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Research Article

Utilization of indigenous phosphate-solubilizing bacteria to optimize the use of coal fly ash for increasing available P in an Ultisol

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Abstract

Coal fly ash (CFA) is a coal-burning by-product containing macro and micronutrients, and it is the potential material for improving available P in Ultisols. Phosphate-solubilizing bacteria (PSB) play a role in phosphorus solubilization. This study aimed at elucidating the potential use of phosphate-solubilizing bacteria to optimize the use of coal fly ash for increasing soil available P. This study was conducted in two stages, namely isolation of indigenous PSB from an Ultisol and application of the PSB and CFA to improve soil available P. Five indigenous PSB isolated from the soil had the ability to dissolve phosphate. Isolate B5 could dissolve 9.89 ppm P and had a 99.57% closeness to *Pseudomonas stutzeri*. The application of 20 and 40 t CFA ha^{-1} increased the soil pH by 4.2% and 7.2%, respectively. Increasing the dose of CFA decreased the content of available P by 50.6%. However, the combination of PSB and 20 t CFA ha^{-1} increased soil available P, plant growth, plant dry biomass, and P-uptake by plant.

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Introduction

Phosphorus (P) is one of the macro-nutrients limiting agricultural production on acid soils in the tropics (Thao et al., 2008; Setiawati and Handayanto, 2010; Vitousek et al., 2010). Low P availability is found to be one of the main problems of Ultisols, where its presence ranges from 3.4-19 ppm (Hilman et al., 2007). In soil with low pH, phosphorus is fixed by Al^{3+} and Fe^{3+} ions that makes phosphorus becomes insoluble, and it cannot be used by plants. Coal fly ash (CFA) can be used as an ameliorant to reduce P fixation and increase the availability of P in Ultisols (Hermawan et al., 2014). Fahrunsyah et al. (2018) reported that CFA could increase soil pH and P availability in an Ultisol of East Kalimantan. CFA is a by-product of the coal burning process in steam power plants. CFA production in Indonesia reached 4 million ton in 2012 (Hermawan et al., 2014). Several studies showed that the P content in CFA varies from 553.3 mg kg^{-1} to 1,197.3 mg kg^{-1} (Mupambwa et al., 2015), 1,378 mg kg^{-1} (Fahrunsyah et al., 2018), and 3,140 mg kg^{-1} (Masto et al., 2013).

However, phosphorus in CFA is not optimally available (Masto et al., 2013; Mupambwa et al., 2015; Fahrunsyah et al., 2018) even though the total P is high. Masto et al. (2013) reported that 55% P in coal fly ash was associated with Ca^{2+}. An effort that can be made to increase available P in CFA is the application of phosphate-solubilizing bacteria (PSB) (Setiawati and Handayanto, 2010). Phosphate-solubilizing bacteria produce organic acids, including citric, malic, oxalate and acetate, which function to release P from Ca fixation (Tamad et al., 2013). Organic acids react with phosphate binders such as Al^{3+}, Fe^{3+}, Ca^{2+}, or Mg^{2+} to become stable organic chelates so that phosphate ions are released and can be absorbed by plants (Walpola and Yoon 2012). Several species of *Pseudomonas* and *Bacillus* can dissolve P through organic acid production (Kumawat et al., 2017). The research by Bakhshandeh et al. (2014) showed that several PSB isolates were able to dissolve 172,263 and 254 µg mL^{-1} of phosphate in $Ca_3(PO_4)_2$ after five days of growth. This indicates that the potential of P availability in CFA can be increased with the PSB application so that the use of CFA at low doses can still provide sufficient amounts of P for plants and can also reduce heavy metal contamination from CFA.

The ability of PSB to dissolve phosphate is influenced by several factors, one of which is soil conditions. The diversity and activity of PSB are influenced by the physical, chemical and biological characteristics of the soil. The use of indigenous phosphate-solubilizing bacteria has more advantages because the bacteria are already compatible with the soil conditions. Identification of indigenous phosphate-solubilizing bacteria in Ultisols can optimize the performance of PSB in increasing the availability of P in Ultisols. Indigenous phosphate-solubilizing bacteria is expected to increase the availability of P in the application of CFA in Ultisol.

