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Effect of Fly Ash on the Distribution of P Fractions in Acid Soil at Different Stages

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The incubation study was conducted to know the extent of P solubility by microorganisms in fly ash added soil. This experiment was done by adding microbial culture *ie, Pseudomonas straita* and PGPR along with fly ash to soil. Significantly higher saloid-P and Ca-P fractions were recorded in the T_9 treatment (Fly ash @ 80 t ha^{-1} + PGPR) which was followed by T_7 treatment (Fly ash @ 20 t ha^{-1} + PGPR) as compared to control. Application of fly ash with *Pseudomonas straita* also increased saloid-P fractions in acid soil but its effectiveness is less than the inoculation of PGPR with fly ash. Higher values of Al-P, Fe-P, reductant-P, occluded-P, organic-P and total-P fractions were recorded in treatment that received only RDF and FYM (T_1) as compared to the application of fly ash with P solubilizers. The T_9(Fly ash @ 80 t ha^{-1} + PGPR) recorded lower value of Al-P, Fe-P, reductant-P, occluded-P, organic-P fractions and total-P in acid soil which was on par with T_{13} (Fly ash @ 40 t ha^{-1} + *Pseudomonas straita*).

Introduction

Fly ash is a by-product of the Thermal Power Station (TPS), where coal energy is converted into electrical energy. Fly ash is an amorphous mixture of ferroaluminosilicate minerals generated from the combustion of coal at a temperature of 400 to 1500°C.

Its physical and chemical characteristics depend on the composition of parent coal, combustion conditions, the efficiency and type of emission control devices and the disposal methods used.

The production of a vast quantity of fly ash will create dumping and management problems. The management of this huge amount of solid waste at both regional and global levels is a prime concern for the present and coming future (Ahmaruzzaman, 2010; Kishore *et al.*, 2009). Therefore agricultural utilization and wastes management techniques have emerged as prime utilization methods for solving fly ash related problems.

Since it is a store house of readily available plant macro and micronutrients, in conjunction with organic manure, microbial inoculants or fertilizers, the fly ash can be used to design a soil benefaction strategy, which can help in improving soil properties and enriching its nutrient status. The presence of almost all essential plant nutrients in ionic form and the ameliorating effect on the physical, chemical and microbial nature of soil makes fly ash an important input for biomass production, especially on variously degraded soils and wastelands.

Phosphorus is deficient in most acid soils because soluble inorganic P is fixed by Al and Fe. This reaction contributes to less availability of P for crops which is a critical nutritional element in the early stages of plant growth and development. The availability of P is influenced by soil organic matter, pH, and exchangeable and soluble Al, Fe, and Ca. Likewise fly ash has been widely recommended as a soil amendment to acid soil which modifies the physical properties of soil, but the major concern is the P, which is not in available form.

Fly ash is alkaline in nature which increases the soil pH and neutralizes the acid soils. By application of FA to the acid soils, improves physical properties (WHC, BD, etc.), neutralizes the soil pH and increases the availability of phosphorus by reducing P with Fe and Al fixation and increase the microbial population. Plant Growth Promoting Rhizobacteria (PGPR) isa group of bacteria that actively colonize plant roots and enhance plant growth and yield. Some common examples of PGPR genera exhibiting plant growth-promoting activity are *Azospirillum, Pseudomonas straita* and *Bacillus mucilaginous* which helps in nitrogen fixation, P-solubilization and K mobilization respectively and increase nutrient availability. The use of efficient plant growth-promoting rhizobacteria (PGPR) inoculants biofertilizer along with a fly ash would be another sustainable route to increase nutrient availability especially phosphorus which leads to better performance in terms of crop yield.

By knowing all the beneficial effects of fly ash on acid soil, the incubation study was conducted to know the effect of fly ash on P availability

Materials and Methods

Laboratory incubation study

This was a preliminary study conducted to know the extent of the solubility of P by microorganisms in fly ash added to soil. This experiment was done by adding microbial culture *i.e.*, *Pseudomonas straita* and PGPR along with fly ash to high build up soil to know the role of P solubilizer in P solubility and available P in the soil.

Two kilo gram of soil was filled in separate plastic boxes (2 kg capacity) and treatments were imposed as per the following treatment details (Plate 1) and replicated thrice and was laid out in a completely randomized design (CRD). With basic objective, an incubation study was conducted.

To study the effect of different levels of fly ash with and without PGPR and *Pseudomonas straita* on phosphorus fractions of acid soil.

Experimental details

Design : CRD (completely randomized design)

Treatments : 13

Replications : 3

Fly ash levels : 04 (10, 20, 40, 80 t ha^{-1})

Treatment details

T_1: Control (RDF + FYM)
T_2: Fly ash @ 10 t ha^{-1}
T_3: Fly ash @ 20 t ha^{-1}
T_4: Fly ash @ 40 t ha^{-1}
T_5: Fly ash @ 80 t ha^{-1}
T_6: Fly ash @ 10 t ha^{-1} + PGPR
T_7: Fly ash @ 20 t ha^{-1} + PGPR
T_8: Fly ash @ 40 t ha^{-1} + PGPR
T_9: Fly ash @ 80 t ha^{-1} + PGPR
T_{10}: Fly ash @10 t ha^{-1} + *Pseudomonas straita*
T_{11}: Fly ash @ 20 t ha^{-1} + *Pseudomonas straita*
T_{12}: Fly ash @ 40 t ha^{-1} + *Pseudomonas straita*
$T_{13:}$ Fly ash @ 80 t ha^{-1} + *Pseudomonas straita*

Note: Recommended dose of FYM was added commonly to all the treatments.

Analysis of soil samples for phosphorus fractions

Saloid bound phosphorus (Saloid-P)

One gram of soil was taken in a 50 ml polythene centrifuge tube and 25 ml of 1M NH_4Cl solution was added and shaken for 30 minutes. Supernatant solution after centrifugation was taken for saloid-P determination. Saloid bound P was determined by molybdophospheric acid reagent, using stannous chloride as reductant after taking the extract in 10 ml isobutyl alcohol. The intensity of the blue colour was measured at 660 nm wavelength using a spectrophotometer (Peterson and Corey, 1966).

