Effect of Coal fly ash On Growth, Biochemistry, Cytology and Heavy Metal Content of *Allium cepa L.*

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Abstract: To assess possible impacts of fly ash on edible crops, this investigation examined the changes in growth, biochemistry, cytology, heavy metal content of *Allium Cepa*. Results of field experiments reveals that fly ash applied to soil at the rate of 5 T/H increased germination, shoot height, Leaf number, Root number, Root length number of bulbs, peroxidase Activity, cell division process etc. Further it was not genotoxic in the Allium micronucleus test.The observed beneficial effects of fly ash on crop growth & its yield performance may be attributed to its contents of plant nutrients especially the trace elements in poor or marginally deficient soil. Because of the presence of heavy metals in the edible parts, it may be concluded that fly ash can be used in agriculture as soil amendment with caution. [New York Science Journal 2010;3(5):10-16]. (ISSN: 1554-0200).

Key words: Fly ash, Onion, Growth, Biochemistry, Cytology, Soil and Heavy metal

1. Introduction

Thermal power stations generate fly ash (FA) with varying concentrations of contaminating cations depending on the quality & the source of used coal (Pluss and Ferrel, 1991). In India, FA is generated in a higher proportion (to the amount of used coal) compared with station data in USA; (Canada Warren and Dudas, 1984). For production of one mega watt of energy, in India 5-6 tons of coal are generally used (Sen and Kumar, 1995), while the American figures are almost the half (Warren and Dudas, 1984). Consequently, the dumping sites of FA grew faster by 80 million ha/ year in India (Sen and Kumar, 1995), of course, eco-friendly methods of disposal of FA grew faster by 80 million ha/ year in India (Sen and Kumar, 1995). Of course, eco-friendly methods of disposal of FA have been followed, but heaps of FA lie unclaimed and contaminate adjacent crop fields.

Fly ash has a potential in agriculture and related applications. Physically Fly Ash occurs as very fine particles, having an average diameter of < 10 mm, low to medium bulk density, high surface area and very light texture. Chemically the composition of Fly Ash varies depending on the quality of coal used and the operating conditions of the Thermal Power Stations. Approximately on an average 95 to 99% of Fly Ash consists of oxides of Si, Al, Fe and Ca and about 0.5 to 3.5% consists of Na, P, K and S and the remainder of the ash is composed of trace elements. In fact, Fly Ash consists of practically all the elements present in soil except organic carbon and nitrogen. Thus it was found that this material could be used as an additive / amendment material in agriculture applications. In view of the above, some agencies/ individuals/ institutes at dispersed locations conducted some preliminary studies on the effect and feasibility of fly ash as an input material in agricultural applications. Some amount of experience was been gained in the in the country and abroad regarding the effect of fly ash utilization in agriculture & related applications.

Further, higher levels of macro and micro-nutrients in FA have been shown to affect growth and yield of several crops in USA, Europe and India adversely, i.e. maize and soybean (Mishra and Shukla, 1986), barley and cabbage (Koracak, R.F, 1995); apples and Soybean (Fail and Wochok, 1977). But there is no report on growth yield, cytology, metabolism and elemental composition of onion grown in costal lands of India.

2. Material and Methods

Electro statically precipitated FA obtained from talcher thermal station, Orissa in an un-weathered condition consists of the following (%): sand 82.0, silt 10.0, clay 8.0, pH 7.0, & the following elements (mg / kg soil) Na 1180, K 3900, P 45.5, Fe 325, Mn 103, Ni 5.8, Co 5.15, Zn 36.0, Cu 5.06, Pb 8.3, Cr 0.0, and Cd 0.0, generating weight amounts of 30 - 40% of the used coal (Sen and Kumar, 1995).1x 1 m experimental fields were prepared by repeated ploughings and soil samples were collected for analysis. Equal amount of organic compost (7.5 T/H)

was applied to all the fields, watered properly & left for a day. Then fields amended with required concentrations of FA (1,2.5, 5, 10,15 & 0 T/H) by ploughing and control is maintained uniformly without FA application (0T/H).Bulbs of *Allium cepa* were obtained from local Horticulture office & used in this study.100 bulbs / plot, were presoaked overnight. The soaked bulbs were sown in the fields so that the distance between the plants & rows is kept as per agronomic practice.

