Journal of Environmental Biology **January 2006,** 27(1) 43-47 (2006)

Physiological changes in certain test plants under automobile exhaust pollution

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(Received: 22 April, 2004 ; Accepted: 25 October, 2004)

Abstract: *Plants are the only living organisms which have to suffer a lot from automobile exhaust pollution because they remain static at their habitat. But such roadside plants like Nerium indicum Mill., Boerhaavia diffusa L., Amaranthus spinosus L., Cephalandra indica Naud., and Tabernaemontana divaricata L. can easily avoid the effects of air pollution by altering their physiological pathways pertaining to photosynthesis and respiration. Stomatal closure in Boerhaavia, Amaranthus, Cephlandra and stomatal clogging in Nerium and Tabernaemontana help these plants in preventing the entry of poisonous gases. The increased activity of the enzyme Phosphoenol Pyruvate Carboxylase (PEPCase) belonging to C_4 pathway helps Nerium and Boerhaavia (both C_3 plants) in carbon fixation under stress condition. Photorespiration is favoured in Amaranthus, Cephalandra and Tabernaemontana to compensate for the over production of ATP in them. Owing an inefficient gaseous exchange in Boerhaavia and Tabernaemontana, the activity of Glucose 6 - Phosphate Dehydrogenase (G6-PD) also increases for the preferential shift to Pentose Phosphate Pathway to produce excess NADPH+H^+ which are likely to re-oxidize by metabolic reactions not linked to electron transport chain.*

Key words: *Automobile exhaust, Plants, Anaerobic respiration, Fructose 1,6-bis phosphate aldolase, Posphoenol pyruvate carboxylase, Glucose 6 phosphate dehydrogenase.*

Introduction

Rapid industrialization, urbanization and traffic density cause atmospheric pollution all over the country. Vegetation plays the role of major sink of atmospheric dust containing a fair amount of highly toxic heavy metal particles. Air pollution may affect plant tissues either directly (leaf necrosis) or indirectly (acid rain), but the tolerant plants possess strong defense mechanisms which enable them to survive in critical conditions.

Plants are to suffer a lot from the automobile exhaust pollution because they cannot move from the source of pollution. For this reason it is likely that plants exposed to such pollution show many abnormalities in their general appearance, which are termed as "visible injury" in literature. Actually these visible injuries on plant reflect the physiological changes which occur by the impact of pollutants. These physiological changes may be regarded as "hidden injury". These plants are able to avoid these senescing symptoms and can live a normal life. In this paper, a few such alternative mechanisms are described in five roadside plants which are growing in an atmosphere containing an excess of sulfur dioxide (SO_2), oxides of nitrogen (NO_x), suspended particulate matter (SPM), hydrocarbon (HC), lead (Pb), cadmium (Cd) and manganese (Mg).

Materials and Methods

The plants under study (*Nerium indicam* Mill., *Boerhaavia diffusa* L., *Amaranthus spinosus* L., *Cephalandra indica* Naud. and *Tabernaemontana divaricata* (L) R.Br. were collected at random in flowering condition from the edges of both sides of Delhi Road (NH_2) at Rajchandrapur, near Kolkata, having a traffic volume of at least 20 vehicles per minute. The same plants, collected from roadside served as control. The twigs of the polluted and control plants having more or less same age were brought to the laboratory for the following experiments.

The photosynthetic CO_2 was measured by an Infra Red Gas Analyzer (IRGA, ADC-225 MK_3). The samples were assayed for phosphoenol pyruvate carboxylase (Ting and Osmond, 1973), glycolate oxidase (Zelitch, 1955), fructose 1,6-bisphosphate aldolase (Sibley and Lehninger, 1949), succinate dehydrogenase (King, 1976), glucose 6 phosphate dehydrogenase (Upadhayay *et al.,*1981). Total ATP content (Transtschold *et al.,*1985), the oxygen uptake rate during respiration using Warburg Respirometer (Umbreit, 1972), the liberated ethyl alcohol and acetaldehyde using Gas chromatograph- Pye Unicam GC 103 fitted with SP427 OX integrator system (Kimmeror and Kozlowski, 1982) were also estimated from the leaves of the plants under study.

The air quality monitoring was done using a High Volume Air Sampler manufactured by M/s Vayu Bodhan Upkaran Pvt. Ltd., New Delhi (Model Envirotech APM 410-411). The samples were analyzed following the methodology given by Central Pollution Control Board, India (Table 1). The metal concentration of the soil was measured by Atomic Absorption Spectrophotometer (Varian AA575) (Table 2). The nitrogen content of the soil was estimated following the Micro Kjeldalh Method

Results and Discussion

Stomata have long been recognized as an important modifier of plant responses to air pollution. Stomatal area is seen to decrease significantly in *Nerium, Boerhaavia, Cephalandra* but increases in *Amaranthus* and *Tabernaemontana* under pollution (Table 3). Number of

Table – 1: Results of air quality monitoring at Rajchandrapur.

