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Application of fly ash on the growth performance and translocation of toxic heavy metals within *Cajanus cajan* L.: Implication for safe utilization of fly ash for agricultural production

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ABSTRACT

The present study was undertaken to examine the influence of the application of fly ash (FA) into garden soil for *Cajanus cajan* L. cultivation and on accumulation and translocation of hazardous metals from FA to edible part. Numerous studies have been reported on the growth and yield of agricultural crops under FA stress; however, there is a dearth of studies recommending the safe utilization of fly ash for crop production. Pot experiments were conducted on *C. cajan* L., a widely cultivating legume in India for its highly nutritious seeds. *C. cajan* L. were grown in garden soil and amended with varying concentrations of FA in a weight/weight ratio (0%, 25%, 50% and 100%; w/w). Incorporation of fly ash from 25% to 100% in garden soil increases the levels of pH, particle density, porosity and water holding capacity from 3.47% to 26.39%, 3.98% to 26.14%, 37.50% to 147.92% and 163.16% to 318.42%, respectively, than the control while bulk density decrease respectively from 8.94% to 48.89%. Pot experiment found that accumulation and translocation of heavy metals in tested plant depends on the concentration of FA. Addition of FA at lower concentration (25%) had shown positive results in most of the studied parameters of growth and yield (14.23% than control). The experimental results confirmed that lower concentration of FA (25%) is safe for *C. cajan* cultivation, which not only enhanced the yield of *C. cajan* L. significantly but also ensured the translocation of heavy metals to edible parts within the critical limits.

1. Introduction

Increasing dependence on coal as an energy source in thermal power plants resulted in vast quantities of coal residues known as fly ash (FA). FA has been regarded as a problematic solid waste all over the world. In India, more than 100 million ton of FA is generated annually [1,2] from coal-based thermal power plants [3]. The production of FA (including both fly ash and bottom ash) may likely to exceed 140 million ton per annum by 2020 [4,5]. Percentage ash utilization in different countries amounts to more than 85% in west Germany, 73% in Denmark, 60% in France and UK, 50% in Poland, 32% in US, 25% in China. However, in India, a minor percentile of FA (<15%) is being used for preparing bricks, ceramics and cements and a vast amount of FA remains unutilized which requires an additional 100 ha of land each year for disposal.

FA is alkaline in nature having high sorptive capacity, mainly composed of ferroaluminosilicates. Chemically, 90–99% of fly ash is comprised of Si, Al, Fe, Ca, Mg, Na, and K with Si and Al forming the major matrix [6,7]. Al in FA is mostly bound in insoluble aluminosilicate structures, which considerably limits its biological toxicity [8]. FA also contains radioactive elements like ^{222}Ru and ^{220}Ru [9]. However, most of FA is not significantly contain radioactive elements [10]. Tadmore [11] reported radionuclides of uranium (U) and thorium (Th) series in FA. Fly ashes increase the surface area available for element adsorption, improve the physical properties of soil [12], and neutralize the pH of soils [13]. FA contains alkaline (K) and alkaline earth (Ca, Mg) metals that are important plant nutrients. In addition, it may also improve the physico-chemical properties of the soil such as pH, texture and water holding capacity (WHC). The use of FA in agriculture has been based on its liming potential and supply of nutrients such as Ca, Na, K, Mg, B, S and Mo, which promote growth of the plants and also alleviate the condition of nutrient deficiency in soils [3,14,15].

Apparent high economic cost of disposal and subsequent environmental management necessitate the safe utilization and disposal of FA. In view of this, utilization of FA in agriculture could be a viable option. The present work has been aimed to (i) assess the effective utilization of FA for crop production, (ii) to check the

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Table 1
Physico-chemical properties of garden soil (GS) and fly ash (FA).