Aluminium bound phosphorus (Al-P)

The soil residue left in the centrifuge tube after saloid - P estimation was shaken for one hour with 25 ml 0.5M NH_4F (pH 8.2). Al-P in the supernatant centrifuged suspension was determined by choloromolybdic boric acid reagent and chloro-stannous reductant. The intensity of blue colour developed was measured using a spectrophotometer at 660 nm wavelength (Peterson and Corey, 1966).

Iron bound phosphorus (Fe - P)

The soil residue from Al-P estimation was washed twice by shaking and centrifuging with a 25 ml portion of the saturated NaCl solution. The soil was then treated with 0.1M NaOH for 17 hours and centrifuged. The supernatant solution was then treated with five drops of concentrated H_2SO_4. Phosphorus free activated carbon was used to remove colour and filtered to remove suspended organic matter. Fe-P content in the filtrate was determined by the same method as followed in the determination of Al-P (Peterson and Corey, 1966).

Reductant soluble phosphorus (Reductant-P)

The soil residue from the Fe-P estimation was washed twice with 25 ml of saturated NaCl solution by shaking and centrifuging. The soil was then suspended in 15 ml of 0.3 M sodium citrate solution and shaken for 15 minutes with 0.5 g sodium dithionate. The suspension was heated in a water bath at 80 ^{o}C for a few minutes and the clear supernatant solution was decanted into a 50 ml volumetric flask after centrifuge. The soil was then washed twice with saturated NaCl and the washings returned to sodium citrate dithionate extract, which was taken for Red-P determination. Excess of citrate and dithionate was oxidized by adding 1.5ml of 0.25 $KMnO_4$ solution and Red - P was estimated by molybdophospharic acid reagent, using stannous chloride as reductant after taking the extract into a 10 ml isobutyl alcohol. The blue colour intensity measured at 660 nm wavelength using a spectrophotometer (Peterson and Corey, 1966).

Occluded phosphorus (Occluded-P)

The soil residue left out after the estimation of reductant-P was washed twice with 25 ml of saturated NaCl solution, 25 ml of 0.1 N NaOH is added and shaken for one hour.

Supernatant solution after centrifugation was taken for the estimation of occluded-P. Phosphorus in the solution was determined by chloromolybdic-boric acid method using stannous chloride as reductant.

Calcium phosphorus (Ca-P)

The soil sample after extraction of occluded-P was washed twice with 25 ml saturated NaCl and washings are discarded after centrifuging. Ca-P was then extracted by using 0.25 M H_2SO_4 and shaken for one hour and centrifuged for 5 minutes. The P in supernatant solution after centrifugation was estimated as in occluded-P.

Organic phosphorus (Org-P)

Organic phosphorus content in soil was calculated as the difference between the total P content and the total mineral P content of the soil.

Total phosphorus (Total-P)

Total phosphorus in the soils was estimated by digesting 1g of finely powdered soil with perchloric acid as outlined by Jackson (1967).

The results given in Table from 21 to 24 indicates the effect of higher levels of fly ash application with PGPR and *Pseudomonas straita* on the distribution of P fractions in fly ash amended soil.

Results and Discussion

Saloid-P

The result of changes in the saloid-P fraction in the soil at the different period of incubation is presented in Table 1. Application of fly ash with PGPR and *Pseudomonas straita* has significantly increased the saloid-P content in acid soil as compared to control from 30 DAI to 90 DAI. Higher values of saloid-P fractions were recorded in the treatments involving higher levels of fly ash with PGPR application over the treatments with lower levels.

At 30 DAI, treatment T_9 (Fly ash @ 80 t ha^{-1} + PGPR) recorded significantly higher saloid - P fraction with 24.39 mg kg^{-1} which found on par with T_{13} (Fly ash @ 80 t ha^{-1} + *Pseudomonas straita*) with 23.47 mg kg^{-1}, T_5 (Fly ash @ 80 t ha^{-1}) with 22.33 mg kg^{-1} and this was followed by treatment T_7 (Fly ash @ 20 t ha^{-1} + PGPR) which recorded 19.38 mg kg^{-1}, T_{11} and T_3 (18.52 and 16.77 mg kg^{-1}, respectively) as compared to other treatments. The lower value of saloid-P fraction was recorded in T_1 treatment with 9.74 mg kg^{-1} where fly ash not was added.

A similar trend was observed at 60 and 90 DAI. At 60 DAI, significantly higher saloid - P fraction of 27.78 mg kg^{-1} was observed with T_9 (Fly ash @ 80 t ha^{-1} + PGPR) which found on par with T_{13} (Fly ash @ 80 t ha^{-1} + *Pseudomonas straita*) with 26.28 mg kg^{-1}, T_5 (Fly ash @ 80 t ha^{-1}) 25.06 mg $kg^{-1}$and this was followed by treatment T_7 (Fly ash @ 20 t ha^{-1} + PGPR) which recorded 21.11 mg kg^{-1}, T_{11} and T_3 (19.39 and 18.85 mg kg^{-1}, respectively) as compared to other treatments. The lowest value of saloid - P fraction in soil was recorded in T_1 treatment (11.21 mg kg^{-1}).

It is apparent from the data presented in Table 20 that the application of higher levels of fly ash along with PGPR recorded highest saloid-P at 90 DAI. Treatment T_9 which received Fly ash @ 80 t ha^{-1} + PGPR recorded significantly higher saloid-P fraction in soil with 31.97 mg kg^{-1} as compared to other treatments and it was on par with T_{13} (Fly ash @ 80 t ha^{-1} + *Pseudomonas straita*) with 30.05 mg kg^{-1}, T_5 (Fly ash @ 80 t ha^{-1}) 29.21 mg kg^{-1} and followed by treatment T_7 (Fly ash @ 20 t ha^{-1} + PGPR) which recorded 23.69 mg kg^{-1}, T_{11} and T_3 (22.12 and 21.19 mg kg^{-1}, respectively) as compared to other treatments. The lower value of saloid-P fraction in acid soil was recorded in T_1 treatment with 13.47 mg kg^{-1}.