Materials and Methods

Isolation of indigenous phosphate-solubilizing bacteria

Isolation

The isolation was made from an Ultisol of Neglasari Village, Jasinga District, Bogor, Indonesia (S 06°27'20.125", E 106°26'56.278"). Soil sampling was carried out at five diagonal points, not in productive agricultural areas and not under the shade of trees (Husen et al., 2007). Soil composite samples were taken at a depth of 20 cm. A total of 10 g of soil was dissolved in 250 mL of sterile NaCl to obtain an equivalent ratio of 1:10 as a 10^{-1} dilution. Furthermore, serial dilutions of up to 10^{-7} were carried out by taking 1 mL of the first dilution solution into 9 mL of sterile NaCl. A total of 0.1 mL of the dilution series was then spread on Pikovskaya agar media. Pikovskaya media used for this study had composition of 10 g glucose L^{-1}; 5.0 g $Ca_3(PO_4)_2$ L^{-1}, 0.2 g NaCl L^{-1}, 0.2 g KCl L^{-1}, 0.1 $MgSO_4$, 2.5 mg L^{-1} $MnSO_4$, 2.5 mg L^{-1} $FeSO_4$, and 0.5 g $(NH_4)_2SO_4$ L^{-1}. The presence of phosphate-solubilizing bacteria is indicated by forming colonies surrounded by clear zones on the Pikovskaya agar medium (Nautiyal, 1999).

Phosphorus solubilization

A qualitative phosphate solubilization ability test was carried out after bacterial purification on Pikovskaya media. The phosphate-solubilizing bacteria was pricked into the centre of Pikovskaya agar medium with a loop needle, then incubated for 48 hours at room temperature. The ability of bacteria to dissolve phosphate is indicated by the clear zone around the colony (holozone). The results of measurements of the holozone diameter and colony diameter are calculated as SI (Solubility Index) using the following equation (Mursyda et al., 2015). The measurements of solubility index were carried out at 3, 4, 5, 6, 7 days after isolation.

$$SI = \frac{\text{Holozone diameter-Colony diameter}}{\text{Colony diameter}}$$

A quantitative phosphate solubilization test was carried out using liquid Pikovskaya media. One millilitre of inoculum suspension was added to 15 mL of liquid media solution. The inoculated liquid medium was then put into a shaker incubator at 37° C and at a speed of 100 rpm for seven days. Phosphate solubilization measurements were carried out by inserting 1.5 mL of inoculated liquid media into a centrifuge at a speed of 10,600 rpm for 10 minutes. One millilitre of supernatant was taken and added with 3.5 mL of P reagent. The P reagent consisted of 2.5 mL of 2.5% sodium molybdate and 1 mL of 0.3% hydrazine sulfate. The reagent solution must always be fresh or well used 3 hours before. After 30 minutes or when the colour changes to blue, the absorbance of P is measured by UV-vis at a wavelength of 830 nm (Lynn et al., 2013; Mursyida et al., 2015).

Identification of phosphate-solubilizing bacteria

Identification of selected bacteria was started by extracting the DNA with Quick-DNA Fungal or Bacterial Kit (Zymo Research Corp., Tustin, USA). Pure DNA was visualized using PCR to determine which bands were formed. After the bands were formed according to the provisions, pure DNA was sent to Genetics Science Indonesia then forwarded to 1st Base Malaysia for the identification process. The results of 16s rRNA screening were entered into the Basic Local Alignment Search Tool (BLAST) through the National Center for Biotechnology Information (NCBI) website to identify matches with existing references. The final result of BLAST was then looked at the similarities with the names or species in GenBank (Sanjay et al., 2018).

A test of the effect of CFA, PSB and organic fertilizer application on growth, yield and P uptake by maize

The isolated phosphate-solubilizing bacteria were then used in a pot experiment carried out from September to December 2020. The treatments tested consisted of the combined application of coal fly ash (CFA = A) (0, 20, and 40 t ha^{-1}), phosphate-solubilizing bacteria (PSB = B) (0 and 5 L ha^{-1}), and organic fertilizer (P) (0 and 5 t ha^{-1}). Twelve treatments were arranged in a randomized block design with three replications. The plant used was sweet maize. The planting medium contained 10 kg of Ultisol and a combination of CFA, PSB and organic fertilizer according to the treatments. Characteristics of the soil, CFA and organic fertilizer used for this study are shown in Table 1. The planting medium was incubated for ten days, after which the soil pH, available P and total bacteria were observed at 10 DAI (days after incubation). Two maize kernels were planted in each plot and thinned to one plant at 7 DAP (days after planting). Basal fertilizers (150 kg Urea ha^{-1} and 150 kg KCl ha^{-1}) were given at 35 DAP. Plant height, number of leaves and stem diameter were observed at 2, 3, 4, 5, 6, 7, and 8 WAP (weeks after planting). Plant dry biomass and P uptake were observed at 8 WAP.