Station	SPM μgm^{-3}	NO x μgm^{-3}	SO_2 μgm^{-3}	Pb μgm^{-3}	Cd μgm^{-3}	HC μgm^{-3}
In roadside air	1794.0	556.2	67.8	0.9	< 0.03	70.2
In village air	1171.2	533.4	< 21.0	0.9	< 0.03	93.6

SPM = Suspended particulate matter; NOx = Oxides of nitrogen; SO_2 = Sulphur dioxide; Cd = Cadmium; Pb = Lead; HC = Hydrocarbons.

Table – 2: Results of soil quality analysis at Rajchandrapur.

Station	pH	N mg/g	P ppm	K ppm	Fe ppm	Cu ppm	Zn ppm	Mn ppm	Pb ppm	Cd ppm
Roadside soil	8.2	5.37	3.08	13.67	2.83	1.85	1.11	7.54	7.53	0.43
Village soil	8.1	5.00	4.51	14.33	6.71	1.90	0.77	5.72	5.84	0.52

N = Nitrogen; P = Phosphorous; K = Potassium; Fe = Iron; Cu = Copper; Zn = Zinc; Mn = Manganese; Pb = Lead; Cd = Cadmium.

Table – 3: Stomatal parameters.

Plants		Stomatal area μm^{-2}	T	P	Number of stomata remained open / mm^{-2} of leaf	T	P
Nerium indicum	I	5299.60± 3.09	450.07*	$< 10^{-6}$	-		
	II	2194.40± 6.08			-		
Boerhaavia diffusa	I	715.58± 0.48	603.52*	$< 10^{-6}$	56 ± 4	10.47*	$< 10^{-5}$
	II	376.52± 0.29			8 ± 2		
Amaranthus spinosus	I	247.06± 0.41	71.85*	$< 10^{-6}$	69 ± 4	14.92*	$< 10^{-6}$
	II	282.76± 0.28			11 ± 1		
Cephalandra indica	I	452.16± 0.22	995.37*	$< 10^{-6}$	47 ± 4	9.01*	$< 10^{-4}$
	II	150.58± 0.24			8 ± 2		
Tabernaemontana divaricata	I	489.72± 0.16	150.96*	$< 10^{-6}$	105 ± 4	17.88*	$< 10^{-6}$
	II	527.46± 0.25			23 ± 2		

Foot note : *Nerium indicum* possesses sunken stomata and thus stomatal openings are not detected.

stomata remaining open per mm of leaf surface decreases in all the plants under study, except in *Nerium* which, however, possesses sunken stomata. The total area for gaseous exchange of the leaves is restricted by two factors, stomatal closure and stomatal clogging. Stomatal closure is an inherent adaptation of plants to avoid the automobile exhaust entry. It was reported further that in case of onion leaves, exposed to ozone (O_3) tends to close the stomata of resistant cultivers (Engle and Globleman, 1966). Sarkar (1985) also reported the presence of such avoidance mechanism in *Cassia sophera*. In the present work simple stomatal closure is revealed in case of *Boerhaavia, Amaranthus, Cephalandra* under pollution and stomatal clogging is evident in *Nerium* and *Tabernaemonatana* leaves.

The photosynthetic rate in terms of CO_2 uptake decreased in roadside samples of N*erium, Boerhaavia* and *Amaranthus*. A similar pattern of reduction in Hill Reaction is also observed in thses plants (Table 4). Lichens like *Evernia and Ramalina* showed reduced photosynthetic rate when they were fumigated with SO_2 (Sanz *et al.,* 1992). Pb pollution was reported to inhibit photosynthesis in *Chlorella pyrenoidosa* (Poskuta *et al.,* 1996).

The impaired gaseous exchange, on one hand, is offering hindrance to normal photosynthetic activity; on the other hand, it is promoting the wasteful process, photorespiration. The increase in activity of glycolate oxidase, a key enzyme of photorespiration in *Amaranthus, Cephalandra* and *Tabernaemontana* tends to support to this view (Table 4). Again in *Nerium* and *Boerhaavia* which are regarded as C_3 plants, phosphoenol pyruvate carboxylase (PEPCase), key enzyme for C_4 pathway of photosynthesis is showing an increase in activity under pollution (Table 4). The increase in activity of PEPCase indicates the carbon fixation mechanism in these plants in possibly shifting from C_3 to C_4 pathway and subsequent decarboxylation of C_4 acid facilitates to concentrate the CO_2 level inside the mesophyll cells, which is revealed here. Similar result was obtained in the pea leaves fumigated by SO_2 (Rao *et al.,* 1983). In *Amaranthus, Cephalandra* and *Tabernaemontana,* on the other hand, the photorespiration is favoured under pollution probably to use the excess amount of ATP (Table 4), produced in the light reactions to prevent the damage of photosynthetic apparatus due to over production of ATP

Table – 4: Photosynthetic alterations.