The germination counts were recorded. Within uniform time gap period morphological parameters like seedling height, shoot height, leaf number etc were recorded. After harvest the dry weights and fresh weights of plant parts were recorded.

2.1 Metal Analysis

Detailed methods of study of soil characteristics have been described previously (Harper et al. 1989). Digestions of soil samples were done in 20 ml of mixed acids (10N HNO_3, 12 N H_2SO_4, 60% $HCIO_4$ in the ratio (5:5:1) (Shaw P.J.A,1992). Harvested roots were thoroughly washed & oven-dried at 100^0C for 40h, and the dried plant parts ground to powders. Lots of 1g of the powders were digested with $10NHNO_3$ (Sen and Kumar, 1995), and subjected to elemental analysis using an atomic absorption spectrophotometer (Model AA 1475, at regional research laboratory, Bhubaneswar), whereas Na and K contents were analyzed by flame photometry.

2.2 Pigment and Enzyme Study

Fresh leaves of *Allium cepa* and were collected time to time at different ages (in days) and pigment contents like ch1.a, ch1.b, carotenoid content etc. were recorded. Chlronphyll was extracted in 80% acetone and the absorption at 490, 645, 663 nm were read in a spectrophotometer, using the absorption coefficients, the amount of chlorophyll was calculated (Arnon D.I, 1949).

Freshly weighed (100 mg) samples of *Allium cepa* leaves in triplicate for each treatment were homogenized in cold 0.1 M sodium phosphate buffer at pH-7 and centrifuged at 4^0C for 10 minutes at 10, 000 rpm. The supernatant was used as the enzyme extract for peroxidase assay by (Maehly and Chance, 1967), with minor modifications made by (Subhadra and panda, 1992).

The abbreviations and values for different soil parameters mentioned in table 7 are as follows : All values except pH, electrical conductivity (EC) cation exchange capacity (CEC), are in percent values (%). EC:micro mhos cm^{-1}, CEC; cmols kg^{-1}. OC= organic carbon; OM= organic matter; WHC= water holding capacity.

2.3 Cytological Study

a) Mitotic index Test

The bases of the bulbs were scrapped exposing the root eyes and kept in surface touch with water in specimen tubes. About 8-15 roots emerged within 2-3 days. Meristems of 20 -30 mm long roots which give the highest cell division were used in our experiment.1000 cells are scored for mitotic cells with division and the percentage of cells in division was calculated as Mitotic Index in the root meristems.

b) Clastogenicity Test

Roots were processed for clastogenicity studies according to the method of (Kihlman, 1971). primary roots of *Allium cepa* were treated for 1 hour in the experimental solution & recovered in knopp's solution for 6,12,24 & 48 hours in order to detect the delayed and non delayed effects. In every recovery the roots were pretreated in colchicine (0.05%) 2 hrs prior to the closure of the recovery schedule to facilitate metaphase studies. Each series of experiments included control with treated set & processed in an identical way but without the test compound. All treatments and recoveries were carried out in darkness & at room temperature. Roots were fixed & squashed according to the procedure described earlier. 10 slides were prepared from 5 meristems derived from different bulbs.

C) Micronucleus Test

The classical Allium test originally developed by (Levan, 1938), for studying the effect of chemicals on chromosome and cell division, is one of the simplest and most validated cytogenetic assay systems presently available (Grant, 1982). This system has further been recommended as a standard in environmental monitoring (Fiskes jo, 1970). The end points usually measured in Allium test so far, have been mitotic aberrations or turbogenic effects (Kihlman, 1966), and chromosomal aberrations or clastogenic effects (Kihlan, 1971). An attempt has been made to increase the sensitivity as well as the versatility of the Allium test following a new protocol of exposure of root meristems to contaminated water or soil. Unlike the earlier studies (Ramel, 1967, 1969; Fiskesjo, 1969, 1970), in the present study the end point measured was the frequency of cells with micronucleus.