Parameters	Garden soil	Fly ash	't'-test
pH	7.20 ± 0.39	9.10 ± 0.46	5.46**
Electrical conductivity ($dS\,m^{-1}$)	1.20 ± 0.06	7.60 ± 0.38	28.81**
Cation exchange capacity [$meq\,(100\,g)^{-1}$]	1.59 ± 0.08	1.25 ± 0.06	5.89**
Total nitrogen (%)	1.39 ± 0.07	0.02 ± 0.001	33.90**
Total phosphorus (%)	0.76 ± 0.03	0.11 ± 0.04	22.52**
Organic carbon (%)	1.47 ± 0.07	1.16 ± 0.06	5.82**
Metals ($\mu g\,g^{-1}$ dw)			
Cu	14.50 ± 0.73	58.43 ± 2.92	25.28**
Cr	36.11 ± 1.81	40.32 ± 2.02	2.69ns
Cd	27.46 ± 1.40	42.51 ± 2.13	10.23**
Al	111.29 ± 5.57	4851.00 ± 232.0	35.38**
Pb	40.44 ± 2.05	40.17 ± 1.99	0.16ns
B	10.22 ± 0.51	28.95 ± 1.41	21.64**
Mo	40.32 ± 2.02	33.32 ± 1.67	4.63**
Fe	603.58 ± 28.46	4017.00 ± 2.3.0	28.84**
Mn	204.00 ± 10.20	69.36 ± 3.47	21.64**
Zn	108.60 ± 5.31	82.27 ± 4.06	6.82**
Ni	24.67 ± 1.24	204.86 ± 10.20	30.37**

Values are mean ± S.D. (n = 3), ns = (p > 0.05), ** = (p < 0.01).

safe level translocation of heavy metals present in FA to the agricultural commodities. For this, application of FA was tested on the growth, yield and heavy metal translocation potential of a leguminous plant, i.e. pigeon pea (*Cajanus cajan* L.), which is widely used in India for its high protein content.

2. Materials and methods

Unweathered FA samples were collected from the FA-dykes of National Thermal Power Plant (NTPC), Tanda, and garden soil (GS) from the garden of M.L.K. (P.G.) College, Balrampur, (Uttar Pradesh), India. FA and GS samples were air dried and analyzed for pH and electrical conductivity (EC) using a pH meter (Orion, 960) and a conductivity meter, respectively. Organic carbon (OC) was measured by modified method of Walkley and Black [16]; total nitrogen by micro-Kjeldahl digestion [17] and total phosphorus using the molybdenum blue method [18]. Chlorophyll (a, b and total) estimation was done by the method of [19] and protein content by [20] method. For metal analysis FA and GS samples (1 g) were digested with a mixture of nitric, sulphuric and perchloric acid (6:1:2 by volume) at 100 °C. Digested material was diluted with deionized water and Fe, Zn, Mn, Cu, Cr, Cd, Pb, B, Al, Mo and Ni contents were detected using a PerkinElmer 2380 Atomic Absorption Spectrophotometer (AAS) [21].

2.1. Experimental lay-out

Seeds of *Cajanus cajan* L. var. ICP 8863 (Maruti; dwarf variety) were obtained from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India. Selected seeds were soaked in deionized water for 2 h and were surface sterilized with 0.1% mercuric chloride for 2 min and sown in earthen pots, which were filled with FA and GS in a weight/weight ratio (0% + 100%, 25% + 75%, 50% + 50%, 100% + 0% FA + GS; w/w). GS without FA amendment were taken as the control. For each amendment, 15 replicates were prepared and in each pot (12 in.) one seed was sown and watered with deionized water during alternate days. At day 90, and day 160 (at maturity) three randomly selected plants were uprooted from each treatment for the growth, biochemical and heavy metal evaluation.

2.2. Statistical analysis

Physico-chemical properties of GS and FA were compared by Student's t-test. Physical properties; growth; biochemical; metals in roots, shoots and seeds; and yield parameters of four treatment groups (amendments) were compared by one way analysis of variance (ANOVA) and significance of mean difference between control group (GS) and other treatment groups were done by Dunnett's test.

