the cement pollution is considered to be a localized problem. Nevertheless, it is assuming a mammoth proportion in recent times particularly in several parts of Tamilnadu, India, in view of the mushrooming of cement factories (*2,3*). In the present study, the cement exhaust dust jetting out from the stacks of the Tamilnadu Cements Corporation Limited (TANCEM), Ariyalur Works, located at Ariyalur, Tiruchirapalli was collected at regular intervals for experimental purposes. Thus far, the effects caused by exhaust gases were not clearly demarcated from the effects caused by continuous fallout of dust from the cement factory. Hence, the electrostatic precipitator (ESP) dust was collected from the factory, as and when required, and employed on leguminous crops raised in earthern pots containing the garden soil.

It has become a conventional practice these days to understand the effects of gaseous pollutants through simulation study to pinpoint the effects caused and delineating them from other environmental effects that might operate at the pollution site. Similarly, the present simulation study was conducted in the university botanical garden in open conditions to understand precisely the effects of application of ESP dust from that of gaseous kiln exhaust pollutants (O_2, N_2, SO_2, NO, NO_2, CO, H_2S, and Peroxy acyl nitrates [PAN]) that operate at the location of the cement factory. In the natural conditions, dusting occurred both on the soil and on the plant surfaces in and around the cement plant. Therefore, in the present study, soil application of the dust has been coupled with the foliar spray of the dust. This study is to find out whether soil plus foliar application of the dust could affect the root growth, nodulation, root nodule biochemistry of certain legume crops like *Vigna radiata, Vigna mungo, Vigna catjung, Cajanus cajan,* and *Glycine max* most popularly grown in Tamilnadu, India, and the association of beneficiary microbes of biofertilizer origin.

MATERIALS AND METHODS

In the present simulation study, the cement exhausts directly collected from the electrostatic precipitator (ESP) of the TANCEM, Ariyalur Works, Tamilnadu, India were used in all experiments. The ESP dusts were mixed thoroughly with the garden soil at a concentration of 200 g/kg garden soil, since even this highest concentration of dust was not found to be inhibitory to the growth of crop plants (*3*). The seeds of *Cajanus cajan* (L.) Millsp var. SA-1, *Vigna mungo* L. var. Vamban-1, *Vigna radiata* L. var. CO-123, *Vigna catjung* L. var. CO-4, and *Glycine max* L. var. CO-1 were collected from the National Pulses Research Centre, Vamban, Tamilnadu, India. About 20 healthy seeds were selected and sown in earthern pots (20 cm in height and 19 cm in diameter) containing garden soil with and without the ESP dust. The seedlings were raised under a natural photoperiod (26 ± 1 W/m^2) with day and night temperatures of 28–32°C and 22–25°C, respectively. The seedlings raised in the dust-free soil were treated as control. The soil physical and chemical parameters were carried out by the following standard methods (*4*). The available soil nutrients were N (10.36 mg/g), P (0.63 mg/g), and K (0.88 mg/g) with total organic matter of 3.12% and total organic carbon of 1.99%. The soil had a bulk density of 1.33 g/cc and water-holding capacity of 21.87% with percentage pore space of 36.67. After germination, the seedlings were thinned down to 10/pot to permit the healthy growth of the seedlings. When the seedlings attained 7 d of growth, foliar application of the dust (10 g dust/pot) was resorted to at 2-d intervals using a hand sprayer. Considering the prevailing concentration of the dust in the natural environment in the crop fields around the cement plant, the present usage of 200 g/kg soil plus 10 g of the dust as foliar spray/pot at 2-d intervals is considered the highest. The root nodules were collected from 80-d (*C. cajan*) and 50-d-old (*V. radiata, V. mungo, V. catjung* and *G. max*) plants. Leghemoglobin (LHb) was extracted in Drabkin's solution, and quantification was carried out by the method of Wilson and Reisenauer (*5*).

Assays of ascorbate peroxidase (AP), catalase, glutathione reductase (GR), superoxide dismutase (SOD), acid and alkaline phosphatases, nitrogenase (N_2-ase), nitrate reductase (NR), nitrite reductase (NIR), and glutamine synthetase (GS) were carried out by following standard methods (*6*). The intermediary N compounds, like total ureides, allantoin, and allantoic acid, as well as the basic biomolecules, like free amino acids, total soluble sugars, phenols, proteins, starch, sucrose, SH compounds, free proline, nitrate and nitrite, and nitrogen, were quantified by following the methods described by Malik and Singh (*7*). Assessment of nodule and soil rhizobial population was determined by most probable number (MPN) counts following the method suggested by Vincent (*8*). Screening for other beneficial microbes, like *Azotobacter, Azospirillum*, and

Vesicular arbuscular mycorrhizal (VAM) spores, was carried out by following the standard methods. All the data were statistically analyzed using Student's *t*-test (*9*).