Roots of Allium were exposed to soil amended with different concentrations of fly ash. In this experiment soil samples containing soil and sand (1:2) were used. This ratio of soil and sand was found suitable for best root growth. In order to keep the moisture content of the experimental soil uniform throughout the experiment, 100 ml of distilled water

was daily added to each pot. 5 bulbs per exposure were used. Each exposure had 3 replications. On the 5th day of exposure the bulbs were removed and washed thoroughly. The root lengths were recorded. Subsequently 10-15 root meristems, chosen at random from 15 bulbs were excised & fixed in acetic acid: ethanol (1:3) for cytological analysis by (Darlington and lacour, 1976). The cytological slides were examined under a microscope to score cells with micronuclei.

3. Results and Discussions

Soil amended with different doses of FA induced better growth, metabolism, pigment synthesis, enzyme activity, cytology and yield in comparison to untreated plants in our experiments.

Ex situ Germination Pattren of *Allium cepa* is not affected by the graded application of fly ash, however higher dose (15 t/h) is slightly toxic to the germination process. FA amendments in soil caused a gradual increasing trend of each growth parameters viz: Root number, Root length, Shoot length, Leaf number, Fresh wt, Drive wt till 5t/h but decrease at higher doses like 10 and 15 t/h (Table-1). Statistical analysis of the data supported this conclusion at levels of significance as indicated in tables.

Contents of both chlorophyll a and b of *Allium Cepa* leaves had increasing trends with increasing FA amendments up to 5t/h but at higher level of FA Chlorophyll steadily decreased. Carotene content decreased in all concentration. Total chlorophyll followed the same trend of chlorophyll a and chlorophyll b (Table-2).

All the concentration Of FA increased peroxidase activity measured after 15 days of sowing except 1 and 15t/h. The highest peroxidase activity was observed in 5 t/h FA treatment. None of the concentrations were significant from its control (Table 3).

Mitotic index percentages were counted 4 days after sowing the bulbs. Mitotic index percentage increased progressively with increase in the concentration of FA. 5, 10, 15 t/h showed increase in percentage of mitotic index significantl from the control. FA treatments vs mitotic index showed positive correlation (Table-4).

In our clastogenicit test, fly ash water did not induce any kind of chromosomal aberrations in all the recover periods of *Allium cepa* (Table-5).

Allium cepa was grown in the soil amended with different amounts of fly ash to investigate the genotoxic effect in the root meristems. Fly ash did not induce micronucleus in the somatic as well as germinal cells. There are few micronuclei and their frequency is very low for consideration (Table-6).

The present result with fly ash demonstrated that it is micronucleus negative in our allium micronucleus test (table-6). Also the fly ash water did not cause chromosome aberration in comparison with its control in *Allium cepa* root meristems (table-13). On the other hand fly ash water and fly ash amended soil induced increased root number, root length, seedling height accompanied by higher mitotic index from its control. Further the biochemical analysis (peroxidase assay) in the levels of *Allium cepa* grown on fly ash amended soil shows adoption and stress tolerance to fly ash with higher catalase activity. Thus the absence of mutagenic events in our test system may be due to protective action of peroxidase activity which checks the free radical oxygen mediated oxidative DNA damage/clastogenesis / micronucleus formation in our test system during replication / repair process by (Carlson et al., 1988).

FA amendment in soil caused a gradual decrease in sand, but increase in clay percentages. The pH value increased from natural to 8.3 due to FA amendments where as electrical conductivity (EC) and cation exchange capacity (CEC) remained almost unchanged. Values of organic carbon & organic matter increased with increasing FA amendments. The water holding capacity (WHC) of soils also increased steadily with FA amendments (table -7).

Elemental analysis of soil with different grades of FA revealed that the net content of Na was not affected where as a gradual increase of K level in soil was noticed. The P content of soil increased steadily by from 45.5 (control) to 70. 0 mg /kg. The Fe content of soil remained almost unchanged due to FA amendments. Micro elements Mn, Ni, Ca, Zn, Cu had progressive increases due to increasing FA amendments and contaminating elements Pb, Cr and Cd had too (table -8).

Elemental analysis of onion bulb revealed that Na was unaffected, while K and P contents gradually increased but Fe remained unchanged due to FA amendments. Microelements and heavy metals accumulated at higher concentrations in comparison to control (table-9).

