Amending triple superphosphate with chicken litter biochar improves phosphorus availability

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Abstract
The reaction of $\text{H}_2\text{PO}_4^-$ and $\text{HPO}_4^{2-}$ with Al and Fe in acid soils to form a precipitate reduces P availability. Chicken litter biochar has been used to improve soil P availability for maize production but with limited information on optimum rates of biochar and Triple Superphosphate (TSP) to increase P availability. This study determined the optimum amount of chicken litter biochar and TSP that could increase P availability. Different rates of chicken litter biochar and TSP were evaluated in an incubation study for 30, 60, and 90 days. Selected soil chemical properties before and after incubation were determined using standard procedures. Soil pH, total P, available P, and water soluble P increased in treatments with 75% and 50% biochar. Total acidity, exchangeable Al$^{3+}$, and Fe$^{2+}$ were significantly reduced by the chicken litter biochar. The chicken litter biochar also increased soil CEC and exchangeable cations (K, Ca, Mg and Na). The use of 75% and 50% of 5 t ha$^{-1}$ biochar with 25% TSP of the existing recommendation can be used to increase P availability whilst minimizing soil Al and Fe content. This rates can be used to optimize chicken litter biochar and TSP use in acid soils for crop production especially maize and short term vegetables.

Keywords: Incubation period, interaction, optimization, phosphorus fertilizers, phosphorus fixation, tropical acid soils.

Introduction
Orthophosphates are essential macronutrients which when taken up by plants as soluble inorganic P regulate protein synthesis (Mkhabela and Warman, 2005). Phosphate availability and use efficiency is poor in acid soils (Oxisols and Utisols) because of Al and Fe ions. Aluminium and Fe ions have been implicated in P fixation (Adnan et al. 2003; Ch'ng et al. 2014a,b, 2016a,b,c). The reaction of $\text{H}_2\text{PO}_4^-$ and $\text{HPO}_4^{2-}$ with Al and Fe ions to form a precipitate reduces diffusion of P into plant roots (Adnan et al., 2003) and conventionally, large amounts of lime and P fertilizers such as triple super phosphate (TSP) and rock phosphates are applied to acid soils to saturate Al and Fe ions (Ch'ng et al. 2014a; Rahman et al. 2014). Although this approach is to maintain sufficient supply of plant-available P (Myers and De Pauw, 1995; Ch'ng et al. 2014a; Rahman et al., 2014) it is uneconomical and environmentally unfriendly. Moreover, it leads to wastage of the limited P resources and the Ca from liming may also fix P in the soil thereby compounding the problem of P fixation. Hence there is a need for more sustainable and environmentally friendly methods for improving tropical acid soil P availability.
In recent times, attempts have been made to increase soil available P using organic amendments (Ch’ng et al. 2014a,b, 2016a,b,c). Ch’ng et al. (2014a) reported that amending tropical acid soils with biochar does not only improve soil total P, available P, organic P, and inorganic fractions of P (soluble-P, Al-P, Fe-P, redundant soluble-P, and Ca-P) but it also reduces soil exchangeable acidity, Fe, and Al. This is possible because biochar fixes Al and Fe ions instead of P. Although Ch’ng et al. (2014a) used chicken litter biochar to improve P availability of TSP, their study did not optimize the use of both biochar and TSP as these materials were not varied. Therefore, this present study was focused on optimizing biochar and TSP to increase P availability by understanding the reaction between biochar and TSP. Hence, an incubation study was conducted over a period of 90 days in a controlled environment to determine the optimum rates of biochar and TSP vis a vis reduction of P fixation by Al and Fe ions. It was hypothesized that the use of the right amounts of chicken litter biochar and TSP will significantly increase soil available P by reducing P-fixation by Al and Fe ions. The objectives of this study were to determine: (i) the optimum amounts of biochar and TSP that will not only increase P availability in an acid soil but will also reduce P-fixation by Al and Fe ions and (ii) how time affects P availability following application of chicken litter biochar and TSP.

Material and Methods

Soil sampling and preparation
The soil (Nyalau Series, Typic Paleudults) used in this study was taken from an uncultivated secondary forest at Universiti Putra Malaysia, Bintulu Sarawak Campus. Although this soil is high in Al and Fe, it is one of the most cultivated soils in Sarawak, Malaysia. The soil samples were taken at 0-20 cm using a shovel. The soil samples were air dried, ground, and sieved to pass through 2 mm after which they were bulked. A 300 g of soil was taken for each treatment with nine replications based on the bulk density.