Plants		1	T	P	2	T	P	3	T	P	4	T	P	5	T	P
Nerium indicum	I	1.565 ±.007	8.24*	$<10^{-4}$	0.097 ±.004	4.41*	$<10^{-3}$	2.083 ±.005	58.74*	$<10^{-6}$	0.008 ±.003	6.45*	$<10^{-3}$	1.815 ±.007	70.16*	$< 10^{-6}$
	II	1.485 ±..006			0.073 ±.003			1.665 ±.005			0.045 ±.004			1.087 ±.007		
Boerhaavia diffusa	I	1.335 ±.007	61.47*	$<10^{-6}$	0.242 ±.005	24.18*	$<10^{-6}$	33.16 ±.008	317.13*	$<10^{-6}$	0.037 ±.005	2.04**	0.07	1.818 ±.006	4.88*	$< 10^{-6}$
	II	0.707 ±.008			0.061 ±.005			30.032 ±.007			0.264 ±.003			1.735 ±.015		
Amaranthus spinosus	I	1.724 ±.007	132.2*	$<10^{-6}$	0.233 ±.004	7.15*	$<10^{-4}$	23.013 ±.003	285.87*	$<10^{-6}$	0.077 ±.003	41.19*	$<10^{-6}$	1.819 ±.005	51.89*	$<10^{-6}$
	II	0.473 ±.006			0.185 ±.004			25.246 ±.005			0.063 ±.003			2.513 ±.007		
Cephalandra indica	I	0.773 ±.004	974.53*	$<10^{-6}$	0.195 ±.005	35.64*	$<10^{-6}$	1.200 ±.006	304.68*	$<10^{-6}$	0.043 ±.003	3.37*	$<10^{-3}$	1.824 ±.006	24.26*	$<10^{-6}$
	II	9.775 ±.008			0.517 ±.007			4.159 ±.007			0.026 ±.003			1.614 ±.006		
Tabernaemontana divariceta	I	0.236 ±.007	8.26*	$<10^{-4}$	0.170 ±.005	4.37*	$<10^{-3}$	1.526 ±.007	661.54*	$<10^{-6}$	0.024 ±.003	0.65**	0.53	1.527 ±.004	42.62*	$<10^{-6}$
	II	0.155 ±.007			0.202 ±.005			7.908 ±.006			0.021 ±.003			1.817 ±.004		

1) mgCO_2 uptake per dm^2 of leaf per hr
2) Hill activity μ mole DCPIP reduced/mg chlo/hr.
3) Glycolate oxidase unit enzyme /mg protein
4) PEP case μM NADH oxidized/ min/mg protein
5) ATP content μmole ATP/g fresh tissue

(Taiz and Zeiger, 2001).

To determine the effect of air pollution on the respiratory pathway of the plants under study two key enzymes of respiration namely, fructose 1,6- bisphosphate aldolase (F 1,6-BPaldolase) from glycolytic pathway and succinate dehydrogenase (SD) from Krebs Cycle are assayed. The results (Table 5) show an increase in the activity of F 1,6-BPaldolase in *Boerhaavia* and *Amaranthus* whereas it shows no difference in activity in *Nerium* under pollution. At the same time, the activity of SD shows a declining effect in all the roadside plants except in *Cephalandra* (Table 5). Most surprisingly it is revealed that the oxygen uptake rate increases to a great extent in *Amaranthus* and *Tabernaemantana* and decreases in other three plants under pollution. Previous reports also showed a reduction in oxygen uptake rate in tobacco plants under ozone fumigation (Lee, 1967). But under present study the large amount of oxygen uptake in *Amaranthus* may be attributed to the manifold increase in F1,6-BP aldolase activity, which may produce in subsequent reactions a large amount of reducing power ($NADH+H^+$) to pass through the electron transport chain efficiently. But in case of *Tabernaemontana*, the situation is little different, as in this plant the activity of glucose 6- phosphate dehydrogenase (G6PD) from the oxidative pentose phosphate pathway (PPP) increased (Table 5). It is quite possible that this plant under stress is employing PPP unusually to an appreciable extent for the purpose of glucose oxidation and production of reducing power ($NADH+H^+$) (Taiz and Zeiger, 2001).