3. Results

3.1. Physicochemical properties of FA and GS

Physico-chemical properties of GS and FA were shown in Table 1. GS and FA used in the present experiment have alkaline pH (7.20 and 9.10). The mean values of pH, EC, Cu, Cd, Al, B, Fe and Ni were significantly (p < 0.01) high in FA whereas CEC, N, P, OC, Mo, Mn and Zn were significantly (p < 0.01) high in GS while level of Cr and Pb in the GS and FA were found the same, i.e. statistically not significant (p > 0.05).

Physical properties of four treatment groups were summarised in Table 2. Mean values showed that pH, particle density (PD), porosity and water holding capacity (WHC) increases with increasing ratio of FA whereas bulk density (BD) decreases. ANOVA revealed that mean physical properties in all treatments differed significantly (p < 0.01). Porosity, PD, and WHC in 100% and 50% FA were found significantly higher (p < 0.05) than the control whereas

Table 2
Physical properties of different amendments.

Amendments	pH	BD ($g\,cc^{-1}$)	PD ($g\,cc^{-1}$)	Porosity (%)	WHC (%)
100% GS	7.20 ± 0.39	1.34 ± 0.06	1.76 ± 0.09	24.00 ± 1.20	29.00 ± 2.90
100% FA	9.10 ± 0.46**	0.90 ± 0.05**	2.22 ± 0.11**	59.50 ± 2.90**	79.50 ± 3.80**
50% FA + 50% GS	8.00 ± 0.42ns	1.15 ± 0.07*	1.98 ± 0.09*	42.00 ± 2.10**	58.00 ± 2.80**
25% FA + 75% GS	7.45 ± 0.35ns	1.23 ± 0.06ns	1.83 ± 0.07ns	33.00 ± 1.60**	50.00 ± 2.70**
ANOVA ($F_{3,11\ DF}$)	12.90**	28.74**	14.97**	163.80**	198.50**

Values are mean ± S.D. (n = 3), ns = (p > 0.05), * = (p < 0.05), ** = (p < 0.01).

Table 3
Growth responses of *Cajanus cajan* L. in different amendments at day 90.

Amendments	Root length (cm)	Shoot length (cm)	Plant length (cm)	Total leaf area (cm^2)	No. of nodules/plant	Biomass (g dw)
100% GS	16.47 ± 0.83	43.06 ± 2.15	59.53 ± 2.95	31.05 ± 1.70	19.12 ± 0.96	9.79 ± 0.49
100% FA	12.56 ± 0.63**	34.31 ± 1.72**	46.87 ± 2.34**	23.64 ± 1.18**	12.21 ± 0.54**	8.08 ± 0.41**
50% FA + 50% GS	16.83 ± 0.84ns	44.99 ± 2.26ns	61.82 ± 3.12ns	30.23 ± 1.49ns	17.00 ± 0.90*	9.10 ± 0.46ns
25% FA + 75% GS	17.00 ± 0.90ns	43.33 ± 2.17ns	60.33 ± 2.97ns	29.33 ± 1.49ns	18.67 ± 0.95ns	9.58 ± 0.48ns
ANOVA ($F_{3,11\ DF}$)	20.63**	6.01**	17.51**	15.49**	40.98**	8.19**

Values are mean ± S.D. (n = 3), ns = (p > 0.05), * = (p < 0.05), ** = (p < 0.01).