Incubation set up
The percentages of chicken litter biochar and phosphorus fertilizer rates are summarized in Table 1. The soil, chicken litter biochar, and TSP were thoroughly mixed after which the mixture was incubated in a transparent polypropylene container of 800 cm³ volume. The treatments were arranged in a Complete Randomized Design (CRD) with 3 replicates at the Research Centre, Universiti Putra Malaysia Bintulu Sarawak Campus, Malaysia. The mixture was moistened to 60% of moisture content based on the soil field capacity after which the TSP rates in Table 1 were surface applied. The lids of the polypropylene containers were perforated to allow good aeration. When necessary, the soil moisture content was maintained using distilled water. The incubated soil was sampled using destructive sampling at 30, 60, and 90 days of incubation. The recommended rate of P fertilizer used was 60 kg P₂O₅ ha⁻¹ (130 kg ha⁻¹ TSP) for maize production and this was scaled down to per plant basis (Table 2) from the standard fertilizer recommendation by Malaysian Agriculture Research and Development Institute (1993). The chicken litter biochar rate was 5 t ha⁻¹ and but it was scaled down to per plant basis (Table 2).

Table 1. Percentages of chicken litter biochar and phosphorus fertilizer rates

| Treatment code | Treatment | Soil | Biochar (5 t h⁻¹) | TSP (60 kg h⁻¹) |
|----------------|-----------|------|------------------|----------------|
| T1             | Soil      | 0%   | 0%               | 0%             |
| T2             | Soil      | 0%   | 100%             |                |
| T3             | Soil      | 100% | 0%               |                |
| T4             | Soil      | 75%  | 25%              |                |
| T5             | Soil      | 50%  | 25%              |                |
| T6             | Soil      | 25%  | 25%              |                |
| T7             | Soil      | 75%  | 50%              |                |
| T8             | Soil      | 50%  | 50%              |                |
| T9             | Soil      | 25%  | 50%              |                |
| T10            | Soil      | 75%  | 75%              |                |
| T11            | Soil      | 50%  | 75%              |                |
| T12            | Soil      | 25%  | 75%              |                |

Soil chemical analysis before and after incubation
Soil samples were characterized for physical and chemical properties before and after the incubation study. Soil pH in water and KCl were determined in a 1:2.5 (soil: distilled water / KCl) using a digital pH meter (Peech, 1965). Soil texture was determined using the hydrometer method (Bouyoucus, 1962). Soil total carbon was calculated as 58% of the organic matter determined using loss of weight on ignition method.
The amount of the soil used in this study was based on soil bulk density method (Dixon and Wisniewski, 1995). The cation exchange capacity (CEC) was determined using leaching method (FAO, 1980) followed by steam distillation (Bremner, 1965). Exchangeable cations were extracted with 1 M NH₄OAc, pH 7.0 using the leaching method (FAO, 1980). Afterwards, the cations were determined using Atomic Absorption Spectrometer (AAnalyst 800, Perkin Elmer Instruments, Norwalk, CT). Total N was determined using Kjeldhal method (Tan, 2005).

Exchangeable acidity, H⁺, and Al³⁺ were determined using acid-base titration method (Rowell, 1994).

Table 2. Scale down of chicken litter biochar and phosphorus fertilizer rates in incubation study

| Treatments | Soil Biochar rate | TSP rate |
|------------|------------------|----------|
| T1         | 300              | 0        | 0        |
| T2         | 300              | 0        | 4.8      |
| T3         | 300              | 7.7      | 0        |
| T4         | 300              | 5.8      | 3.6      |
| T5         | 300              | 3.9      | 3.6      |
| T6         | 300              | 1.9      | 3.6      |
| T7         | 300              | 5.8      | 2.4      |
| T8         | 300              | 3.9      | 2.4      |
| T9         | 300              | 1.9      | 2.4      |
| T10        | 300              | 5.8      | 1.2      |
| T11        | 300              | 3.9      | 1.2      |
| T12        | 300              | 1.9      | 1.2      |

Statistical analysis

Analysis of variance (ANOVA) was used to test treatment effects whereas treatments means were compared using Tukey’s Test. Statistical Analysis Software version 9.3 was used for the statistical analysis (SAS, 2011).

Results and Discussion

Initial soil chemical properties

The physico-chemical properties of the soil used in this study (Table 3) were within the range reported by Soil Survey Staff (2014) for Bekenu series (Typic Paleudult). The pH values, and C, N, P, K, Ca, Al, Fe, Mg and Na contents of the chicken litter biochar were also within the range reported by the Black Earth Company in North of Bendigo Victoria, Australia (Table 4).