RESULTS AND DISCUSSION

The present study critically analyzes the combined effects of the cement exhaust (ESP) dust through both soil and foliar application on root nodule formation and its biochemistry on a few economically important legumes popularly cultivated in and around the location of the cement factory. After addition of the dust, the soil nutrient status was found to be altered. The soil nutrients, such as N, P, and K varied, by 12.46, 2.63, and 1.93 mg/g, respectively, with a change in total organic matter of 21.04% and total organic carbon of 13.99%. Similarly, the treated soil had a bulk density of 0.87 g/cc and water-holding capacity of 14.31% with percentage pore space of 32.17. The dusted soil had an initial pH of 9.4 ± 0.1 and an EC of 0.53 ± 0.02 mS/cm. However, after the plants attained 50-d of growth, the rhizosphere soil showed the pH and EC values of 8.6 ± 0.1 and 0.34 ± 0.01 mS/cm, respectively (*3*).

The microbial population of the ESP-dusted soil is compared with that of the untreated control soil. From the perusal of the data (not shown), it is evident that the population of the soil microbes (*Rhizobia, Azotobacter, Azospirillum,* and mycorrhizal spores) was not found to be affected at all by the addition of the ESP dust. No significant difference was observed in the microbial population of the control and treated soils at 5 and 1% levels. Since the ESP dust had no effect on rhizosphere soil rhizobial population, experiments were further carried out to find out whether the added dust could have any effect on the nodular rhizobial population. In all the leguminous crops, the root nodule rhizobial population was not found to be affected at all, even at highest concentration (200 g dust/kg soil) of the dust. Colonization of rhizosphere zone by microbes is not affected by the dust.

In the present study, the number of root nodules of the treated plants on an average was 37% higher than the control. The possible explanation for the ESP dust not interfering with the formation of root nodule symbioses as well as the growth of *Rhizobia* is that the dust contained many elements (Ca, Fe, Mg, Mn, K, P, S, N, Cu, and Zn) needed for the growth of *Rhizobia* as well as for the efficient functioning of the root nodules (*3*).

The root nodule pigment leghemoglobin (LHb) is considered to be more important in creating a microaerobic environment at the site of N_2-ase action in all legume *Rhizobium* symbioses. From Table 1, it is inferred that the combined application of the dust increased the level of LHb by 10–20% (Table 1). As regards total nitrogen, nitrites, and free amino acids, there

Table 1
Root Nodule Biochemistry of the Control and Dusted Plants[a]

Crop legumes	Total leghemoglobin	Total nitrite	Free proline	Free amino acids	Total soluble proteins	Total phenols	Total soluble sugars	Total soluble starch	Total nitrogen
	mg/g fresh wt				mg/g dry wt				
Vigna radiata									
Control	29.25	0.09	6.28	24.87	101.21	0.43	403.28	18.81	225.17
Treated	32.25	0.10	9.27[c]	34.66[b]	128.14[c]	1.28[c]	443.26	34.28[c]	280.24[b]
	(110)	(111)	(148)	(140)	(127)	(298)	(110)	(182)	(124)
Vigna mungo									
Control	30.75	0.07	7.31	23.42	137.41	0.84	312.23	24.21	202.17
Treated	36.75[b]	0.07	10.37[c]	35.26[b]	145.41[c]	1.68[c]	394.24[c]	37.26[c]	248.65[b]
	(120)	(100)	(142)	(151)	(106)	(200)	(126)	(154)	(123)
Vigna catjung									
Control	18.75	0.08	7.21	24.81	136.24	0.95	395.16	23.28	210.16
Treated	21.51[b]	0.10[c]	10.21[c]	39.83[b]	159.11[c]	1.93[c]	476.28[c]	37.82[c]	253.15[b]
	(115)	(125)	(142)	(161)	(117)	(203)	(121)	(162)	(120)
Cajanus cajan									
Control	40.54	0.12	6.23	24.52	143.21	0.75	396.16	23.16	196.13
Treated	46.25[b]	0.14[c]	9.21[c]	38.62[b]	147.63[b]	2.25[c]	452.18[c]	35.18[b]	258.63[b]
	(115)	(117)	(148)	(158)	(103)	(300)	(114)	(152)	(132)
Glycine max									
Control	36.75	0.12	5.53	26.81	152.18	0.53	312.17	16.80	282.36
Treated	42.24[b]	0.15[c]	9.21[c]	38.62[b]	172.24[c]	1.61[c]	382.19[c]	28.92[c]	312.24[b]
	(115)	(125)	(167)	(144)	(113)	(304)	(122)	(172)	(111)