Field experiments carried out with *Allium cepa* grown in FA amenements clearly indicated that growth and yield of the crop was significantly increasing at 5t/h FA. Similar results of growth and yield enhancements by FA were recorded for rice in this laboratory, however, 15 Mg FA/ ha was the highest level tolerated by rice by (Mishra M et al., 2005). Similar FA-related growth enhancements have been recorded for several crops, grown in other countries (Koracak R.F,1995) and (FailJ.L and Wochock Z.S, 1977), (Allen S.E et al., 1974). Ecological studies on effects of FA contaminating terrestrial & aquatic habitats have also been well-

documented (Mishuntinand E.N and Shilinikova V.K, 1971) and (Twardowska, 1990). All these reports recorded heavy metal pollutions due to FA & observed.

Although, fly ash has many benefits as an in put material for agriculture applications, in view of the fear in the minds of many (regarding the levels of natural radioactivity in Fly Ash and/ the characteristic presence of some amounts of heavy and toxic elements in it) there may be some cautions which have to be taken for the time being while using Fly Ash in agriculture. From the information available till now, there appears to be not much ground for concern on these accounts (heavy metals, radioactivity etc) however further confirmatory studies at the ICAR centers would be helpful in bringing out recommendations in this field. Meanwhile there appears to be sufficient ground now for the cautious and judicious use of this useful material, which is otherwise being wasted/ underutilized.

Table-1 : Effect of Coal-Fly ash on Growth & yield Parameters of *Allium cepa*.Probability levels: - * P<0.05, ** P<0.01, *** P<0.0001

FaApplication (In t/h)	Ch 1 a	Ch 1 b	Ch1 a+b	Carotenoid
0	0.019± 0.011	0.020± 0.011	0.039± 0.022	1.216 ± 0.702
1	0.010± 0.005	0.018 ± 0.010	0.028 ± 0.016	0.820 ± 0.473
2.5	0.011± 0.006	0.007± 0.004	0.019± 0.011	2.127± 1.228
5	0.023± 0.013	0.018± 0.010	0.041± 0.023	3.352 ± 0.935
10	0.018± 0.010	0.016± 0.009	0.034 ± 0.020	2.771± 0.599
15	0.013± 0.007	0.013 ± 0.007	0.026 ± 0.015	1.402 ± 0.809

Table 2: Pigment (Ch1 a, ch1 b, ch1 a+b, Corotenoid) content (mg g^{-1} FW) in the leaves of *Allium cepa*. Grown on soil treated with different conc. of coal fly ash amended soil.

FaApplication (In t/h)	Germination (%)	Root number	Root length	Shoot length	Leaf number	Totalbulbs	TotalFW	TotalDW
0	77.5	7.2 ± 0.6 **	5.2± 0.3 *	22.0 ± 0.4	24.7 ± 1.2	79	212	99
1	100.0	12.6 ± 1.7 **	6.1 ± 0.3	23.3± 1.1	24.5± 1.1 **	109	292	116
2.5	92.5	13.3± 1.9 ***	5.4 ±0.3	23.1± 0.7 *	30.8 ±1.7 ***	109	236	102
5	97.5	17.7± 2.4 ***	5.6± 0.6	23.7± 0.6	31.8 ±1.5	116	302	123
10	92.5	17.9 ± 1.9 ***	5.0 ± 0.5	23.0± 0.6	26.9 ±1.7	105	224	101
15	77.5	16.9± 1.4	6.3± 0.5	23.0± 0.8	27.0± 2.3	83	188	65

Table 3: Peroxidase activity in the leaves of *Allium cepa*: grown in different conc. of fly ash amended soil.