The highest saloid-P fraction was recorded in the treatments which received increased levels of fly ash with PGPR and FYM. This progressive increased in saloid-P fraction might be due to a more solubility of P due to fly ash application and also due to the complexing of P with metallic cations and the organic acids released from microorganisms which preventing P to be adsorbed on soil particles.

Thus, it helped in the extraction of loosely bonded NH_4Cl extractable fraction of P. Similar results were reported by Singaram and Kothandaraman (1992). Another possible reason for increasing saloid-P fraction in acid soil might be due to silicon present in fly ash which might have helped more P solubilization from added fertilizer as well as in soil. Chang *et al.,* (2007) reported that the application of fly-ash significantly increased saloid -P fraction in soil.

Aluminium bound-P (Al-P)

Application of different levels of fly ash with and without PGPR and PSB on changes in aluminium bound – P at different intervals of incubation study is presented in Table 1.

Application of fly ash with PGPR and *Pseudomonas straita* has significantly decreased the Al-P fraction in acid soil from 30 DAI to 90 DAI. With an increase in the dose of fly ash with and without PGPR and *Pseudomonas straita*, there was a maximum decline in Al-P fraction in acid soil as compared to control treatment.

At 30 DAI, treatment T_1 recorded significantly higher Al - P fraction which recorded 57.25 mg kg^{-1} which was followed by T_2 (Fly ash @ 10 t ha^{-1}) with 53.02 mg kg^{-1} which found statistically on par with T_6 (Fly ash @ 10 t ha^{-1} + PGPR) with 49.66 mg kg^{-1}, respectively as compared to the application of fly ash with P solubilizers. Among different treatments, T_9 (Fly ash @ 80 t ha^{-1} + PGPR) recorded significantly lower Al - P fraction of 33.81 mg kg^{-1} which found on par with T_{13} (Fly ash @ 80 t ha^{-1} + *Pseudomonas straita*) with 35.26 mg kg^{-1}, T_5 (Fly ash @ 80 t ha^{-1}) 36.55 mg kg^{-1} and followed by treatment T_7 (Fly ash @ 20 t ha^{-1} + PGPR) which recorded 40.76 mg kg^{-1}, T_{11} and T_3 (42.17 and 43.55 mg kg^{-1}, respectively)

A similar trend in the variation of Al-P fraction in soil was observed at 60 and 90 DAI. At 60 and 90 DAI, treatment T_1 recorded significantly higher Al-P fraction which recorded 54.67 and 52.38 mg kg^{-1}, respectively which found on par with T_2 (Fly ash @ 10 t ha^{-1}) with 50.86 and 46.25 mg kg^{-1}, respectively. Application of fly ash with PGPR in T_9 treatment (Fly ash @ 80 t ha^{-1} + PGPR) recorded significantly lower value of Al - P in soil with 29.76 mg kg^{-1} and 26.90 mg kg^{-1}, respectively and found on par with T_{13} (31.90 and 28.34 mg kg^{-1}, respectively) which was followed by T_7 (Fly ash @ 20 t ha^{-1} + PGPR) which registered 36.39 and 33.29 mg kg^{-1}, respectively.

The higher Al-P fraction was recorded in the treatments which received FYM and RDF as compared to the application of fly ash with PGPR and *Pseudomonas straita*. Among different treatments, significantly higher Al-P fraction was observed in T_1 as compared to other treatments. The higher Al-P fraction might be due to lower pH. At low pH, the activity of aluminium was found to be high which precipitate P into Al-P. It corroborates with the findings of Hong *et al.,* (2018). However, the treatments receiving PGPR inoculation with fly ash application (T_9) showed further enhancement in phosphorus availability by neutralization of soil acidity by the alkali oxides present in the fly ash. At neutral pH, the availability of phosphorus will be more due to the suppression of the activity of potential ions of Fe, Al, and Ca that are responsible for P fixation in soils and also by reducing Al-P fixation in soil due to their ability to solubilize insoluble phosphorus. Similar results were reported by Masto *et al.,* (2013).

Iron-P (Fe-P)

The result of changes in Fe-P fractions in acid soil at different intervals are presented in Table 2. The results reveal that higher values of Fe-P fractions were recorded in treatments that received the RDF and FYM. Application of fly ash with PGPR and *Pseudomonas straita* has significantly decreased the Fe-P fraction in acid soil from 30 DAI to 90 DAI. With an increase in the dose of fly ash with and without PGPR and *Pseudomonas straita*, there was a maximum decline in Al-P fraction in acid soil as compared to control treatment and there was a slight increase Al-P fraction of soil and no significant effect was found due to application of fly ash with PGPR and PSB as compared to the application of fly ash as alone.

At 30 DAI, treatment T_1 recorded significantly higher Fe-P fraction which recorded 70.27 mg kg^{-1}, which was followed by T_2 (Fly ash @ 10 t ha^{-1}) with 68.51 mg kg^{-1}. Significantly lower value of Fe-P fraction was recorded in T_9 (Fly ash @ 80 t ha^{-1} + PGPR) treatment with 45.68 mg kg^{-1} which found on par with T_{13}(47.11 mg kg^{-1}), T_5 (48.24 mg kg^{-1}) and followed by treatment T_7 (Fly ash @ 20 t ha^{-1} + PGPR) which recorded 55.30 mg kg^{-1}, T_{11} and T_3 (58.17 and 63.42 mg kg^{-1}, respectively)

A similar trend in the variation of Fe-P fraction in acid soil was observed due to the application of fly ash with PGPR at 60 and 90 DAI. At 60 and 90 DAI, treatment T_1 recorded significantly higher Fe-P fraction which recorded 69.18 and 68.59 mg kg^{-1}, respectively which found on par with T_2 (Fly ash @ 10 t ha^{-1}) with 67.51 and 65.41 mg kg^{-1}, respectively. Application of fly ash with PGPR in T_9 treatment (Fly ash @ 80 t ha^{-1} + PGPR) recorded significantly lower value of Al-P in soil with 40.68 and 38.95 mg kg^{-1}, respectively and found on par with T_{13} (42.06 and 41.23 mg kg^{-1}, respectively) which was followed by T_7 with PGPR (Fly ash @ 20 t ha^{-1} + PGPR) which registered 49.25 and 46.13 mg kg^{-1}, respectively.