[a]The data are mean values of three different experiments with two replicates. The data in parentheses indicate % control values.
[b]Significant at 5% level.
[c]Significant at both 5% and 1% levels.

was a marginal increase, whereas in soluble starch, soluble proteins, soluble sugars, phenols, and free proline, a significant increase in their levels was observed. The levels of total nitrates, soluble sucrose, and SH compounds of the nodule of the control and treated plants did not show any significant difference (data not shown). From the overall survey of the levels of various chemical components from Table 1, phenol level is significantly increased in the nodules of the dusted plants by about three-fold over the control. It may be construed from the levels of various chemical constituents that nodular functioning is absolutely not affected, as evidenced by unaltered levels of LHb and marginal increase in the levels of total nitrogen coupled with uninhibited concentrations of other chemical constituents for the normal functioning of the root nodule. Hooda et al. (*10*) reported that the water-stressed plants showed greater efficiency in terms of the amount of nitrogen fixation in relation to carbon assimilation in spite of reduced growth, which might be the result of dark CO_2 fixation by PEP carboxylase in nodules (*11*). SOD and catalase are reported to protect the overall nitrogen-fixation process against O_2^- radicals (*12,13*). Since the dust did not affect the nodular biochemistry, assay of enzymes more innately connected to nitrogen metabolism, like N_2-ase, NIR, NR, and GS, as well as oxygen-scavenging enzymes, such as AP, catalase, SOD, and GR, was carried out (Table 2). The levels of N_2-ase, NR, NIR, and GS were significantly higher in the nodules of the treated plants than the control, indicating that the nodular nitrogen metabolism is active and is not curbed by the dust at the level investigated. The fact that the nitrogen metabolism is active or at least remains unaltered is evident from the higher activity of N_2-ase in the treated plants over the control. The fact that oxygen-scavenging enzymes, such as AP, catalase, SOD, and GR, inclusive of LHb are more active in the treated plants makes it distinctly clear that the nodule oxygen-scavenging property is not affected (*14–16*). This observation derives indirect support from the work of Tanaka et al. (*17*), which in some other context reported that the test plants fumigated with 2 ppm SO_2 had higher superoxide dismutase activity than the control, indicating that SOD participates in counteracting SO_2 toxicity. Similarly Rao and Dubey (*18*) observed that peroxidase and SOD activities were higher in wheat plants exposed to SO_2, indicating detoxifying mechanism against SO_2. This is only supportive of the present observation. Had these been affected, the LHb content would have been altered with a concomitant reduction in N_2-ase activity. Higher activities of GS also favor the above conclusion and the conclusion that more amides and amino acids could be formed under the duress of the cement dust. The concurrent increases in the concentrations of LHb and glutathione, and activities of N_2-ase, AP, and catalase in the nodule indicate increased capacity of H_2O_2 scavenging, associated with the process of nitrogen fixation (*14*). From the appreciable levels of alkaline and acid phosphatases (data not shown), it may be concluded that the dust did not affect the phosphate metabolism as well. Peroxidase activity was deter-

Table 2
Levels of Activity of Root Nodule Enzymes in the Control and Dusted Plants[a]

