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Studies on the effect of fly ash amelioration on amino acids and protein content of the seeds of *Helianthus annuus*

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ABSTRACT

Fly ash is an industrial waste of thermal power plant and has been regarded as a problematic solid waste all over the world. The conventional disposal methods of fly ash lead to degradation of aerable land and contamination of the ground water as well. However, fly ash is useful ameliorant that improve the physical, chemical and biological properties of problem soil and is readily available source of macro and micronutrients for plants. *Helianthus annuus* is the good source of essential fatty acids, phosphatids, protein and vitamins. There are amazing effects of fly ash on different parameters of *H. annus*.

Key words: Fly ash, Amino acids, Protein content, *Helianthus annuus*.

INTRODUCTION

Fly ash closely resembles volcanic ashes used in production of the earliest known hydraulic cements about 2,300 years ago. Those cements were made near the small Italian town of Pozzuoli - which later gave its name to the term “pozzolan.”

A pozzolan is a siliceous or siliceous / aluminous material that, when mixed with lime and water, forms a cementitious compound. Fly ash is the best known, and one of the most commonly used, pozzolans in the world.

Fly ash is a fine, glass powder recovered from the gases of burning coal during the production of electricity. These micron-sized earth elements consist primarily of silica, alumina and iron.

Oil of *H. annuus* is gaining attraction as edible oil due to its good essential fatty acid (EFA) contents. It is also a rich source of phosphatids proteins and vitamins. In addition to protein and linoleic acid (EFA), sunflower seeds are also an excellent source of dietary fiber, Vitamin E, Vitamin B and minerals such as magnesium, iron, phosphorus, selenium and zinc. Additionally, seeds are rich in cholesterol-lowering phytosterols. They are also a good source of calories.[3]

But the plant is very much pH sensitive. It can not grow on acidic soil. In acid soil nutrient availability reduces as at low pH, nutrients convert into their non-available and non-absorbable forms.

Its oleic to linoleic acid ratio is also affected by climatic condition, plant location and genetics [4].

Current studies on these and other derived acids have showed that they play a critical role in the body such as inhibiting the growth of malignant cells without interfering normal cells.[6] In the present study we have observed effect of fly ash on different parameters of *H. annus* (physical, physiochemical and chemical aspects).

MATERIAL AND METHODS

For the accomplishment of the objectives experiment was started with sowing the seeds of *H. annuus* in control and different combination of soil and fly ash (table-I). Initially, fly ash and soil were dried and mixed homogenously and analyzed for pH, electrical conductivity and nutrients (table-2 & 3). Percent germination, Plant height, number of branches per plant was studied timely. After full growth, leaf area was determined by planimetry, Chlorophyll content by the method of Loomis and shull[7,8] and organic carbon content was by Walkley and Black method[9]. Method of Subbiah and Asija[10] was taken as reference for estimation of nitrogen while Phosphorus was determined by oleson's method. [11] Other plant nutrients such as Manganese, Molybdenum and zinc were estimated as per standard procedure.[12]

Seeds obtained from differently treated plants were dried and weighed. After preliminary observation, seeds were subjected to soxhlation for the extraction of oil. Exhausted seeds were re-extracted for protein for several hrs with 10% brine solution for the isolation of proteins. Isolated protein was hydrolyzed by the action of mixture of concentrated hydrochloric acid and formic acid to form hydrolysate and was studied by HPLC. Oil of seeds obtained was saponified and fatty acid mixture was isolated. Isolated mixed fatty acids were then treated with methanol and concentrated sulfuric acid to from methyl esters of the same. [13]

RESULTS

Pre and post harvest studies of various plants obtained from different treatment was carried out. Pre harvest study included plant growth parameter like percent germination, plant height, leaf area and chlorophyll content and the post harvest studies comprised of grain yield and seed analysis.[14]

The change in physico-chemical properties of soil was observed on increasing fly ash content