The increase in higher Fe-P fraction in the soil might be due to the application of soluble phosphatic fertilizers to acid soils which leads to fixation and transformation of added phosphate into Fe - P. Similar results were reported by Chang and Jackson (1957) and Yuan *et al.,* (1960). Significantly lower Fe-P was observed in treatment which received fly ash application with PGPR and fly ash. However, application of fly ash with *Pseudomonas straita* reduced the Fe-P fraction in acid soil but its effectiveness is less than PGPR inoculation along with fly ash application. This might be due to the, fly ash application was found to enhance the pH of acid soil by the release of alkaline compounds from fly ash, which neutralized the soil acidity and thus increased the soil pH. At neutral pH, the availability of P will be more due to the suppression of the activity of potential Fe, Al, and Ca ions that are responsible for P fixation in soils which was reported by Masto *et al.,* (2013). There was a significant correlation between Fe-P and soil pH value.

The increase of soil pH value leads to a decreased in the formation of precipitation of Fe^{3+} in soil. As a result, the soil loses many highly active P-adsorption sites (Hong *et al.*, 2018) which resulted in a decrease of Fe-P content in soils. The reduction of Fe-P with P solubilizers (PGPR and *Pseudomonas straita)* is ascribed to the dissolution of iron oxide coatings with organic acids produced by P solubilizers causing the reduction in Fe - P. These results are corroborate with the findings of Sheela (2006).

Another possible reason for decreasing Fe-P fraction in acid soil might be because of silicon present in fly ash might have reduced the adsorbed phosphate from its anion with Fe and Al and also competing against P for the place on the surface of hydrated sesquioxides Sihag *et al.*, (2005).

Reductant soluble-P (Red-P)

The results obtained about the effect of fly ash with and without PGPR and *Pseudomonas straita* on reductant soluble-P in acid soil at different intervals during incubation study are presented in Table 2.

It was noticed from the results presented in Table 22 that all the treatments which received fly ash along with PGPR and *Pseudomonas straita* reduced the reductant soluble – P fraction as compared to control treatment from 30 DAI to 90 DAI. However, higher values of reductant soluble-P fraction were recorded in the control treatment (T_1) which received only FYM and RDF as compared to the fly ash with P solubilizers. At 30 DAI, significantly higher reductant soluble-P was recorded in treatment which received RDF and FYM (T_1) which noticed 48.28 mg kg^{-1} which was followed by T_2 with 45.61 mg kg^{-1} (Fly ash @ 10 t ha^{-1}). A lower value of reductant soluble -P fraction was recorded in treatments with the application of PSB and PGPR. Significantly lower reductant soluble -P fraction was recorded in T_9 (Fly ash @ 80 t ha^{-1} + PGPR) with 26.68 mg kg^{-1} which found on par with T_{13} (27.73 mg kg^{-1}), T_5 (29.08 mg kg^{-1}) and followed by treatment T_7 (Fly ash @ 20 t ha^{-1} + PGPR) which recorded 33.84 mg kg^{-1}.

Even at 60 DAI, significantly higher reductant soluble-P values were recorded in control (T_1) which noticed 47.61 mg kg^{-1} which was followed by T_2 with 43.18 mg kg^{-1} (Fly ash @ 10 t ha^{-1}) without PSB and PGPR application. A lower value of reductant soluble-P fraction was recorded in treatments with *Pseudomonas straita* and PGPR application. However, significantly lower reductant soluble -P fraction was recorded in T_9 (Fly ash @ 80 t ha^{-1} + PGPR) with 24.84 mg kg^{-1} which found on par with T_{13} (26.65 mg kg^{-1}), T_5 (27.88 mg kg^{-1}) and followed by treatment T_7 (Fly ash @ 20 t ha^{-1} + PGPR) which recorded 31.19 mg kg^{-1}.

A similar trend was followed in the variation of reductant soluble- P fraction in the soil at 90 DAI. Significantly higher reductant soluble-P values were recorded in control treatment with 45.05 mg kg^{-1} where fly ash and PGPR were not applied which was followed by T_2 (Fly ash @ 10 t ha^{-1}) which noticed 42.21 mg kg^{-1}.

A significantly lower reductant soluble -P was recorded in T_9 (Fly ash @ 80 t ha^{-1} + PGPR) treatment with 22.59 mg kg^{-1} which found on par with T_{13} (24.44 mg kg^{-1}), T_5 (25.11 mg kg^{-1}) and followed by treatment T_7 (Fly ash @ 20 t ha^{-1} + PGPR) which recorded 30.63 mg kg^{-1}.

Significantly higher reductant soluble -P fraction was recorded in the treatment which received only RDF and FYM might be due to a major portion of the added P through fertilizers and manures got fixed by the Fe (III) and Al oxides. Since applied fertilizer, readily reacts with ferric hydroxides, leading to the conversion of water-soluble form to insoluble form (Singaram and Kothandaraman, 1991). A significant decrease in reductant-P with P solubilizers might be due to the release of an unavailable form of nutrients to available form. There will be a gradual reduction in reductant-P content with an increase in fly ash application that was observed with the lapse of time. This might be due to silicic acid that may compete against phosphate for a place on the surface of hydrous oxide and thereby transforming reductant-P into the available pool. This might be attributed to the hydrolysis of Fe-P and Al-P with the advancement of time. These results are in agreement with the findings of Sheela (2006) and (Sihag *et al.*, 2005).

Occluded-P

The results obtained with the effect of fly ash with and without PGPR and *Pseudomonas straita* on occluded-P in acid soil at different intervals during incubation study are presented in Table 3.It was noticed from the results presented in Table 23 that all the treatments which received fly ash along with PGPR and *Pseudomonas straita* reduced the occluded – P fraction as compared to control treatment from 30 DAI to 90 DAI.

However, higher values of occluded – P fraction was recorded in the control treatment (T_1) which received only FYM and RDF compared to the fly ash with P solubilizers.