Specific activities of enzymes	*Vigna radiata*		*Vinga mungo*		*Vigna catjung*		*Cajanus cajan*		*Glycine max*	
	C	T	C	T	C	T	C	T	C	T
Nitrogenase (nmol C_2H_4 produced/g fresh wt/h)	2565	3696[b] (144)	5775	8654[c] (150)	2702	3218[b] (142)	1194	2183[c] (183)	3584	5678[c] (158)
Nitrate reductase (μmol NO_2 released/mg protein/h)	3.17	4.51[b] (142)	3.62	5.01[b] (138)	2.95	4.79[c] (162)	3.11	5.71[c] (184)	5.32	7.39[b] (139)
Nitrite reductase (μmol NO_2 disappeared/mg protein/h)	6.34	8.39[b] (132)	3.54	5.30[b] (150)	4.04	5.47[b] (135)	4.40	6.95[b] (158)	4.68	6.72[b] (144)
Glutamine synthetase (OD U/mg protein/min)	0.59	0.97[c] (164)	0.41	0.69[c] (168)	0.68	0.97[b] (143)	0.51	0.72[b] (141)	0.55	0.84[b] (153)
Ascorbate peroxidase (nmol of ascorbate consumed/ mg protein/min)	103.98	168.91[c] (162)	107.02	209.32[c] (196)	120.12	209.58[c] (174)	108.72	202.41[c] (186)	75.78	121.32[c] (160)
Catalase (U/mg protein/min)	17.81	33.47[c] (188)	17.66	29.72[c] (168)	15.04	28.73[c] (191)	20.18	31.77[c] (157)	18.12	27.28[b] (154)
Glutathione reductase (OD U/mg protein/min)	0.29	0.45[c] (155)	0.31	0.44[b] (142)	0.29	0.43[b] (148)	0.42	0.63[c] (150)	0.34	0.54[c] (159)
Superoxide dismutase (U/mg protein/min)	63.79	95.54[c] (150)	66.14	97.27[c] (147)	86.17	123.06[c] (143)	74.18	101.13[c] (137)	79.75	104.27[b] (131)

[a]The data are mean values of two different experiments with three replicates. The data in parentheses indicate % control values. (C—control; T—treated).
[b]Significant at 5% level.
[c]Significant at both 5 and 1% levels.

mined to evaluate the suitability of this enzyme as an indicator of stress (*19,20*) imposed by the dust.

The combined effects of foliar as well as soil application of the dust on relative distribution of nitrogenous compounds, like total allantoin, total allantoic acid, and total ureides, were studied in different organs, like nodule, root, stem, leaf, and seed, of the different legume crops (Table 3). A comparative study of the distribution of these compounds in different organs of the plants clearly indicates that the compounds are predominantly gorged into the nodules. In soybean, cowpea, and *Phaseolus* species, the major transportable form of nitrogen is ureide (up to 90%) (*21*), although very little is known about the ureide metabolism in higher plants (*22*). The data in Table 3 indicates that the concentration of total ureides was always more in nodule, followed by leaf, root, stem, and seed, and such a distribution only indicates that the stored nitrogenous compounds are transported from the nodules to the root, stem, and finally to the leaf. This routine form of transport is not affected by application of the dust. Compared to the level of allantoin, allantoic acid level was always higher in both the control and treated plants (data not shown), although total ureides are the principal storage compounds. The highest concentration of ureides in leaves (Table 3) of the control (58–162 mg/g dry wt) and treated (70–191 mg/g dry wt) plants is quite understandable in view of the fact that ureide catabolic products are subsequently used as a primary source of nitrogen for synthesis of amino acids and proteins (*22*). The overall conclusion that can be drawn from the data is that the root nodules are the primary synthesizers of ureides for which carbon is supplied through photosynthesis, and the transport of ureides through root, stem, and leaf and finally to the sink (seed) is not affected at all by the combined treatment of the dust.

The regular supply of photosynthates to the nodules is not affected by the dust at the concentration used, which is evident from the heavy accumulation of starch grains in the nodules (Table 1). The rate of assimilates translocation has been reported to be correlated positively with the rate of CO_2 fixation and leaf sucrose concentration (*23*). In the treated plants, the level of total ureides increased significantly (up to 81%).

It may be surmised from the present study that the application of the ESP dust at the concentration used (200 g dust/kg soil) directly to the soil coupled with foliar spray (10 g dust/pot) did not affect the beneficial soil microbes, root nodule initiation, nodular biochemistry, N_2-ase, and nitrogen metabolism in general.

ACKNOWLEDGMENTS

We gratefully acknowledge the general manager and the chief chemists of Tamilnadu Cements Corporation Limited (TANCEM), Ariyalur, India

Table 3
Effect of Soil cum Foliar Application of the ESP Dust on Total Ureides[a]

	Plant Organs									
	Nodule		Root		Stem		Leaf		Seed	
Crop legumes	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
	mg/g dry wt									
Vigna radiata	59.26	81.24[c] (138)	52.45	59.57[b] (114)	38.78	40.89 (105)	58.11	70.19[c] (121)	26.22	29.38[b] (112)
Vigna mungo	71.22	122.28[c] (172)	78.27	87.27[c] (111)	24.21	26.24[b] (108)	112.24	141.79[c] (126)	32.16	38.46[c] (120)
Vigna catjung	59.26	100.07[c] (169)	55.85	61.90[b] (111)	44.61	48.77[b] (109)	69.29	77.61[c] (112)	29.12	31.74[b] (109)
Cajanus cajan	72.31	130.78[c] (181)	49.32	51.38 (104)	75.22	94.33[c] (125)	162.32	191.23[c] (118)	42.50	47.85[c] (113)
Glycine max	74.08	119.58[c] (161)	56.26	68.73[c] (122)	42.61	52.45[c] (123)	77.29	99.23[c] (128)	37.82	44.25[c] (117)