Table 1. Characteristics of soil, coal fly ash and organic fertilizer used for the study.

Parameter	Soil (Ultisol)	Coal Fly Ash	Organic Fertilizer
pH H_2O	4.0	4.9	6.7
Total N (%)	0.26	0.01	1.11
Total P (%)	211.08	0.05	0.27
Available P (mg kg^{-1})	1.53	0.72	
Available K (me 100 g^{-1})	0.12	0.16	1.69
CEC (me 100 g^{-1})	47.43	1.02	50.44
Organic C (%)	2.04	0.11	11.35
C/N	8	18	10
Exchangeable Al (me 100 g^{-1})	17.36	-	-
Fe (ppm)	253.55	-	-
Pb (ppm)	-	270.6	-
Total Cd (ppm)	-	1.44	-
Exchangeable Ca (%)	-	22.02	-

Soil and plant analyses

Soil samples were dried and sieved (2 mm and 0.5 mm) for analysis of pH H_2O (1: 1) and available P (Bray 1). Total microbial analysis (Total Plate Count in Plate Count Agar media) was made using fresh soil samples. Harvested plant samples were dried at 70°C then weighed as dry plant biomass (roots, stems, leaves) and ground. Plant powders were analyzed for P uptake (dry ashing).

Statistical analyses

Data were subjected to analysis of variance at the 5% level, followed by the Duncan's Multiple Range Test (DMRT) at the 5% level. Correlation analysis was used to determine the relationship between parameters observed.

Results and Discussion

Identification of indigenous phosphate-solubilizing bacteria

The results of bacterial isolation showed that there were five types of bacteria (isolates B1, B2, B3, B4, and B5) that had the potential to dissolve phosphate. Isolate B1 had the highest solubility index (SI) compared to other isolates (Figure 1). A further test was carried out on liquid Pikovskaya media to see the ability of bacteria to dissolve P more accurately (Baig et al., 2010). Isolate B5 (9.89 ppm) had a higher dissolution rate compared to other isolates (Figure 2). Isolate B5 was identified using 16S rRNA sequencing analysis. DNA was extracted from pure isolate B5, then observed for electrophoresis on an agarose gel. The electrophoresis results showed that isolate B5 was seen as gram-negative, and there was a band of 1391 bp. The reading showed that isolate B5 was 99.57%, similar to the *Pseudomonas stutzeri* (Figure 3).

Effect of inoculation of PSB on soil properties, plant growth and P-uptake

Soil properties

The treatments had a significant effect on soil pH. The results showed that CFA was able to increase the soil pH (Figure 4). Increasing the CFA dose was followed by the increase in soil pH. The application of 40 t CFA ha^{-1} yielded higher soil pH than that due to the application of 20 t CFA ha^{-1}. The ability of coal fly ash in increasing pH of acid soils because it contains Ca and Mg silicates, aluminosilicates and oxides of Ca and Mg (Yunusa et al., 2006; Murugan and Vijayarangam, 2013). Fahrunsyah et al. (2018) reported that the application of CFA could reduce the solubility of Al and H in the soil, thereby increasing soil pH. The addition of 5 L PSB ha^{-1} tended to decrease soil pH. The secretion of organic acids resulting from the activity of microorganisms will be accompanied by a decrease in soil pH (Khan et al., 2009; Kumar et al., 2018). Provision of PSB and organic fertilizer at 20 t CFA ha^{-1} tended to reduce soil pH. The addition of organic matter affects the enzymatic activity in the soil and can stimulate microbial activity (Lee et al., 2004). The addition of coal fly ash (CFA), phosphate-solubilizing bacteria (PSB) and organic fertilizer significantly affected the available P of the soil studied (Figure 5).

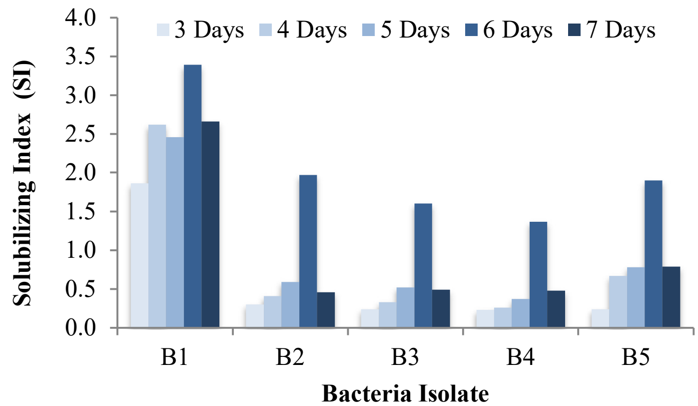

Figure 1. The phosphate-solubilizing activity of selected strains on Pikovskaya media (in terms of solubility index).

