IMPAIRMENT OF CHLOROPHYLL CONTENT IN LEAVES OF *DIOSPYROS MELANOXYLON* BY FLUORIDE POLLUTION

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Abstract. The effects of fluoride pollution on the chlorophyll content of leaves of *Diospyros melanoxylon*, an economically important tree, have been determined under field conditions. Chlorophyll *a*, chlorophyll *b*, total chlorophyll, individual leaf biomass and size were reduced in polluted plants compared to unpolluted ones. The rate of increase of chlorophyll content up to September was comparatively less in polluted plants. Reduction in individual leaf biomass and size was about 32.3 and 27% respectively.

1. Introduction

Fluoride is an important phytotoxic air pollutant emitted by aluminium, steel, glass, petroleum, and phosphate industries. It causes injury to vegetation growing near the emission source and injury may extend for a considerable distance down wind. Several workers (Hitchcock *et al.*, 1962; Jacobson *et al.*, 1966; Brewer *et al.*, 1969; Leonard and Graves, Jr., 1969; Tourangeau *et al.*, 1977; Temple *et al.*, 1978) have studied its detrimental effects on vegetation. Workers like McCune and Weinstein (1971), Chang (1975) and Weinstein (1977) have reviewed the biochemical and morphological changes caused by exposure of terrestrial vegetation to fluoride.

Plants exposed to airborne fluoride show foliar damage, grow less vigorously and accumulate significant amounts of fluoride in the foliage.

The chlorophyll concentration of a plant is of ecological significance because it governs the absorption of solar radiation which ultimately affects the organic production of a plant. Therefore the present study was conducted to determine the effect of fluoride pollution on chlorophyll content, individual leaf biomass and size of individual leaves of *Diospyros melanoxylon*-a deciduous forest tree. This is an economically important tree because young leaves of this plant are used for wrapping tobacco to make an Indian cigarette called 'Biri'.

2. Materials and Methods

2.1. Study Sites

Three sites were selected near the Hindustan Aluminium Company (HINDALCO) situated at Renukoot in the Mirzapur district of Uttar Pradesh (India). HINDALCO commenced operations in May, 1962, and presently has an annual production capacity of 95 000 tonnes. Every tonne of aluminium produced results in the discharge of 40 kg of fluoride and 55 to 100 kg of particulates into the atmosphere (Ott, 1963; Stern, 1968).

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The three sites, selected on the basis of the concentration of fluoride pollutant in the area are:

Site I – Highly polluted site at a distance of 1 km from the aluminium factory.

Site II – Lesser polluted site at a distance of 3.5 km from the aluminium factory.

Site III – Control site (beyond the reach of pollutant at a distance of 12 km).

Aluminium is produced in HINDALCO by using bauxite ore. A large quantity of fluoride is reported to be given out by the HINDALCO into the atmosphere in the form of gas and particulate by Pal (1974) who has found the ambient concentration of fluoride to be 692 and 379 ppm at a distance of 1 and 3.5 km respectively from the HINDALCO. Lal and Ambasht (1981) have found the foliage fluoride concentration to be 280, 150, and 10.5 $\mu g\ g^{-1}$ dry weight of leaves of *D. melanoxylon* collected from I, II, and III sites respectively.

Leaves were sampled bimonthly from the selected sites.

2.2. Chlorophyll estimation

Leaves were sampled from the center and periphery of the top, middle, and base of the crown of the studied plants. Leaf discs were made from the leaves with the help of a cork borer (18 mm diam) avoiding the midrib area. 500 mg of leaf discs was weighed accurately. Chlorophyll was extracted by crushing leaf discs using 80% acetone, centrifuging the suspension and making the supernatant to a volume of 50 ml. The concentrations of chlorophyll *a* and *b* were estimated by measuring the optical density of the extract with a photospectrocalorimeter at 663 and 645 nm, respectively. The chlorophyll content was calculated by using Arnon's formula (1949).

The amount of pigments in mg g^{-1} was calculated by multiplying the value in mg l^{-1} with the fraction obtained by dividing the volume of the solutions (l) by the dry weight of the samples.

2.3. Dry weight determination

To determine the dry weight per leaf, 50 leaves of different sizes were sampled and dried in an oven at 80 °C for about 48 h. The dried leaves were weighed and the average weight of a leaf was calculated.

2.4. Leaf area determination

Leaf area was determined according to a method outlined by Kemp (1960).

3. Result and Discussion

The chlorophyll content (mg g^{-1}) of *D. melanoxylon* was much reduced due to fluoride emitted by HINDALCO in gaseous and particulate forms. Fluoride concentrations in leaves were reported to be 280, 150, and 10.5 $\mu g\ g^{-1}$ dry weight of leaves of *D. melanoxylon* collected from I, II, and III sites respectively (Lal and Ambasht, 1981). In the beginning chlorophyll *a*, chlorophyll *b* and total chlorophyll followed an increasing trend up to September (fully expanded and matured leaf stage) after which time a decreasing

TABLE I

Effects of fluoride on chlorophyll content (mg g^{-1} dry wt of leaves) of leaves of *Diospyros melanoxylon*