Application FYM and RDF (T_1) recorded a significantly higher value of 35.67 mg kg^{-1} of occluded-P at 30 DAI, which was followed by treatment T_2 (Fly ash @ 10 t ha^{-1}) with 33.08 mg kg^{-1}.

There was a slight increase in reductant soluble-P fraction of soil and non-significant effect was found due to the application of fly ash with PGPR and PSB as compared to the application of fly ash as alone. However, significantly lower value of occluded-P was recorded in T_9 (Fly ash @ 80 t ha^{-1} + PGPR) treatment with 15.63 mg kg^{-1} which found on par with T_{13} (17.06 mg kg^{-1}), T_5 (18.27 mg kg^{-1}) and followed by treatment T_7 (Fly ash @ 20 t ha^{-1} + PGPR) which recorded 23.04 mg kg^{-1}.

A similar trend was observed in the variation of occluded-P fraction in the soil at 60 and 90 DAI. At 60 DAI, treatment T_1 (RDF and FYM) recorded a significantly higher occluded-P value of 34.58 mg kg^{-1} which was on par with T_2 (Fly ash @ 10 t ha^{-1}) with 31.74 mg kg^{-1}.

Significantly lower value of occluded-P fraction in acid soil was recorded in T_8 (Fly ash @ 40 t ha^{-1} + PGPR) with 14.86 mg kg^{-1} which found on par with T_9 (Fly ash @ 80 t ha^{-1} + PGPR) treatment with 14.86 mg kg^{-1} which found on par with T_{13} (16.20 mg kg^{-1}), T_5 (17.15 mg kg^{-1}) and followed by treatment T_7 (Fly ash @ 20 t ha^{-1} + PGPR) which recorded 18.55 mg kg^{-1}.

At 90 DAI, the same trend was followed for occluded-P. Treatment that received RDF and FYM (T_1) recorded significantly higher occluded-P (31.29 mg kg^{-1}) which was followed by treatment T_2 (29.60 mg kg^{-1}) as compared to other treatments. Treatment T_9 (Fly ash @ 80 t ha^{-1} + PGPR) recorded significantly lower value of occluded-P fraction with 13.59 mg kg^{-1} which was on par with T_{13} (Fly ash @ 80 t ha^{-1} + *Pseudomonas straita*) with 14.11 mg kg^{-1} and followed by T_7 (Fly ash @ 20 t ha^{-1} + PGPR) which registered 19.51 mg kg^{-1}.

Table.1 Effect of different levels of fly ash on Saloid bound P and Aluminium bound P fraction of acid soil at different days of incubation

Treatment details	Saloid - P (mg kg^{-1})			Aluminium - P (mg kg^{-1})		
	30 DAI	60 DAI	90 DAI	30 DAI	60 DAI	90 DAI
T_1: Control (RDF+FYM)	9.74	11.21	13.47	57.25	54.67	52.38
T_2 : Fly ash @ 10 t ha^{-1}	11.24	13.27	16.81	53.02	50.86	46.25
T_3 : Fly ash @ 20 t ha^{-1}	16.77	18.85	21.19	43.55	40.00	38.25
T_4 : Fly ash @ 40 t ha^{-1}	20.54	22.26	25.40	39.5	35.18	32.44
T_5 : Fly ash @ 80 t ha^{-1}	22.33	25.06	29.21	36.55	32.91	29.40
T_6 : Fly ash @ 10 t ha^{-1} + PGPR	14.37	17.62	18.77	49.66	47.11	44.37
T_7: Fly ash @ 20 t ha^{-1} + PGPR	19.38	21.11	23.69	40.76	36.39	33.29
T_8 : Fly ash @ 40 t ha^{-1} + PGPR	21.12	24.38	28.58	37.66	33.73	30.62
T_9 : Fly ash @ 80 t ha^{-1} + PGPR	24.39	27.78	31.97	33.81	29.76	26.90
T_{10}: Fly ash @ 10 t ha^{-1} + *Pseudomonas straita*	13.25	15.12	19.07	51.03	49.04	45.03
T_{11}: Fly ash @ 20 t ha^{-1} + *Pseudomonas straita*	18.52	19.39	22.12	42.17	38.64	35.68
T_{12}: Fly ash @ 40 t ha^{-1} + *Pseudomonas straita*	21.08	23.88	26.65	37.85	33.78	31.06
T_{13}: Fly ash @ 80 t ha^{-1} + *Pseudomonas straita*	23.47	26.28	30.05	35.26	31.9	28.34
S. Em±	**1.12**	**1.20**	**1.30**	**1.31**	**1.36**	**1.27**
C. D. at 1%	**3.29**	**3.52**	**3.83**	**3.86**	**4.01**	**3.74**

RDF: Recommended dose of fertilizer, **PGPR:** Plant growth promoting rhizobacteria, **FYM:** Farm yard manure, **DAI:** Days after incubation
Note: FYM is common to all the treatments

Table.2 Effect of different levels of fly ash on Iron bound P and Reductant soluble P fraction of acid soil at different days of incubation

Treatment details	Iron - P (mg kg^{-1})			Reductant soluble - P (mg kg^{-1})		
	30 DAI	60 DAI	90 DAI	30 DAI	60 DAI	90 DAI
T_1: Control (RDF+FYM)	70.27	69.18	68.59	48.28	47.61	45.05
T_2 : Fly ash @ 10 t ha^{-1}	68.51	67.51	65.41	45.61	43.18	42.21
T_3 : Fly ash @ 20 t ha^{-1}	63.42	60.38	59.91	38.55	36.88	35.62
T_4 : Fly ash @ 40 t ha^{-1}	53.88	47.09	45.2	32.28	30.88	28.05
T_5 : Fly ash @ 80 t ha^{-1}	48.24	42.51	41.35	29.08	27.88	25.11
T_6 : Fly ash @ 10 t ha^{-1} + PGPR	64.64	61.83	61.55	41.79	38.74	37.15
T_7: Fly ash @ 20 t ha^{-1} + PGPR	55.3	49.25	46.13	33.84	31.19	30.63
T_8 : Fly ash @ 40 t ha^{-1} + PGPR	50.01	44.89	43.18	30.42	28.38	26.34
T_9 : Fly ash @ 80 t ha^{-1} + PGPR	45.68	40.68	38.95	26.68	24.84	22.59
T_{10}: Fly ash @ 10 t ha^{-1} + *Pseudomonas straita*	67.74	64.82	63.57	43.03	41.08	40.69
T_{11}: Fly ash @ 20 t ha^{-1} + *Pseudomonas straita*	58.17	52.32	49.43	35.49	33.35	31.83
T_{12}: Fly ash @ 40 t ha^{-1} + *Pseudomonas straita*	50.66	45.53	42.39	30.4	29.03	27.13
T_{13}: Fly ash @ 80 t ha^{-1} + *Pseudomonas straita*	47.11	42.06	41.23	27.73	26.65	24.44
S. Em±	**1.92**	**1.56**	**1.45**	**1.27**	**1.22**	**1.28**
C. D. at 1%	**5.66**	**4.61**	**4.27**	**3.76**	**3.59**	**3.78**