[a]The data are mean values of three different experiments. The data in parentheses indicate % control values.
[b]Significant at 5% level.
[c]Significant at both 5 and 1% levels.

for generously providing the ESP dust throughout the period of investigation. One of the authors (V. C. S.) is grateful to the Ministry of Environment and Forestry, New Delhi, India for financial assistance. We thank V. Karthikeyan, II M. Sc. (Life Sciences) student of Bharathidasan University for statistical analysis. We thank R. Balasubramanian for neat manuscript typing.

REFERENCES

1. Smith, W. H. (1991), *Chem. Eng. News* **69,** 30-43.
2. Prasad, M. S. V., Subramanian, R. B., and Inamdar, J. A. (1991), *Ind. J. Environ. Health* **33,** 11-21.
3. Saralabai, V. C. and Vivekanandan, M. (1992), *Appl. Biochem. Biotechnol.* **36,** 35-46.
4. Piper, C. S. (1966), in *Soil and Plant Analysis.* Hans, Bombay, India.
5. Wilson, D. O. and Reisenauer, H. M. (1963), *Anal. Biochem.* **6,** 21-30.
6. Mahadevan, M. and Sridhar, R. (1986), in *Methods in Physiological Plant Pathology,* Sivakami, Madras, India.
7. Malik, C. P. and Singh, M. B. (1980), in *Plant Enzymology and Histo-Enzymology—A Text Manual.* Kalyani, Ludhiana, India.
8. Vincent, J. M. (1970), *A Manual for the Practical Study of the Root Nodule Bacteria.* (IBP Handbook 15), Blackwell, Oxford, p. 164.
9. Gomez, K. A. and Gomez, A. A. (1984), in *Statistical Procedures for Agricultural Research.* Wiley, New York.
10. Hooda, R. S., Sheoran, I. S., and R. Singh, (1990), *Ann. Botany* **65,** 111-120.
11. Streeter, J. G. and Salminen, S. D. (1985), in *Nitrogen Fixation Research Progress,* Evans, H. J., Bottomley, P. J., and Newton, W. E., eds., Martinus-Nijhoff, Dordrecht, pp. 277-283.
12. Puppo, A. and Rigaud, J. (1986), *FEBS Lett.* **201,** 187-189.
13. Puppo, A., Dimitrijevic, L., and Rigaud, J. (1989), *Physiol. Plant.* **77,** 308-311.
14. Dalton D. A., Russell, S. A., Hanus, F. J., Pascoe, G. A., and Evans, H. J. (1986), *Proc. Natl. Acad. Sci. USA* **83,** 3811-3815.
15. Puppo, A. and Halliwell, B. (1988), *Planta* **173,** 405-410.
16. Vivekanandan, M. and Saralabai, V. C. (1994), *SOD—An Indicator of Air Pollution Tolerance?* Excerpta Medica International Congress series, in press.
17. Tanaka, K. Furusawa, I., Kondo, N., and Tanaka, K., (1988), *Plant Cell Physiol.* **29(4),** 743-746.
18. Rao, M. V. and Dubey, P. S. (1991), *Indian J. Env. Toxicol.* **1(1),** 39-45.
19. Markkola, A. M., Ohtonen, R., and Tarvainen, O. (1990), *Water Air Soil Pollution* **52,** 149-156.
20. Agrawal, M., Singh, S. K., Singh, J., and Rao, D. N. (1991), *J. Environ. Biol.* **1,** 211-222.
21. McClure, P. R., Israel, D. W., and Volk, R. J. (1980), *Plant Physiol.* **66,** 720-725.
22. Rice, C. F., Lukaszewski, K. M., Walker, S., Blevins, D. G., Winkler, R. G., and Randall, D. D. (1990), *J. Plant Nutr.* **13(12),** 1539-1553.
23. Kerr, P. S., Israel, D. W., Huber, S. C., and Rufty, T. W. (1986), *Can. J. Bot.* **64,** 2020-2027.