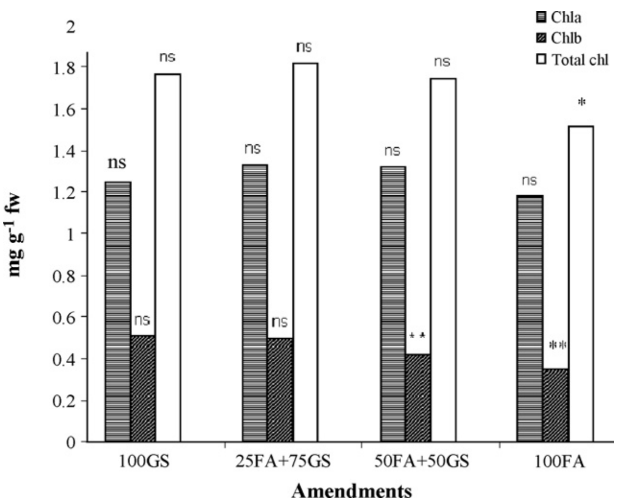

Fig. 1. Changes in photosynthetic pigments in *Cajanus cajan* L. grown in different amendments at day 90.

BD in 100% FA and 50% FA were found significantly lower (p < 0.05) than the control. The mean values of pH, BD and PD in 25% FA and control were found the same, i.e. did not differed significantly (p > 0.05).

3.2. Growth response

Growth and photosynthetic pigments of four treatment groups were depicted in Table 3 and Fig. 1, respectively. Mean values showed that growth variables (root length, shoot length, plant height, total leaf area, number of nodules/plant and biomass) were increased with a decreasing ratio of FA incorporation. ANOVA revealed that the mean levels of all the growth variables and photosynthetic pigments (chlorophyll-a, chlorophyll-b and total-chlorophyll) differed significantly (p < 0.05) between the groups. The mean values of all the growth variables and photosynthetic pigments in 100% FA decreased significantly (p < 0.05) than the control whereas the same variables in control and lower concentration of FA incorporated soils (50% and 25%) were did not differ significantly at 95% confident level.

3.3. Heavy metal accumulation and translocation

Concentrations of metals in roots, shoots and seeds of four treatment groups are presented in Table 4. In all the four treatments, mean concentration of Fe was maximum followed by Cu, Zn, Cr and Cd. Between plant parts (roots, shoots and seeds), accumulation of Fe, Zn and Cu were higher in roots than the shoots followed by seeds while Cr and Cd were higher in shoots than the roots followed by seeds. Among treatments, mean concentrations of all the metals were high in 100% FA, followed by 50% FA, 25% FA, and 100% GS. Mean concentration of Zn, Cu, Cr and Cd in edible parts (seeds) were found below the respective critical value of 100–900, 20–100, 2–30 and 0.7–200 $\mu g\ g^{-1}$ dw [22]. ANOVA revealed that between groups mean concentrations of all the metals in roots, shoots and seeds dif-

Table 5
Average translocation of heavy metals from roots seeds of *Cajanus cajan* L. in different FA amendments.

Amendments	TF value				
	Fe	Zn	Cu	Cr	Cd
100% GS	0.27	0.65	0.66	0.93	0.75
100% FA	1.26	1.23	1.06	1.86	2.01
50% FA + 50% GS	0.30	1.67	1.02	1.05	1.36
25% FA + 75% GS	0.24	0.54	0.57	0.49	0.52

Table 4
Accumulation of metals ($\mu g\ g^{-1}$ dw) in roots, shoots and seeds of *Cajanus cajan* L. in different FA amendments.