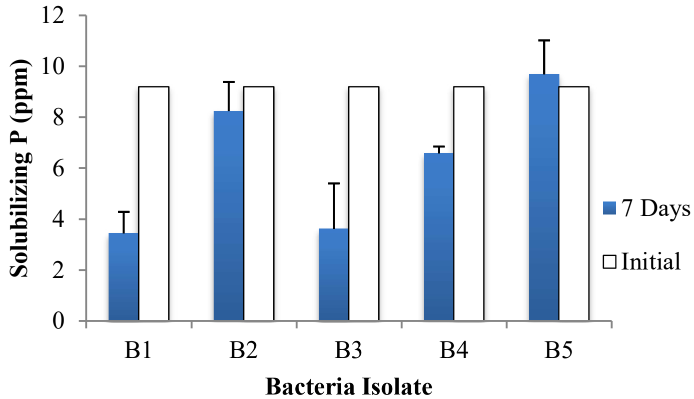

Figure 2. Phosphate-solubilizing concentration. Initial P concentration (9.20 ppm) from Pikovskaya media that source P by 5 g $Ca_5(PO_4)_3OH$ L^{-1}.

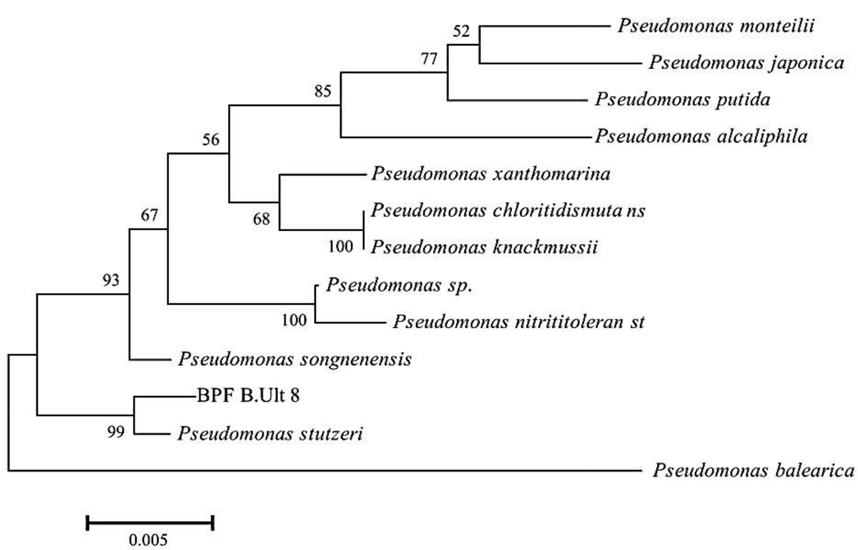

Figure 3. Construction of phylogenetic tree of B5 isolate.

CFA applications tended to decrease the available P of the soil. Increasing doses of CFA was followed by the decrease in the soil available P. This is presumably due to the high Pb content in CFA, which can fix P into an unavailable form. Phosphorus fixation results in stable Pb-P complex bonds (Cao et al., 2009; Waterlot et al., 2011). Phosphorus has been widely used in Pb remediation with pyromorphite formation as a barrier to Pb transfer and fixation efforts (Seshadri et al., 2017; Andrunik et al., 2020). Application of 40, 80 and 120 t ha^{-1} CFA resulted in a decrease in the Pb concentration to 7.23 mg kg^{-1}, 6.26 mg kg^{-1} and 5.57 mg kg$^{-1,}$ respectively, which was due to the high available P content in CFA (Lee et al., 2006). The application of PSB and organic fertilizer did not significantly affect the soil available P. This is presumably because the organic acids produced by phosphate-solubilizing bacteria had not worked well at the dosage used.

Another contributing factor is the presence of heavy metal concentrations resulting from CFA applications which can inhibit the activity of microorganisms in dissolving phosphate. The presence of heavy metals in high concentrations can inhibit the respiration rate and soil microbial activity (Chu, 2018). There was an increase in PSB and organic fertilizer applications in 20 t CFA ha^{-1} (A20B5P5) by 100% compared to A20B0P0.