Table 1: Symbols for treatment

S. No.	Symbol	Treatment
1	A	Control
2	B	N.P.K.
3	C	N.P.K.+10 %Fly ash
4	D	N.P.K.+20 %Fly ash
5	E	N.P.K.+30 %Fly ash
6	F	N.P.K.+40 %Fly ash
7	G	N.P.K.+50 %Fly ash

Table 2: The ph and electrical conductivity

S. No.	Treatment Symbol	pH	E.C. (millimho)
1	A	6.22	.080
2	B	6.31	.088
3	C	6.43	.095
4	D	6.48	.102
5	E	6.89	.127
6	F	6.83	.124
7	G	7.23	.132

Table 3: Nutrient content

S. No	Treatment Symbol	Al_2O_3 (%)	CaO (%)	SiO_2 (%)	Zn (ppm)	Mn (ppm)	Mo (ppm)	N (kg/ha)	K (kg/ha)	P (kg/ha)
1	A	8.54	1.201	78.99	2.46	11.4	0.038	457.6	169.8	2.91
2	B	9.91	1.208	80.23	2.49	11.4	0.039	458.7	171.2	3.03
3	C	10.21	1.232	81.62	2.52	11.7	0.041	460.1	172.7	3.22
4	D	12.82	1.247	83.09	2.55	11.9	0.045	461.4	174.0	3.37
5	E	14.54	1.272	84.48	2.61	12.1	0.048	463.2	176.1	3.51
6	F	16.90	1.295	86.12	2.69	12.2	0.052	464.8	177.5	3.62
7	G	18.30	1.317	87.39	2.72	12.4	0.046	466.0	179.2	3.76

of the mixture viz. pH has been increased 6.22-7.23 , conductivity has been increased 0.080-0.132 (Kg/ha) in available Nitrogen(table-2), 2.46-2.72 in zinc, 0.038-0.046 in Molybdenum (21.50%) and 11.4-12.4(8.77%) in Manganese content (table-3).

During this experiment no toxicity (or adverse effect) of metallic nutrients were seen. A significant increase in percent germination was observed in treatment E (20.9%, $p<0.05$) over control (table-4). Plant height showed an increase of 30.24% over control in the same treatment. Number of branches per plant also increased and was maximum (76%, $p<0.01$) in treatment G. leaf area and chlorophyll content has also been maximized in treatment E and found 96.17 (27.59%, $p<0.01$) and 94.23 (33.35%, $p<0.05$) respectively (table-5)

Grain yield was maximum in 30% fly ash treated soil and was 25.09 % ($p<0.05$) greater than control. Seed size and density showed a liner significant increase over control and was 11.8% and 8.29% more in treatment F and G respectively (table-6).

Protein content of the seed linearly increased up to treatment F (31.82%). Simultaneously, amino acid content was found to increased in same concentration (table 7 and 8)

Table 4: Percent germination in posts

S. No.	Treatment Symbol	Germination	Change (%)
1	A	62*	-
2	B	65**	4.8387
3	C	67***	8.064
4	D	70***	12.903
5	E	75***	20.967
6	F	71***	14.5161
7	G	68***	9.677

(*** = P<.05, ** = P<.01, * = P+n.s.)

Table 5: Preharvest study of plants

S. No.	Treatment Symbol	Germination	Change (%)	Leaf Area (Cm^2)	Change (%)	Chlorop-hyll Content(mg)	Change (%)
1	A	62	-	75.36	-	70.66	-
2	B	65	4.083	83.14	10.31	74.82	5.88
3	C	67	8.06	87.32	15.85	80.34	13.69
4	D	70	12.90	90.32	19.71	89.67	26.89
5	E	75	20.96	96.17	27.59	94.23	33.35
6	F	71	14.51	89.34	18.53	90.78	28.46
7	G	68	9.67	84.12	11.61	88.32	24.98

Table 6: Seed size

S. No.	Treatment Symbol	Seed Size (cm)	Weight (100 Seeds)	Volume (100 Seeds)	Density (gm/cc)
1	A	1.106	6.6028	16.1	.4101
2	B	1.113	6.8134	17.0	.4007
3	C	1.165	6.9945	17.8	.3929
4	D	1.191	7.3221	18.7	.3915
5	E	1.216	7.6936	19.8	.3885
6	F	1.237	7.9267	19.7	.4023
7	G	1.221	8.3932	18.9	.4441

Table 7: Protein content

S. No.	Treatment Symbol	Protein	Change (%)
1	A	32.7*	-
2	B	34.3*	4.89
3	C	37.6***	14.99
4	D	40.1***	22.64
5	E	40.6***	30.29
6	F	43.1**	31.82
7	G	42.9**	31.21

(*** = P<.05, ** = P<.01, *= P= n.s.)