RDF: Recommended dose of fertilizer, **PGPR:** Plant growth promoting rhizobacteria, **FYM:** Farm yard manure, **DAI:** Days after incubation
Note: FYM is common to all the treatments

Table.3 Effect of different levels of fly ash on Occluded P and Calcium P fraction of acid soil at different days of incubation

Treatment details	Occluded - P (mg kg^{-1})			Calcium - P (mg kg^{-1})		
	30 DAI	60 DAI	90DAI	30 DAI	60 DAI	90DAI
T_1: Control (RDF+FYM)	35.67	34.58	31.29	4.65	5.91	6.64
T_2 : Fly ash @ 10 t ha^{-1}	33.08	31.74	29.6	6.21	7.14	8.09
T_3 : Fly ash @ 20 t ha^{-1}	29.10	27.41	26.51	9.07	10.18	11.29
T_4 : Fly ash @ 40 t ha^{-1}	21.10	19.02	18.54	12.89	13.71	14.76
T_5 : Fly ash @ 80 t ha^{-1}	18.27	17.15	15.23	15.55	15.22	16.63
T_6 : Fly ash @ 10 t ha^{-1} + PGPR	30.32	28.26	27.47	8.10	9.09	10.21
T_7: Fly ash @ 20 t ha^{-1} + PGPR	23.04	21.61	19.51	11.61	12.36	13.59
T_8 : Fly ash @ 40 t ha^{-1} + PGPR	19.48	18.55	17.36	14.43	15.26	16.85
T_9 : Fly ash @ 80 t ha^{-1} + PGPR	15.63	14.86	13.59	18.87	19.43	20.69
T_{10}: Fly ash @ 10 t ha^{-1} + *Pseudomonas straita*	32.43	30.52	28.31	7.15	8.01	9.14
T_{11}: Fly ash @ 20 t ha^{-1} + *Pseudomonas straita*	24.12	22.73	21.3	10.32	11.13	12.34
T_{12}: Fly ash @ 40 t ha^{-1} + *Pseudomonas straita*	20.18	18.22	16.45	14.41	15.19	16.55
T_{13}: Fly ash @ 80 t ha^{-1} + *Pseudomonas straita*	17.06	16.20	14.11	16.60	17.61	18.81
S. Em±	**1.31**	**1.25**	**1.28**	**1.51**	**1.42**	**1.33**
C. D. at 1%	**1.68**	**1.59**	**0.77**	**4.46**	**4.19**	**3.95**

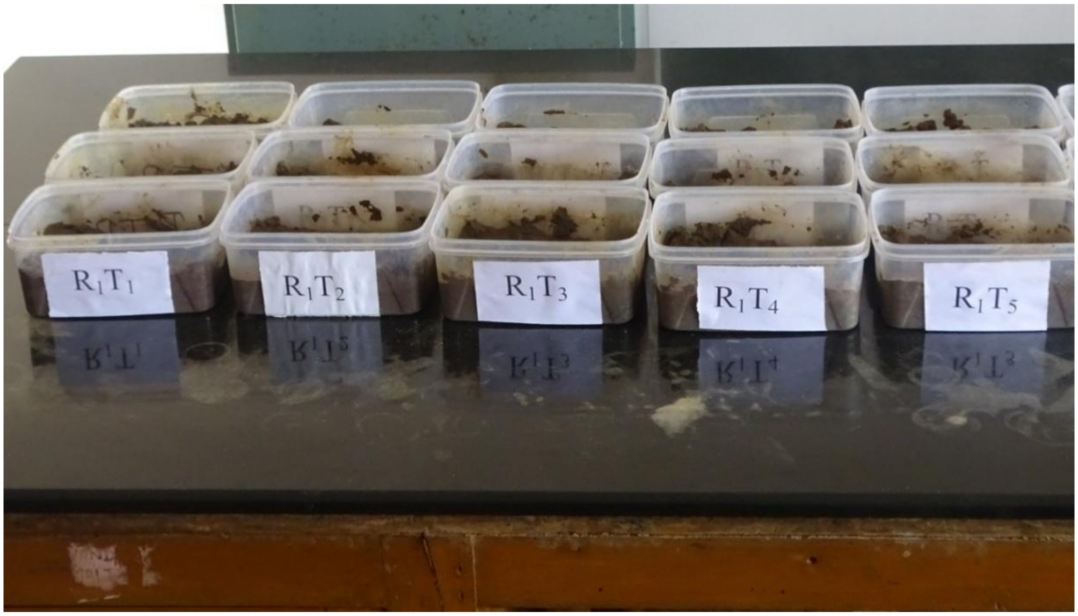
RDF: Recommended dose of fertilizer, **PGPR:** Plant growth promoting rhizobacteria, **FYM:** Farm yard manure, **DAI:** Days after incubation
Note: FYM is common to all the treatments

Table.4 Effect of different levels of fly ash on Organic P and Total P fraction of acid soil at different days of incubation