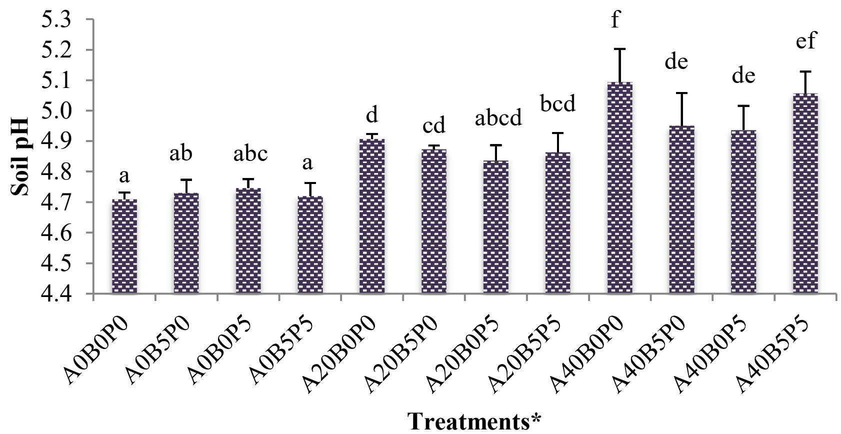

Figure 4. Changes in soil pH due to the application of coal fly ash, phosphate-solubilizing bacteria, and organic fertilizer. Bars represent standard deviations. Numbers followed by the same letter in the same column are not significantly different at α 5% DMRT. Remarks*: A = coal fly ash (0, 20 and 40 t ha^{-1}); B = phosphate-solubilizing bacteria (0 and 5 L ha^{-1}); P = organic fertilizer (0 and 5 t ha^{-1}).

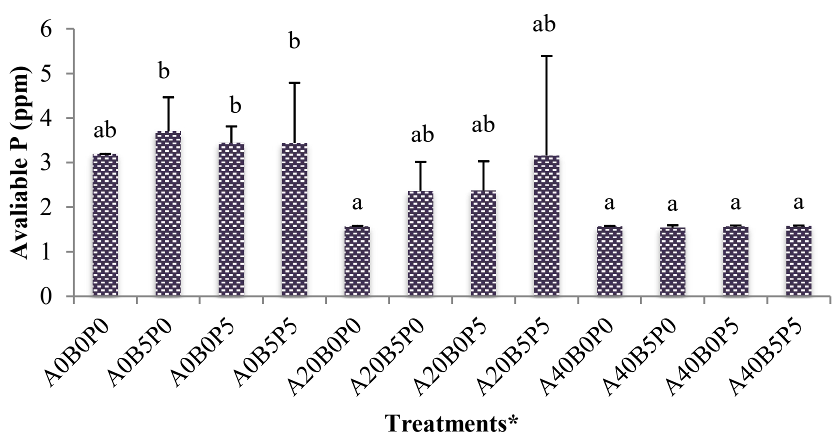

Figure 5. Changes in soil available P due to the application of coal fly ash, phosphate-solubilizing bacteria, and organic fertilizer. Bars represent standard deviations. Numbers followed by the same letter in the same column are not significantly different at α 5% DMRT. Remarks*: A = coal fly ash (0, 20 and 40 t ha^{-1}); B = phosphate-solubilizing bacteria (0 and 5 L ha^{-1}); P = organic fertilizer (0 and 5 t ha^{-1}).

The combination of CFA, PSB and organic fertilizer significantly affected the total soil microbes (Figure 6). The addition of CFA tended to decrease the total soil microbes. This is presumably due to the heavy metal content which affects the microbial population. Heavy metals affect the growth, morphology, and metabolism of soil microorganisms through malfunctioning, protein denaturation or destruction of cell membrane integrity (Leita et al., 1995). High levels of heavy metals in the soil have a significant impact on population size and soil microbial community activity (Xie et al., 2016). Application of CFA can also cause soil compaction, thereby affecting the soil microbial population. Soil physical changes, especially shifting in pore size into smaller pores, alter soil micro-habitat and affect the distribution, activity and diversity of soil microorganisms (Pengthamkeerati et al., 2011). Soil microbes are also sensitive to increased soil density; therefore, increasing soil compaction can inhibit the growth of soil bacteria (Siczek and Frac, 2012).