DISCUSSIONS

Results obtained from the experiments showed that the sunflower responded positively to fly ash amelioration. With the increase in fly ash contents of the mixture, pH of soil also increases. Increased pH values coupled with good micronutrient content of fly ash provides optimum condition for the maximum uptake of micronutrients by plant. Low value of pH has negative effect on growth of plant since acidity deteriorates root tip and converts nutrients in to their unavailable form and thereby their uptake decreases. This is the reason due to which the plant may show a good growth in the presence of fly ash. The fly ash contains Zinc, Manganese, and Magnesium, Molybdenum etc. [18] Therefore on addition of fly ash, concentration of these nutrient increases. Due to increase in concentration of above said nutrients in the plants various hormones and enzymes of the plant may also get activated. This fact has also been supported by the work of Savithri, [14, 15, 16] Singh and Kapoor, who showed that the increased zinc uptake increases the grain yield. Zinc availability in turn influenced by nitrogen concentration in the soil. Increased enzyme activity on fly ash has also been reported by Sarangi *et al.* [18]

Table 8: Amino acid content (gm/100gm protein)

S. No.	Treatment Symbol	LYS	PHE	VAL	LEU	MET	TYR	THR	HIS
1	A	5.13	3.70	3.69	3.10	1.65	2.66	5.9	2.37
2	B	5.33 (3.88%)	3.82 (3.24%)	3.77 (2.17%)	3.43 (10.67%)	1.69 (2.42%)	2.71 (1.88%)	5.97 (1.18%)	2.43 (2.53%)
3	C	5.78 (12.67%)	3.93 (6.21%)	3.84 (4.06%)	3.67 (18.43%)	1.78 (7.87%)	2.78 (4.51%)	6.11 (3.55%)	2.57 (8.43%)
4	D	6.12 (19.20%)	4.06 (9.72%)	3.98 (7.86%)	3.89 (25.55%)	1.86 (12.72%)	2.82 (6.01%)	6.18 (7.74%)	2.62 (10.54%)
5	E	6.40 (24.63%)	4.23 (14.32%)	4.11 (11.38%)	4.51 (45.61%)	1.96 (18.78%)	2.97 (11.65%)	6.25 (5.92%)	2.70 (13.92%)
6	F	6.54 (27.35%)	4.28 (15.67%)	4.18 (13.28%)	4.63 (49.49%)	2.08 (26.05%)	3.20 (20.29%)	6.29 (6.60%)	2.76 (16.45%)
7	G	6.47 (25.98%)	4.12 (11.34%)	4.07 (10.29%)	4.21 (35.90%)	2.02 (22.42%)	3.03 (13.87%)	6.17 (4.57%)	2.71 (17.71%)

Chlorophyll content has been found to be increased which may be due to increased in availability of zinc and magnesium. Zinc is involved in electron transfer system, activation of enzymes and growth hormones and magnesium is central atom of chlorophyll porphyrine ring [19, 20].

Seed analysis results in regular increase in size and weight up to treatment D. Protein content of the seed also increases and can be attributed to the fly ash addition, which ensures good availability of nutrients and their up take. These nutrients play a significant role in the synthesis of proteins by

different mechanisms for example, molybdenum is important in nitrogen fixation, zinc is activator of enzymes and promotes seed maturation, Copper contributes toward proteins metabolism and manganese is essential for photosynthesis and nitrogen assimilation [21]. Amino acid content of seeds is also affected by nutrient content. [22]

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