Table 3. Selected physico-chemical properties of Nyalau Series

| Properties                  | Value Obtained                      |
|-----------------------------|-------------------------------------|
| Bulk density (g cm⁻³)       | 1.12                                |
| Soil texture                | Sand: 67.5%, Silt: 15.5%, Clay: 17%, Sandy Loam |
| pH in water                 | 4.44                                |
| pH in KCl                   | 3.83                                |
| Total Carbon (%)            | 1.20                                |
| Total N (%)                 | 0.08                                |
| Total P (%)                 | 0.005                               |
| Available P (ppm)           | 4.50                                |
| CEC                         | 5.22                                |
| Exchangeable acidity        | 0.51                                |
| Exchangeable Al             | 0.32                                |
| Exchangeable H              | 0.19                                |
| Exchangeable K              | 0.22                                |
| Exchangeable Ca             | 0.25                                |
| Exchangeable Mg             | 0.34                                |
| Exchangeable Na             | 0.22                                |
| Extractable Fe              | 0.19                                |
Table 4. Selected chemical properties of chicken litter biochar

| Properties                      | Chicken little biochar |
|---------------------------------|------------------------|
| pH in water                     | 8.5                    |
| pH in KCl                       | 7.83                   |
| Total Carbon                    | 63.7                   |
| Total N (%)                     | 2.8                    |
| Total P (%)                     | 2.6                    |
| Total K (%)                     | 3.9                    |
| Total Ca (%)                    | 5.9                    |
| CEC (cmol kg\(^{-1}\)biochar)  | 80.5                   |
| Total Mg (g kg\(^{-1}\)biochar) | 15.2                   |
| Total Na (g kg\(^{-1}\)biochar) | 19.5                   |
| Total Fe (g kg\(^{-1}\)biochar) | 2.7                    |
| Total Al (g kg\(^{-1}\)biochar) | 0.0006                 |

Effects of different amounts of chicken litter biochar and phosphorus on soil total C and pH

The interaction between treatments and incubation time significantly affected soil total carbon (TC) (Table 5). At 30 days of incubation, the effect of T3 on TC was higher than those of T1, T2, T4, T5, T6, T7, T8, T9, T10, T11, and T12 (Figure 1). Soil TC at 30 days of incubation of T4, T7, and T10 were not significantly different but higher than those of T1, T2, T5, T6, T8, T9, T11, and T12 (Figure 1). Among the treatments amended with chicken litter biochar, T6, T9, and T12 showed lower soil TC at 30 days of incubation compared with those of T4, T8, and T12. At 30 days of incubation, the TC of T1 and T2 (treatments without chicken litter biochar) were not significantly different but lower than those amended with chicken litter biochar (Figure 1). Regardless of treatment, the soil TC at 30, 60, and 90 days of incubation were not significantly different. The differences in the TC suggest increase in organic matter and humic substances that are known to be effective in fixing Al and Fe instead of P availability (Chen et al. 2004; Ch’ng et al. 2014a).

![Figure 1. Effect of treatments on soil total carbon at 30, 60 and 90 DAI. Means with different letter(s) indicate significant difference between treatments by Tukey’s HSD test at P ≤ 0.05. Bars represent the mean values ± SE.](image-url)

The interaction between treatments and incubation time significantly affected soil pH in water and KCl (Table 6). The soil pH in water (Figure 2) and in KCl (Figure 3) at 30 days of incubation show that, the soils with chicken litter biochar (T3, T4, T5, T6, T7, T8, T9, T10, T11, and T12) had significantly higher effect on soil pH than those of T1 and T2. This was due to the liming effect of the chicken litter biochar as it has high affinity for Al and Fe thus, reducing the hydrolysis of Al and Fe to produce hydrogen ions (Sparling et al. 1999; Ch’ng et al. 2014a). Instead, the reaction enabled the release of OH\(^{-}\) to increase soil pH. The pH of T3 increased at 30 days of incubation compared with T4, T5, T6, T7, T8, T9, T10, T11, and T12 (Figures 1 and 2) because the treatments with higher amounts of chicken litter biochar might have increased the contents of phenolic and humic-like materials in the soil during decomposition (Narambuye and Haynes, 2006) to form organic anions which consumed H\(^{+}\) to increase the soil pH (Haynes and Mokolobate, 2001). The soil pH of T4, T7, and T10 significantly differed in spite of these treatments having the same amount of chicken litter biochar and this was due to the different amounts of the TSP used. The inherent content of Ca of the TSP might have contributed to the increase in the soil pH.
Table 5. Mean square values of analysis of variance (ANOVA) to evaluate the effects of treatments and incubation time on soil total carbon, total nitrogen, exchangeable K, exchangeable Ca, exchangeable Mg, exchangeable Na, exchangeable Fe, and CEC.