Treatment details	Organic - P (mg kg^{-1})			Total - P (mg kg^{-1})		
	30 DAI	**60 DAI**	**90 DAI**	**30 DAI**	**60 DAI**	**90 DAI**
T_1: Control (RDF+FYM)	198.25	193.57	182.56	424.11	414.73	398.98
T_2 : Fly ash @ 10 t ha^{-1}	196.71	189.69	178.12	414.38	402.39	387.49
T_3 : Fly ash @ 20 t ha^{-1}	181.83	170.03	160.74	382.29	361.73	349.70
T_4 : Fly ash @ 40 t ha^{-1}	168.57	157.49	145.26	348.76	323.63	307.65
T_5 : Fly ash @ 80 t ha^{-1}	151.25	143.18	118.11	321.27	302.91	274.04
T_6 : Fly ash @ 10 t ha^{-1} + PGPR	189.63	176.53	164.59	398.51	377.18	362.11
T_7: Fly ash @ 20 t ha^{-1} + PGPR	173.66	162.27	149.71	357.59	332.18	314.55
T_8 : Fly ash @ 40 t ha^{-1} + PGPR	156.61	148.42	134.81	329.73	311.61	295.74
T_9 : Fly ash @ 80 t ha^{-1} + PGPR	143.14	135.10	110.40	308.20	290.45	263.09
T_{10}: Fly ash @ 10 t ha^{-1} + *Pseudomonas straita*	193.10	185.72	174.88	407.73	392.31	379.69
T_{11}: Fly ash @ 20 t ha^{-1} + *Pseudomonas straita*	177.32	166.28	152.2	366.11	341.84	322.90
T_{12}: Fly ash @ 40 t ha^{-1} + *Pseudomonas straita*	162.16	153.34	141.39	336.74	316.97	299.62
T_{13}: Fly ash @ 80 t ha^{-1} + *Pseudomonas straita*	148.77	138.22	120.58	316.00	296.92	275.56
S. Em±	**5.86**	**5.16**	**9.56**	**9.63**	**7.49**	**11.71**
C. D. at 1%	**17.35**	**15.28**	**28.29**	**28.49**	**22.16**	**34.65**

RDF: Recommended dose of fertilizer, **PGPR:** Plant growth promoting rhizobacteria, **FYM:** Farm yard manure, **DAI:** Days after incubation
Note: FYM is common to all the treatments

Plate.1 An over view of incubation study on P release pattern in acid soil due to fly ash with and without PGPR application



The higher occluded-P was recorded in the treatments which received only RDF and FYM as compared to the application of fly ash with PGPR and *Pseudomonas straita*. Among different treatments significantly lower occluded-P was observed in T_8 treatment (Fly ash @ 40 t ha^{-1}+ PGPR).

This might be due to the neutralization of soil pH by releasing alkali compounds present in fly ash and dissolution of Al-P and Fe-P present in the form of oxides of Fe and Al by the action of organic acids released by P solubilizers. These results are following the findings of Goroji (2000). Similar findings were reported by Gaind and Gaur (2002).

Calcium bound-P (Ca-P)

The result of changes in Ca-P fraction in the soil at different intervals are presented in Table 3. Results revealed that higher levels of fly ash with P solubilizer treatments significantly increased the Ca-P fractions in soil.

Application of higher levels of fly ash with PGPR and *Pseudomonas straita* increased the Ca - P fraction in acid soil at different intervals of incubation study from 30 DAI to 90 DAI over control treatment. However, maximum values of Ca-P fractions were recorded in treatment T_9 (Fly ash @ 80 t ha^{-1} + PGPR) (18.87 mg kg^{-1}) which remained statistically on par with T_{13} (Fly ash @ 80 t ha^{-1} + *Pseudomonas straita*) with 16.60 mg kg^{-1}, T_5 (Fly ash @ 80 t ha^{-1}) 15.55 mg kg^{-1} and followed by treatment T_7 (Fly ash @ 20 t ha^{-1} + PGPR) which recorded 14.43 mg kg^{-1}, T_{11} and T_3 (14.41 and 12.89 mg kg^{-1}, respectively) as compared to other treatments.

Significantly lower value of Ca - P fraction in soil was recorded in T_1 treatment (4.65 mg kg^{-1}) where fly ash and P solubilizers were not applied.

A similar trend in the variation of Ca - P fraction was observed at 60 and 90 DAI. At 60 DAI, T_9 (Fly ash @ 80 t ha^{-1} + PGPR) (19.43 mg kg^{-1}) which remained statistically on par with T_{13} (Fly ash @ 80 t ha^{-1} +

Pseudomonas straita) with 17.61 mg kg^{-1}, T_5 (Fly ash @ 80 t ha^{-1}) 16.22 mg kg^{-1} and followed by treatment T_7 (Fly ash @ 20 t ha^{-1} + PGPR) which recorded 12.36 mg kg^{-1}, T_{11} and T_3 (11.13 and 10.18 mg kg^{-1}, respectively) as compared to other treatments.

Significantly lowest value of Ca - P fraction in soil was recorded in T_1 treatment (4.91 mg kg^{-1}) where fly ash and P solubilizers were not applied. The addition of increased levels of fly ash along with P solubilizers increased the Ca-P content at 90 DAI. Treatment T_9 (Fly ash @ 80 t ha^{-1} + PGPR) recorded significantly higher Ca-P fraction in soil (20.69 mg kg^{-1}) compared to other treatments. All the treatments including the application of *Pseudomonas straita* PSB and PGPR recorded higher values of Ca-P compared to no application of *Pseudomonas straita* and PGPR with fly ash. The lower value of Ca - P fraction in soil was recorded in T_1 treatment (5.64 mg kg^{-1}).

Higher values of Ca-P fractions were recorded in treatments involving the application of fly ash with P solubilizer treatment compared to the application of only fly ash. The increase of Ca-P content in soil contributed due to the input of free Ca ion from soil amendments. The fly ash amendment can release more Ca ion into soils. Similar results were obtained by Hong *et al.,* (2018). Chang *et al.*, in 2007 reported that calcium-bound P (Ca-P) fractions were increased significantly with fly ash application at 40 Mg ha^{-1}.