Months	Chlorophyll *a*			Chlorophyll *b*			Total chlorophyll			*a*/*b* ratio		
	A	B	C	A	B	C	A	B	C	A	B	C
March, 1978 (New leaves)	1.55	1.55	1.60	0.96	1.00	1.20	2.50	2.55	2.80	1.60	1.55	1.33
May	1.64	1.70	1.85	1.06	1.12	1.34	2.70	2.85	3.16	1.54	1.52	1.38
July	1.75	1.88	2.10	1.15	1.26	1.50	2.90	3.15	3.60	1.51	1.49	1.40
September	1.80	2.30	2.78	1.20	1.55	1.97	3.00	3.85	4.75	1.50	1.48	1.41
November	1.65	2.20	2.67	1.15	1.50	1.95	2.84	3.70	4.62	1.43	1.46	1.36
January, 1979	1.50	1.95	2.40	1.10	1.46	1.90	2.60	3.42	4.25	1.36	1.33	1.26
March (Old leaves)	1.26	1.70	2.25	0.98	1.30	1.82	2.20	3.00	4.07	1.28	1.30	1.23

trend was observed at all sites (Table I). The increase in chlorophyll concentration up to September was comparatively slower at the polluted sites (Table II). This may be due to less biosynthesis of chlorophyll under the influence of fluoride pollution. Poovaiah and Wiebe (1971) and Willis *et al.* (1974) have reported that fluoride affects the oxidative enzymes which inhibit the biosynthesis of chlorophyll. It is evident from Table III that chlorophyll *a* was more affected than chlorophyll *b*. The gradual decrease in chlorophyll *a*/*b* ratio at the polluted sites also indicates comparatively less synthesis and more destruction of chlorophyll *a* than chlorophyll *b* under the influence of fluoride pollution. Workers like Jamrich (1968), Turck and Mathe (1976), Rao and Pal (1978) have also reported that in case of fluoride pollution chlorophyll *b* is relatively more stable than chlorophyll *a*. Table III also reveals that the chlorophyll degradation at polluted sites was rapid under the influence of fluoride pollution. The greater reduction of chlorophyll *a*, chlorophyll *b* and total chlorophyll contents at the polluted sites is attributed to gradual accumulation of fluoride in the leaves which causes chlorosis. Lal and Ambasht (1981)

TABLE II

Increase (%) in chlorophyll *a*, chlorophyll *b* and total chlorophyll content relative to their previous month values in the leaves of *Diospyros melanoxylon* under the influence of fluoride pollution

Months	Increase (%)								
	A			B			C		
	Chl. *a*	Chl. *b*	Total Chl.	Chl. *a*	Chl. *b*	Total Chl.	Chl. *a*	Chl. *b*	Total Chl.
May, 1978	5.7	10.4	8.0	9.6	12.0	11.7	15.6	11.6	12.8
July	6.6	8.4	7.4	10.5	12.5	10.5	13.5	11.8	13.9
September	2.8	4.3	3.4	22.3	23.0	22.2	32.3	31.3	31.9

Chl. Chlorophyll.
A: Heavily polluted site.
B: Lesser polluted site.
C: Control site.

TABLE III

Decrease (%) in chlorophyll *a*, chlorophyll *b* and total chlorophyll during different months from their previous month values and from their maximum values in the leaves of *Diospyros melanoxylon* under the influence of fluoride pollution

Parameters	Decrease (%)					
	I			II		
	A	B	C	A	B	C
November, 1978						
Chlorophyll *a*	8.3	4.3	3.9	8.3	4.3	3.9
Chlorophyll *b*	4.1	3.2	1.0	4.1	3.2	1.0
Total Chlorophyll	5.3	3.8	2.7	5.3	3.8	2.7
January, 1979						
Chlorophyll *a*	9.0	11.3	8.6	16.6	15.2	13.6
Chlorophyll *b*	4.3	2.6	2.5	8.3	5.7	3.5
Total Chlorophyll	8.4	7.5	8.0	13.3	11.1	10.5
March,						
Chlorophyll *a*	16.0	12.8	6.2	30.0	26.0	19.0
Chlorophyll *b*	10.9	10.9	4.2	18.3	16.1	7.6
Total Chlorophyll	15.3	12.2	4.2	26.6	22.0	14.3

I: Decrease from previous month value. II: Decrease from maximum value.
A: Heavily polluted site. B: Lesser polluted site. C: Control site.

have reported that concentrations of fluoride in the leaves of *D. melanoxylon* increased with the age of leaves and hence the chlorosis percentage also increased. Due to this fact, the rate of reduction in chlorophyll content is greater at polluted sites than that at control site.

Table I also reveals that the chlorophyll content is negatively correlated with the distance of the tree from the pollutant source i.e. chlorophyll content of the leaves of the tree growing nearest to the factory was more reduced than that of the tree growing at distant places.

The individual leaf biomass and size of *D. melanoxylon* trees were also affected due to fluoride pollution (Table IV). Anderson (1960) has reported reduction in leaf area of

TABLE IV

Effects of fluoride on individual leaf biomass and individual leaf area of *D. melanoxylon*

Sites	*Dry wt. of individual leaf (g/leaf)	Reduction in individual leaf biomass (%)	*Leaf area of individual leaf (cm^2)	Reduction in individual leaf area (%)
Polluted	1.61	32.3	259.07	27.0
Control	2.38		355.25	

* Fully expanded and matured leaf stage.

many plants in fluoride polluted areas during his field studies in U.S.A. Table IV shows a 32.3% reduction in individual leaf biomass and about 27% reduction in individual leaf area of *D. melanoxylon* under the influence of fluoride pollution caused by HINDALCO. Pal (1974) and Lal and Ambasht (1981) have reported that ambient and foliar concentrations of fluoride decrease with the increase in distance from the source of pollutant and hence severity of the pollutant is also influenced accordingly.

4. Conclusion

It is evident that fluoride emitted from HINDALCO reduced the chlorophyll content, individual leaf biomass and size of the leaves of *D. melanoxylon* and by the reduction in the size of individual leaves there is an economic loss in the production of 'biris'.

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* Original not seen.