| Source of variations | Degree of freedom | Total Carbon | Total Nitrogen | Exchangeable K | Exchangeable Ca | Exchangeable Mg | Exchangeable Na | Exchangeable Fe | CEC  |
|----------------------|-------------------|--------------|----------------|----------------|-----------------|----------------|----------------|----------------|------|
| Time                 | 2                 | 18.45*       | 0.0340*        | 2.00*          | 17.32*          | 364217799.74* | 0.42*          | 0.81*          | 93.59*|
| Treatments           | 11                | 62.83*       | 0.0685*        | 443.57*        | 21.44*          | 18961253994.00* | 50.05*         | 4.67*          | 83.85*|
| Time*Treatments      | 22                | 0.98*        | 0.0186*        | 0.98*          | 1.02*           | 143477376.03* | 0.18*          | 0.12*          | 33.34*|
| Error                | 70                |              |                |                |                 |                |                |                |      |

Note: Asterisks (*) indicates significant at $P \leq 0.05$

Table 6. Mean square values of analysis of variance (ANOVA) to evaluate the effects of treatments and incubation time on soil pH in water, pH in KCl, total P, available P, water soluble P, total acidity, exchangeable Al, and exchangeable H.

| Source of variations | Degree of freedom | pH in water | pH in KCl | Total P | Available P | Water soluble P | Total acidity | Exchangeable Al | Exchangeable H |
|----------------------|-------------------|-------------|-----------|----------|-------------|-----------------|---------------|----------------|----------------|
| Time                 | 2                 | 0.0936*     | 0.0876*   | 1099444.7* | 9012473* | 1661.82*       | 0.0008        | 0.0041*        | 0.0056*        |
| Treatments           | 11                | 5.7957*     | 8.6147*   | 24028249.4* | 984398.5* | 57298.65*      | 0.1637*        | 0.1641*        | 0.0277*        |
| Time*Treatments      | 22                | 0.0302*     | 0.0109*   | 591783.9* | 357531.9* | 363.8*         | 0.0061*        | 0.0042*        | 0.0023*        |
| Error                | 70                |             |           |          |             |                 |               |                |                |

Note: Asterisks (*) indicates significant at $P \leq 0.05$
It was also possible that the different amounts of the TSP used might have reacted with some of the soluble Al and Fe to reduce production of H⁺ through the hydrolysis of Al and Fe (Jiao et al. 2007; Ch'ng et al. 2014a, b). The soil pH in water (Figure 2) and in KCl (Figure 3) after 60 and 90 days of incubation were also similar to that of 30 days after incubation. A similar finding had been reported by Ch'ng et al. (2014a).

![Figure 2. Effects of treatments on soil pH in water at 30, 60 and 90 DAI. Means with different letter(s) indicate significant difference between treatments by Tukey's HSD test at P ≤ 0.05. Bars represent the mean values ± SE.](image)

![Figure 3. Effects of treatments on soil pH in KCl at 30, 60 and 90 DAI. Means between columns with different letter(s) indicate significant difference between treatments by Tukey's HSD test at P ≤ 0.05. Bars represent the mean values ± SE.](image)

**Effects of different amounts of chicken litter biochar and phosphorus on soil phosphorus**

The interaction between treatments and incubation time significantly affected soil total P, available P, and water soluble P (Table 6). The lower soil total P, available P, and water soluble P of T1 at 30, 60, and 90 days of incubation than those of T2, T3, T4, T5, T6, T7, T8, T9, T10, T11, and T12 (Figures 4, 5, and 6) was due to higher P fixation. The soil total P, available P, and water soluble P of T2 at 30 days of incubation were significantly higher than those of T5, T6, and T9 (Figures 4, 5, and 6) because of the lower rates of chicken litter biochar and TSP, suggesting that the lower rates of chicken litter biochar in particular could not significantly reverse P fixation by Al and Fe (Cheng et al. 2008; Ch'ng et al. 2014a). However, the soil total P, available P, and water soluble P of T2 at 30 days of incubation were lower than those of T3, T4, T8, T10, and T11 (Figures 4, 5, and 6). This was due to the higher rates of the chicken litter biochar as the higher the rates of biochar the higher negative charges to fix Al and Fe ions instead of P (Cheng et al. 2008; Ch'ng et al. 2014a). The soil total P and water soluble P of T10 at 30 days of incubation were higher than those of T4 and T7 although T4 and T7 had the lowest TSP rate but the same amount of biochar (Figures 4, 5, and 6). This observation could be associated with the optimum reaction that occurred in T10 thus, enabling higher release of P into the soil.