Organic-P

The result of changes in organic-P fractions in the soil at different intervals during incubation study is presented in Table 4. The results revealed that higher values of organic – P fraction was recorded in treatment which received the RDF and FYM. With an increase in the dose of fly ash with and without PGPR and *Pseudomonas straita*, there was a maximum decline in organic -P fraction in acid soil as compared to control treatment and there was a slight increased organic -P fraction of soil and non-significant effect was found due to application of fly ash with PGPR and PSB as compared to the application of fly ash as alone.

The treatment T_1 with the application of RDF and FYM recorded significantly higher organic-P with the value of 198.25 mg kg^{-1} which was on par with T_2 (Fly ash @ 10 t ha^{-1}) with 196.71 mg kg^{-1} respectively. A significantly lower value of organic-P was recorded in T_9 (Fly ash @ 80 t ha^{-1} + PGPR) treatment with 143.14 mg kg^{-1} which found on par with T_{13} (148.77 mg kg^{-1}), T_5 (151.25 mg kg^{-1}) and followed by treatment T_7 (Fly ash @ 20 t ha^{-1} + PGPR) which recorded 173.66 mg kg^{-1}, T_{11} and T_3 (177.32 and 181.83 mg kg^{-1}, respectively)

A similar trend in the variation of organic-P fraction in soil was observed at 60 and 90 DAI. At 60 DAI, treatment T_1 with the application of RDF and FYM recorded significantly higher organic-P with the value of 193.57 mg kg^{-1} which was on par with T_2 (Fly ash @ 10 t ha^{-1}) with 189.69 mg kg^{-1}. T_9 (Fly ash @ 80 t ha^{-1} + PGPR) treatment with 135.10 mg kg^{-1} recorded significantly lower organic-P fraction which found on par with T_{13} (138.22 mg kg^{-1}), T_5 (143.18 mg $kg^{-1)}$ and followed by treatment T_7 (Fly ash @ 20 t ha^{-1} + PGPR) which recorded 162.27 mg kg^{-1}, T_{11} and T_3 (160.28 and 170.03 mg kg^{-1}, respectively).

A significantly higher organic-P value of 182.56 mg kg^{-1} was noticed under control treatment where no-fly ash was added and which was on par with T_2 (Fly ash @ 10 t ha^{-1}) with 178.12 mg kg^{-1}. A significantly lower value of organic-P was recorded in T_9

treatment (Fly ash @ 80 t ha^{-1} + PGPR) with 110.4 mg kg^{-1} and on par with T_{13} (120.58 mg kg^{-1}), T_5 (118.11 mg $kg^{-1)}$ and followed by treatment T_7 (Fly ash @ 20 t ha^{-1} + PGPR) which recorded 149.71 mg kg^{-1}.

The higher organic-P was recorded in treatment that received FYM and RDF and the lowest organic-P was noticed in the application of fly ash with P solubilizers. A significant increased in organic P with the application of P fertilizers might be due to excess P may be inhibited phosphorylase activity and consequently inhibited the mineralization processes and favoured a build-up of organic P.

The lowest and decreased in organic-P with P solubilizers might be due to mineralization by micro-organism. Soil microorganisms also play an important role in organic P transformations in the soil through the excretion of enzymes like phosphatase and dehydrogenase. Phosphatase catalyzes the hydrolysis of esters and anhydrides of phosphoric acid and thus its activity indicates the mineralization potential of organic-P in soils. Mehta and Ram Mohan Rao (1996) reported that organic-P content in soil varied with organic carbon and clay content.

Total-P

Application of different levels of fly ash with and without PGPR and *Pseudomonas straita* on changes in total-P in acid soil at different intervals under incubation study is presented in Table 4. Higher values of total-P fractions were recorded in treatments involving the application of RDF and FYM compared to fly ash with P solubilizers treatment.

At 30 DAI, a significantly higher value of 424.11 mg kg^{-1} of total-P was noticed in treatment T_1 (RDF + FYM) which was on par with treatment T_2 (Fly ash @ 210 t ha^{-1}) with 414.38 mg kg^{-1}. Significantly lower value of total-P was recorded in T_9 treatment (Fly ash @ 80 t ha^{-1} + PGPR) with 308.20 mg kg^{-1} and on par with T_{13} (316.00 mg kg^{-1}), T_5 (321.27 mg $kg^{-1)}$ and followed by treatment T_7 (Fly ash @ 20 t ha^{-1} + PGPR) which recorded 357.59 mg kg^{-1}.

A similar trend in the variation of total-P fraction in soil was also observed at 60 and 90 DAI and at harvest. At 60 and 90 DAI, treatment T_1 (control) recorded a significantly higher total-P value of 414.73 and 398.98 mg kg^{-1}, respectively and on par with T_2 (Fly ash @ 210 t ha^{-1}) with 402.39 and 387.49 mg kg^{-1}, respectively. Significantly lower value of total-P fraction in soil (290.45 and 263.36 mg kg^{-1}, respectively) was recorded in T_9 with the application of Fly ash @ 80 t ha^{-1} + PGPR which found on par with T_{13} (296.92 and 275.56 mg kg^{-1}, respectively), T_5 (302.91 and 274.04 mg kg^{-1}, respectively) and followed by treatment T_7 (Fly ash @ 20 t ha^{-1} + PGPR) which recorded 332.18 and 314.55 mg kg^{-1}, respectively.

The result on changes in total-P in the soil at different stages of the incubation period revealed that the application of FYM and RDF significantly increased total P fraction in soil. According to Ranjit (2005) and Sheela (2006), there was a significant increase in Total-P in the soil with the application of P fertilizer. Application of fly ash with P solubilizers decreased total-P fraction in acid soil which might be due to mineralization by micro-organisms.

Fly ash is alkaline in nature which increases the soil pH, neutralizes the acid soils and increases the availability of phosphorus by reducing P with Fe and Al fixation. Application of fly ash with PGPR and *Pseudomonas straita* has significantly increased the saloid-P and Ca-P fractions and lower value of Al-P, Fe-P, reductant-P, occluded-P, organic-P fractions and total-P. Higher values of Al-P, Fe-P, reductant-P, occluded-P, organic-P and total-P fractions were recorded in treatment that received only RDF and FYM (T_1) compared to the application of fly ash with P solubilizers.

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