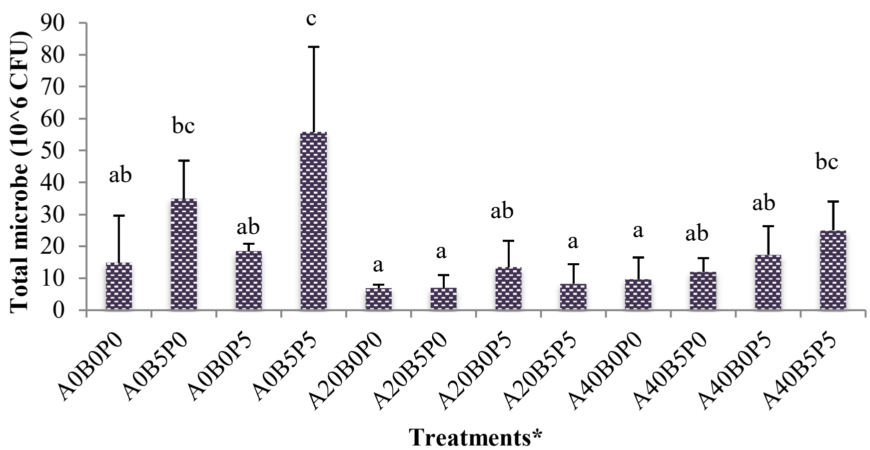

Figure 6. Changes in soil total microbe due to the application of coal fly ash, phosphate-solubilizing bacteria, and organic fertilizer. Bars represent standard deviations. Numbers followed by the same letter in the same column are not significantly different at α 5% DMRT. Remarks*: A = coal fly ash (0, 20 and 40 t ha^{-1}); B = phosphate-solubilizing bacteria (0 and 5 L ha^{-1}); P = organic fertilizer (0 and 5 t ha^{-1}).

There was an increase in soil total microbes at 40 t CFA ha^{-1} compared to 20 t CFA ha^{-1} indicating that total soil microbes after CFA application could utilize the high C / N ratio in CFA. Wan et al. (2014) showed a strong relationship between soil C: N ratio and microbial community structure. Besides soil pH, the C: N ratio also has been shown to be a factor that affects the composition of the soil microbial community (Högberg et al., 2007).

Growth of maize

The application of CFA, PSB and organic fertilizer had a significant effect on the increase in plant height (Table 2), number of leaves at 4, 6 and 8 WAP (Table 3), and stem diameter 2, 4, 6 and 8 WAP (Table 4), and they did not significantly affect the number of leaves at 2 WAP. The application of 20 t CFA ha^{-1} and 40 t CFA ha^{-1} with the combination of PSB and organic fertilizer yielded better plant height, number of leaves and stem diameter than the control (P0). Previous studies reported that the application of CFA increased plant growth and yield because it could improve nutrient supply (Bharti et al., 2000; Reddy et al., 2010; Panda et al., 2015). Muduli et al. (2014) reported that fly ash stimulated growth parameters such as length, fresh and dry weight of roots and shoots, germination rate and chlorophyll content of leguminous crops. Fahrunsyah et al. (2019) reported that the use of CFA and oil palm empty bunches compost on an Ultisol of East Kalimantan had a significant effect on the growth of maize plants, where the best treatment effect was shown in the application of 80 t CFA ha^{-1} and 20 t oil palm empty bunches compost ha^{-1}. Mokolobate and Haynes (2002) stated that the utilization of Ultisols was faced with low nutrients content such as N, P, K, Ca, Mg and Mo and high solubility of Al, Fe, and Mn. These conditions indicate the low soil quality, which could potentially inhibit plant growth and production.

P uptake by maize

The application of CFA, PSB and organic fertilizer significantly increased P uptake by maize (Table 6). The lowest P uptake by maize (88.14 mg P $plant^{-1}$) was observed in control. The highest P uptake by maize (288.35 mg P $plant^{-1}$) was observed in A20B5P5 treatment, although it was not significantly different from the A40B5P5 treatment. Soil pH seemed to be the supporting factor for P uptake by maize plants. The increase in soil pH will be followed by a decrease in the availability of $H_2PO_4^-$ and at the same time, it will increase HPO_4 availability, which is rapidly absorbed by the roots (Chen and Barber, 1990).

Table 2. Effect of coal fly ash, phosphate-solubilizing bacteria, and organic fertilizer on the plant height of maize.