Amendments	Fe	Zn	Cu	Cr	Cd
Roots					
100% GS	90.15 ± 1.29	12.10 ± 0.62	15.35 ± 0.77	1.56 ± 0.06	1.63 ± 0.03
100% FA	665.75 ± 33.31**	51.16 ± 2.56**	74.23 ± 3.71**	28.08 ± 0.41**	21.23 ± 0.06**
50% FA + 50% GS	581.45 ± 28.97**	28.23 ± 1.42**	51.33 ± 2.51**	12.95 ± 0.40**	19.15 ± 0.06**
25% FA + 75% GS	96.35 ± 5.02*	17.31 ± 2.87**	23.96 ± 4.20**	4.51 ± 0.58**	2.75 ± 0.07*
ANOVA ($F_{3,11\ DF}$)	282.80**	304.80**	291.20**	314.90**	136.80**
Shoots					
100% GS	19.15 ± 3.80	5.59 ± 0.43	7.82 ± 0.55	1.23 ± 0.11	0.71 ± 0.03
100% FA	581.61 ± 14.17**	35.55 ± 1.80**	50.52 ± 0.98**	39.10 ± 0.46**	31.51 ± 0.08**
50% FA + 50% GS	267.10 ± 13.30**	30.80 ± 1.55**	36.33 ± 0.91**	9.42 ± 0.48**	16.23 ± 0.06**
25% FA + 75% GS	19.76 ± 1.38ns	6.15 ± 1.98ns	9.69 ± 1.08*	1.70 ± 0.64*	0.96 ± 0.09ns
ANOVA ($F_{3,11\ DF}$)	218.10**	233.90**	82.17**	269.00**	173.00**
Seeds					
100% GS	5.35 ± 2.10	2.33 ± 0.31	2.35 ± 0.41	0.23 ± 0.06	0.52 ± 0.03
100% FA	258.49 ± 7.24**	27.35 ± 1.40**	27.33 ± 0.82**	13.36 ± 0.17**	11.10 ± 0.05**
50% FA + 50% GS	149.22 ± 7.72**	16.58 ± 0.83**	16.67 ± 0.84**	4.23 ± 0.22**	9.82 ± 0.03**
25% FA + 75% GS	3.50 ± 8.73*	3.25 ± 1.61*	4.03 ± 0.96*	0.55 ± 0.31ns	0.48 ± 0.05ns
ANOVA ($F_{3,11\ DF}$)	226.10**	283.60**	116.80**	293.40**	111.20**

Values are mean ± S.D. (n = 3), ns = (p > 0.05), * = (p < 0.05), ** = significant (p < 0.01).

Table 6
Yield of *Cajanus cajan* L. grown in different FA amendments.

Amendments	No. of pods/plant	Total soluble protein (mg g^{-1} dw)	No. of seeds/plant	Weight of 25 seeds (g)
100% GS	35.04 ± 1.75	42.80 ± 2.10	105.68 ± 1.28	2.39 ± 0.11
100% FA	21.64 ± 1.08**	25.83 ± 1.75**	25.00 ± 1.11**	1.89 ± 0.10*
50%FA + 50% GS	30.55 ± 1.80*	34.14 ± 1.70**	92.29 ± 1.79**	2.00 ± 0.12ns
25%FA + 75% GS	37.53 ± 1.88ns	43.99 ± 2.20ns	110.12 ± 1.23*	2.73 ± 0.14*
ANOVA ($F_{3,11\ DF}$)	57.79**	19.17**	2695.00**	13.08**

Values are mean ± S.D. ($n = 3$), ns = ($p > 0.05$), * = ($p < 0.05$), ** = ($p < 0.01$).

fered significantly ($p < 0.01$). Mean comparison showed that in all the plant parts, concentrations of various metals were significantly ($p < 0.01$) higher in 100% and 50% FA amendments than the control. However, in the case of 25% FA amendment, the accumulation pattern was similar to control.

Average translocations (transfer coefficient) of metals from roots to aerial parts are enumerated in Table 5. It could be clear from this table that the translocation of heavy metal from roots to seeds follow a definite pattern, i.e. accumulation of metals are dependent of FA treatments except in the case of Zn translocation in 50% FA amendments. In all the treatments, translocation factor (TF) values of Cd and Cr were slightly higher than any other metals whereas Fe, Zn and Cu followed a similar pattern.