It is quite possible that a relatively high activity of glycolytic enzyme of roadside plants coupled with a corresponding diminished activity of Krebs Cycle enzyme as compared to the control counterparts may indicate a preferential shift from an aerobic to anaerobic metabolism under condition of increased stomatal diffusion resistance. This idea of possible metabolic shift was further strengthened by the observation that the plants like *Boerhaavia* and *Tabernaemontana* under pollution are showing a spectacular burst of ethanol and acetaldehyde evolution (Table 5). Kimmerer and Kozlowski (1982) also obtained a similar result with pine seedlings treated with SO_2 perhaps due to an altered glycolysis.

From the observations discussed above it may be concluded that though the plants under study are not able to escape from the site of pollution but they are quite efficient in escaping the pollution effect by altering their physiological

Table – 5: Respiratory parameters.

		F1,6-B P aldobse (Change in O.D at 560 nm/ min/mg protein)	T	P	SD(m mole succinate oxidized/hr/mg protein)	T	P	Aerobic respiration (μO_2 exchanged/g dry tissue/hr)	T	P	G6PD (n mole gas liberated /g fresh tissue/hr)	T	P	Ethanol liberated (n mole gas liberated / g fresh tissue/hr)	T	P	Acetaldehyde liberated (n mole gas liberated / g fresh tissue/hr)	T	P
Nerium indicum	I	0.016±.0005			0.052±.001			1.263±.007			2.083±.005			56.74±.03			25.39±.03		
			1.83**	0.103		7.58*	$< 10^{-4}$		24.59*	$< 10^{-6}$		218.55*	$< 10^{-6}$		175.97*	$< 10^{-6}$		79.53*	$< 10^{-6}$
	II	0.018±.001			0.035±.001			1.033±.006			1.665±.005			49.16±.03			22.35±.03		
Boerhaavia diffusa	I	0.033±.001			0.046±.001			4.257±.007			33.166±.008			15.46±.03			12.17±.02		
			5.52*	$< 10^{-3}$		11.93*	$< 10^{-5}$		88.63*	$< 10^{-6}$		7.27*	$< 10^{-4}$		530.56*	$< 10^{-6}$		459.77*	$< 10^{-6}$
	II	0.045±.002			0.025±.001			3.333±.007			30.032±.007			42.83±.04			28.34±.02		
Amaranthus spinosus	I	0.015±.001			0.035±.001			0.442±.008			23.013±.003			24.46±.03			19.17±.02		
			25.39*	$< 10^{-6}$		6.97*	$< 10^{-3}$		234.38*	$< 10^{-6}$		15.00*	$< 10^{-6}$		12.30*	$< 10^{-5}$		305.86*	$< 10^{-6}$
	II	0.084±.002			0.023±.001			3.083±.007			25.246±.005			25.08±.04			11.39±.02		
Cephalendra indica	I	0.044±.001			0.044±.02			6.064±.014			1.200±.006			37.83±.03			18.16±.02		
			4.79*	< .05		28.99*	$< 10^{-6}$		148.38*	$< 10^{-5}$		33.64*	$< 10^{-6}$		329.95*	$< 10^{-6}$		20.24*	$< 10^{-6}$
	II	0.035±.001			0.096±.042			3.509±.009			4.159±.007			23.76±.03			14.68±.17		
Tabernaemontana divaricata	I	0.034±.001			0.036±.001			2.142±.006			1.526±.007			20.56±.027			9.36±.02		
			3.15*	< .05		15.54*	$< 10^{-6}$		52.91*	$< 10^{-6}$		6.45*	$< 10^{-3}$		57.229*	$< 10^{-6}$		529.04*	$< 10^{-6}$
	II	0.023±.003			0.007±.001			2.662±.007			7.908±.006			39.63±.018			22.22±.02		

In table 3, 4 & 5 : ± values = Standard errors (five replicates were considered)
T = Results of 'T' tests between polluted (II) and less polluted(I) samples. Degree of freedom 8
P = Probability values(significance of difference)
* = Significantly different
** = Siginificantly not different.

pathways which are regarded as micro evolutionary changes by ecologists. Among the five plants under study, *Boerhaavia* may be regarded as the most flexible in adopting alternative physiological pathways (Mandal and Mukherji, 1997). But the plants like *Nerium, Amaranthus, Cephalandra* and *Tabernaemantana* also demonstrate metabolic strategy designed to cope with the stress effect.

Acknowledgements

The author is thankful for financial support of UGC, New Delhi. Author is also thankful to the Centre for Study of Man and Environment and Bidhan Chandra Krishi Visyavidyalaya for their kind help extended for monitoring air and soil quality at Rajchandrapur respectively. Last but not the least author is also thankful to Professor Subhendu Mukherji for his guidance and supervision for this work.

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