Treatments*	Plant height (cm)			
	2 WAP	4 WAP	6 WAP	8 WAP
A0B0P0	23.17 a	35.67 a	82.33 a	118.00 a
A0B5P0	24.50 abc	47.00 b	101.33 b	130.67 a
A0B0P5	23.67 a	57.33 cd	116.33 cd	151.33 bc
A0B5P5	23.83 a	52.50 bc	109.67 bc	145.67 b
A20B0P0	28.00 cd	62.83 de	116.00 cd	153.00 bc
A20B5P0	27.17 abcd	64.00 de	119.00 cd	159.33 bcd
A20B0P5	27.83 bcd	67.67 de	126.00 de	168.33 de
A20B5P5	29.83 d	70.67 e	129.33 def	165.00 cde
A40B0P0	28.50 cd	69.67 e	129.00 def	163.67 cde
A40B5P0	29.83 d	66.33 de	124.33 de	160.67 cd
A40B0P5	31.00 d	89.33 f	142.33 f	177.67 e
A40B5P5	29.67 d	83.33 f	136.67 ef	168.33 de

Table 3. Effect of coal fly ash, phosphate-solubilizing bacteria, and organic fertilizer on the number of leaves of maize.

Treatments*	Number of leaves (sheet)			
	2 WAP	4 WAP	6 WAP	8 WAP
A0B0P0	2.33 a	5.33 a	8.33 a	12.33 a
A0B5P0	2.67 a	5.67 ab	8.67 ab	13.33 ab
A0B0P5	2.67 a	6.00 bc	9.33 b	14.00 bc
A0B5P5	3.00 a	6.00 bc	9.00 ab	14.33 bc
A20B0P0	3.00 a	6.00 bc	10.00 b	14.00 bc
A20B5P0	3.00 a	6.00 bc	10.00 b	14.33 bc
A20B0P5	3.00 a	6.33 cd	10.00 b	14.67 c
A20B5P5	3.00 a	7.00 ef	10.67 b	14.67 c
A40B0P0	3.00 a	7.00 ef	11.00 b	15.00 c
A40B5P0	3.00 a	6.67 de	11.00 b	14.67 c
A40B0P5	3.00 a	7.00 ef	11.00 b	15.00 c
A40B5P5	3.00 a	7.33 f	11.00 b	14.67 c

Table 4. Effect of coal fly ash, phosphate-solubilizing bacteria, and organic fertilizer on the stem diameter of maize.

Treatments*	Stem diameter (mm)			
	2 WAP	4 WAP	6 WAP	8 WAP
A0B0P0	3.33 a	4.57 a	10.37 a	12.93 a
A0B5P0	3.27 a	6.00 ab	11.47 ab	14.40 ab
A0B0P5	3.53 ab	6.87 bc	13.97 cd	15.23 bc
A0B5P5	3.60 ab	6.37 bc	12.93 bc	14.50 ab
A20B0P0	4.13 bc	7.73 cd	14.23 cde	15.83 bcd
A20B5P0	4.23 c	7.70 cd	15.53 def	16.07 bcd
A20B0P5	4.43 cd	9.07 de	16.73 fg	17.03 cde
A20B5P5	4.37 cd	10.07 e	16.17 f	17.30 cde
A40B0P0	4.63 cd	9.90 e	16.73 fg	17.60 de
A40B5P0	4.40 cd	9.27 de	15.90 ef	17.00 cde
A40B0P5	4.67 cd	12.73 f	18.37 g	18.53 e
A40B5P5	5.00 d	12.30 f	18.07 g	19.00 e

Remarks* for Tables 2, 3 and 4: A = coal fly ash (0, 20 and 40 t ha^{-1}); B = phosphate-solubilizing bacteria (0 and 5 L ha^{-1}); P = organic fertilizer (0 and 5 t ha^{-1}), WAP = weeks after planting. Numbers followed by the same letters in the same column are not significantly different at α 5% DMRT.

Root development affects the absorption of plant nutrients (Minardi et al., 2017). When compared with controls, A20B5P5 showed an increase of P uptake by 227.15%. Application of coal fly ash without PSB and OF in A20B0P0 and A40B0P0 treatments increased P uptake by 40.13% and 191.4%, respectively. Soil pH significantly correlated with plant height (r = 0.715**), number of leaves (r = 0.700**), and plant diameter (r = 0.854**). Furthermore, the uptake of P by maize plants significantly correlated with plant height (r = 0.892**), number of leaves (r = 0.857 **), and plant diameter (r = 0.874**).

Table 6. Effect of coal fly ash, phosphate-solubilizing bacteria, and organic fertilizer on uptake of P.