3.4. Yield

Yield parameters of four treatment groups are summarized in Table 6. Mean values showed that yield parameters increases with decreasing ratio of FA. ANOVA revealed that between groups, yield parameters differed significantly ($p < 0.01$). Mean comparison showed that number of pods/plant and total soluble protein in 100% FA and 50% FA decreased significantly ($p < 0.01$) than the control while in 25% FA it was found similar to control (not significant at 95% confident level). Similarly, mean weight of 25 seeds in control and 100% FA and 50% FA differed significantly ($p > 0.05$) while a significant ($p < 0.05$) increase of 14.23% was observed in 25% FA than the control. This clearly suggests that the most economic level of fly ash incorporation, i.e. at 25%, improved the yield (no. of seeds). At highest dose, i.e. 100% FA though the mean number of pods/plant were 21.64, the most of the pods were found without seeds.

4. Discussion

Incorporation of fly ash from 25% to 100% in GS increases the levels of pH, PD, porosity and WHC from 3.47% to 26.39%, 3.98% to 26.14%, 37.50% to 147.92% and 163.16% to 318.42%, respectively than the respective control while BD decrease respectively from 8.94 to 48.89. Pot experiment found the growth and yield of *C. cajan* L. in FA amended soils depends on the concentration of FA. Addition of FA at lower concentration (25%) to soil had shown positive results in most of the studied parameters of growth and yield. Addition of FA in soils affected its chemical composition due to increased concentration of various elements, which is beneficial for plant growth when applied at low concentrations but becomes toxic at higher doses [23].

Application of FA at higher dose caused severe deficiency of nitrogen in soil, which became an important factor for suppressed growth and yield. The gradual decline in plant growth and yield from 50 to 100% FA amended soil was probably due to the salinity caused by higher levels of sulphate, chloride, carbonate and bicarbonate in FA amended soil [24]. The reduced effect at higher dose (80% FA) on crop growth was also being reported due to the accumulation of elevated levels of inorganic dissolved salts [25]. At higher concentration (100% FA), the most of the pods were found without seeds. This is probably due to the reduction of photosynthetic pigments and low availability of soil nitrogen and phosphorus at higher FA doses [26,27].

In this study, photosynthetic pigments (a, b and total) were found decreased as FA concentration increased. Various studies have concluded that the higher concentration of FA reduced the chlorophyll content [28–30,24] due to the presence of higher level of heavy metals and metalloids in FA [23]. Further, a decline in chlorophyll content suggests that either the chlorophyll synthesizing system or the activity of enzyme chlorophyllase may have been affected by these toxic elements [31,27]. However, at lower concentration (25% FA), increase in chlorophyll-a, b and total chlorophyll content along with root length, shoot length and plant length than the normal (GS) may be attributed to higher primary productivity and metabolic rate due to availability of optimal level of essential macro- and micro-nutrients [32]. Zn is known to maintain higher chlorophyll synthesis through-SH group protection of the oxidation prone δ-aminolevulinic acid dehydratase (ALAD) and protochlorophyllide reductase [33]. ALAD requires Zn for its function of producing porphobilinogen the first precursor of chlorophyll and also known to maintain functioning of PSI and PSII under metal stress [34]. Mn is required for photolysis of water whereas Fe is required as a cofactor for all the three photosynthetic electron transfer chain super complexes and Cu is a component of plastocyanin [35]. Thus increase in chlorophyll synthesis along with proper functioning of photosystems and respiratory processes probably resulted in the better growth of the plants up to 25% FA concentration. Enhanced growth and yield at lower FA doses has been reported earlier in many plants including rice and wheat [36,24,37,38].

5. Conclusion

Land application of FA is currently under consideration as a means of reducing the amount of industrial waste and this study finds *Cajanus cajan* L. is safe and suitable for cultivation in FA amended soils for better crop yields. Pot experiment concluded that fly ash at lower concentration (25% FA) is more beneficial for the growth and yield of *Cajanus cajan* L. than the normal soil which not only enhance the growth and yield (14.23%) but also improves the nutritional quality of the seeds and for this physical and chemical factors collectively played a favorable role. As FA is deficient in nitrogen, cultivation of leguminous plants such as *Cajanus cajan* L., which have the ability to fix atmospheric nitrogen, may be more beneficial than cultivating non-leguminous crops.

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