FaApplication (In t/h)	PEROXIDASE ACTIVITY (In mM of H_2O_2 g^{-1} FW) (Mean ± SEM)
0	1.083 ± 0.625
1	1.066 ± 0.615
2.5	1.183 ± 0.683
5	1.300 ± 0.550
10	1.116 ± 0.644
15	0.800 ± 0.461

Table 4 : Cytological Effect of coal flyash on mitotic index in the root meristems *Allium cepa*.Probability levels :- * P<0.05, ** P<0.01 , *** P<0.0001

FaApplication (In t/h)	MITOTIC INDEX
0	2.205 ± 0.493
1	2.931 ± 0.732
2.5	3.307 ± 0.826 *
5	4.016 ± 1.073 **
10	4.904 ± 1.310 ***
15	5.591 ± 1.443

Table 5 : Clastoganic response in the root meristems of *Allium cepa* exposed to fly ash water for one hr and recovered in tap water for 6, 12, 24 & 48 hrs.

		Cells with					
Test Solution	**Duration of recovery (in hr.)**	**Metaphases scored**	**Gaps (No.)**	**Breaks (No)**	**Exchanges (No.)**	**Total Aberrant cells**	**% Aberrant cells.**
Control	0	100	0(0)	0(0)	0(0)	0	0
Fly ash water	6	100	0(0)	0(0)	0(0)	0(0)	0
	12	100	0(0)	1(1)	0(0)	1(1)	1
	24	100	0(0)	1(1)	0(0)	1(1)	1
	48	100	1(1)	0(0)	0(0)	1(1)	1

Table 6: Micronucleus frequency in the root meristems of *Allium cepa*, exposed to different concentrations of fly ash amended soil for five days

Fa Treatments (In t/h)	**No of days treated**	**No of cells scored**	**No of micronuclei**	**Micronucleus frequency (%)**
0	5	1000	1	0.1
1	5	1000	1	0.1
2.5	5	1000	2	0.2
5	5	1000	2	0.2
10	5	1000	1	0.1
15	5	1000	1	0.1

Table 7: Influence of fly ash on soil physical properties

Fly ash (t/h)	**Sand**	**Silt**	**clay**	**pH**	**EC**	**CEC**	**OC**	**OM**	**WHC**
0	82.0	10.0	8.0	7.0	210	2.35	0.58	0.99	33.68
1	81.0	11.0	8.0	7.5	265	1.78	0.54	0.93	34.69
2.5	80.0	11.5	8.5	8.1	265	256	0.60	1.03	33.75
5	77.0	14.0	9.0	8.0	280	2.48	0.58	0.99	36.13
10	70.0	19.0	11.0	8.10	320	3.0	0.64	1.10	39.93
15	66.0	23.0	11.0	8.2	346	2.82	0.73	1.26	42.93

Table -8: Influence of FlyAsh on soil elemental status.(values in mg/kg)

FA	Na	K	P	Fe	Mn	Ni	Co	Zn	Cu	Pb	Cr	Cd
0	1180	3900	45.5	325	03	5.80	5.15	36.0	5.06	8.3	0.0	0.0
1	1215	4280	36.9	267	100	5.79	6.10	39.6	5.73	9.50	0.02	0.0
2.5	1180	6500	46.0	340	161	6.67	6.68	46.0	5.97	13.46	0.20	0.0
5	900	7050	50.3	263	195	8.50	7.36	49.8	7.50	12.97	1.06	0.005
10	850	8290	46.8	300	211	12.05	10.22	51.0	10.0	17.58	1.39	0.02
15	1025	10150	70.0	310	240	15.37	17.31	67.0	14.38	20.00	1.89	0.06

Table -9 : Elemental Uptake in Onion bulbs Grown in Fly ash amended soil. .(values in mg/kg)

FA	Na	K	P	Fe	Mn	Ni	Co	Zn	Cu	Pb	Cr	Cd
0	800	5060	25.8	48.0	24.4	1.61	0.5	23.00	0.85	0.85	0.06	0.18
1	940	7750	29.0	47.0	23.6	3.64	1.48	32.6	1.69	2.24	1.67	0.64
2.5	968	8060	32.3	50.0	25.3	5.36	3.22	39.0	2.5	3.71	3.0	0..91
5	875	10000	32.5	51.3	23.8	4.10	2.73	36.7	1.58	2.38	1.43	0.45
10	780	10000	42.0	54.2	36.7	8.03	6.40	53.7	4.85	7.81	5.63	2.46
15	845	16200	38.0	50.1	42.3	9.79	5.11	51.2	3.96	6.00	3.86	1.87

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