Treatments*	Uptake of P (mg plant dry weight^{-1})			
	Leave	Root	Stem	Total
A0B0P0	60.95 a	20.86 a	6.33 a	88.14 a
A0B5P0	78.25 a	15.32 a	14.99 ab	108.56 ab
A0B0P5	128.45 a	36.26 a	20.79 ab	185.50 abcde
A0B5P5	77.20 a	30.64 a	31.25 abc	139.09 abc
A20B0P0	86.54 a	19.37 a	19.37 ab	125.28 abc
A20B5P0	111.09 a	22.03 a	32.37 abc	165.49 abcd
A20B0P5	129.45 a	55.34 a	40.73 abc	225.53 bcde
A20B5P5	175.22 a	19.03 a	94.10 d	288.35 e
A40B0P0	181.43 a	27.67 a	48.06 bc	257.17 de
A40B5P0	157.33 a	21.51 a	40.13 abc	218.97 bcde
A40B0P5	143.48 a	27.52 a	90.68 d	261.69 de
A40B5P5	160.96 a	15.48 a	65.67 cd	242.12 cde

Remarks*: A = coal fly ash (0, 20 and 40 t ha^{-1}); B = phosphate-solubilizing bacteria (0 and 5 L ha^{-1}); P = organic fertilizer (0 and 5 t ha^{-1}), WAP = weeks after planting. Numbers followed by the same letters in the same column are not significantly different at α 5% DMRT.

Biomass of maize

The application of CFA, PSB and organic fertilizer showed a significant effect on the total dry biomass of plants. The results showed that A20B5P5 treatment produced the highest plant dry biomass, where it increased by 289.31% compared to the control. This treatment showed better result compared to A20B0P0 and A20B5P0 treatments. Mengel et al. (2001) stated that the impact of P-deficient resulted in reduced RNA synthesis, thereby affecting a decrease in protein synthesis and affecting plant growth and inhibit fruit ripening. The effect of CFA in increasing plant growth was shown in the research of Swamy et al. (2010), which reported higher results than without CFA on plant growth, metabolism, pigment synthesis, enzyme activity, cytology, and plant yield. In addition, Fahrunsyah et al. (2019) reported that the application of 20, 40, and 80 t CFA ha^{-1} increased plant biomass at each CFA dose level, followed by the increase in P uptake by 138.61, 169.63, and 229.72 mg plant^{-1}, respectively compared to that of the control treatment of 71.84 mg plant^{-1}.

Table 7. Effect of coal fly ash, phosphate-solubilizing bacteria, and organic fertilizer on dry biomass.

Treatments*	Dry Biomass (g)			
	Leave	Root	Stem	Total
A0B0P0	12.54 a	9.50 a	1.92 a	23.96 a
A0B5P0	17.83 ab	9.86 a	4.51 ab	32.21 ab
A0B0P5	27.94 abcde	24.94 b	6.62 ab	59.50 bcde
A0B5P5	18.63 ab	15.41 ab	10.27 ab	44.31 abc
A20B0P0	22.80 abc	18.99 ab	6.93 ab	48.72 abcd
A20B5P0	24.68 abcd	19.15 ab	8.72 ab	52.54 abcd
A20B0P5	33.06 bcde	21.88 b	11.44 ab	66.38 cdef
A20B5P5	42.80 de	24.89 b	25.58 c	93.28 f
A40B0P0	45.60 e	19.84 ab	13.30 b	78.74 def
A40B5P0	38.51 cde	16.13 ab	11.27 ab	65.90 cdef
A40B0P5	43.34 e	14.63 ab	28.50 c	86.47 ef
A40B5P5	37.80 cde	20.04 ab	15.58 c	73.41 cdef

Remarks*: A = coal fly ash (0, 20 and 40 t ha^{-1}); B = phosphate-solubilizing bacteria (0 and 5 L ha^{-1}); P = organic fertilizer (0 and 5 t ha^{-1}), WAP = weeks after planting. Numbers followed by the same letters in the same column are not significantly different at α 5% DMRT.

The application of materials produced from empty fruit bunches (phosphate-solubilizing fungi, biochar and compost) to an Ultisol of East Kalimantan significantly increased the growth and yield of maize (Ichriani et al., 2018). The efficiency of uptake and P uptake Plant dry biomass showed a significant positive correlation with P uptake by maize plant ($r = 0.982^{**}$).

Conclusion

Application of coal fly ash with phosphate-solubilizing bacteria and organic fertilizer affected plant growth, P uptake, and plant dry biomass. The increase in pH after the addition of treatment was a factor in increasing plant growth and P uptake. The use of PSB and organic fertilizer at a dose of 20 t CFA ha^{-1} showed non-significant results compared to the dose of 40 t CFA ha^{-1}. The high Pb content in coal fly ash reduced the available P in the soil by forming stable Pb-P complex bonds.

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