At 60 days of incubation, P release was higher in all the treatments amended with chicken litter biochar than those of 30 days of incubation due to the slow release of nutrients from the biochar as biochar is recalcitrant to decomposition (Ch'ng et al. 2014a). At 90 days of incubation, the P release in the soils treated with chicken litter biochar were similar to those at 60 days of incubation, suggesting that the optimum release of P following the application of chicken litter biochar was within 60 days. This observation is related to the increase of P with time due to the increase in pH caused by the increase in net negative charge on Al and Fe oxide surfaces.
Figure 4. Effects of treatments on soil total P at 30, 60 and 90 DAI. Means between columns with different letter(s) indicate significant difference between treatments by Tukey’s HSD test at $P \leq 0.05$. Bars represent the mean values ± SE.

Figure 5. Effects of treatments on soil available P at 30, 60 and 90 DAI. Means between columns with different letter(s) indicate significant difference between treatments by Tukey’s HSD test at $P \leq 0.05$. Bars represent the mean values ± SE.

Figure 6. Effects of treatments on soil water soluble P at 30, 60 and 90 DAI. Means between columns with different letter(s) indicate significant difference between treatments by Tukey’s HSD test at $P \leq 0.05$. Bars represent the mean values ± SE.

Effects of different amounts of chicken litter biochar and phosphorus on soil total acidity, CEC, and exchangeable Al$^{3+}$, H$^+$, Fe$^{3+}$, K$^+$, Ca$^{2+}$, Mg$^{2+}$, and Na$^+$

The interaction between treatments and incubation time significantly affected soil exchangeable Al$^{3+}$, and Fe$^{2+}$ (Tables 5 and 6). Although the interaction between treatments and incubation time did not significantly affect soil total acidity, the soil total acidity, soil exchangeable Al$^{3+}$, and soil exchangeable Fe$^{2+}$ of T1 at 30, 60, and 90 days of incubation were significantly higher than those of T2, T3, T4, T5, T6, T7, T8, T10, T11, and T12 (Figures 7, 8, and 9) because of the inherent contents of Al$^{3+}$ and Fe$^{2+}$ of the soil. This also indicates that the use of chicken litter biochar effectively reduced soil total acidity within 30 days period and can be used
for plants that have short growth period. The total acidity, soil exchangeable Al\(^{3+}\), and soil exchangeable Fe\(^{2+}\) of T2 at 30, 60, and 90 days of incubation were lower than those of T1 because the phosphate of T2 fixed some of the Al\(^{3+}\) and Fe\(^{2+}\) thereby reducing soil acidity. Fe\(^{2+}\) and Al\(^{3+}\) concentrations in the soil. The total acidity, Al\(^{3+}\), and Fe\(^{2+}\) in the soil at 30, 60, and 90 days of incubation were similar in all the soils amended with biochar (T3, T4, T5, T6, T7, T8, T10, T11, and T12) (Figures 7, 8, and 9).

The interaction between time of incubation and treatments significantly affected soil CEC, total N, exchangeable K\(^{+}\), H\(^{+}\), Ca\(^{2+}\), Mg\(^{2+}\), and Na\(^{2+}\) (Tables 5 and 6). The soil exchangeable H\(^{+}\) of T1 and T2 were not significantly different from those of T3, T4, T5, T6, T7, T8, and T9 but lower than those of T10 and T11.
because of the presence of soil exchangeable Fe$^{2+}$. This observation also suggests that the affinity of chicken litter biochar for Al$^{3+}$ was higher than for Fe$^{2+}$. The soil exchangeable H$^+$ of T10 and T11 were significantly higher because of the lowest rate of TSP in these treatments. The differences in H$^+$ might be due to application of TSP which led to the release of H$^+$ as it reacted with water to produce orthophosphate ($\text{Ca(H}_2\text{PO}_4\text{)}_2+$ $2\text{H}_2\text{O} \rightarrow \text{CaHPO}_4 + \text{H}^+ + \text{H}^+\text{PO}_4$). The soil CEC and total N of T1 and T2 were significantly lower than those of T3, T4, T7, T8, and T10 (Figures 11 and 12). However, the exchangeable K$^+$, Ca$^{2+}$, Mg$^{2+}$, and Na$^+$ of T1 and T2 were significantly lower than those of T3, T4, T5, T6, T7, T10, T11, and T12 (Figures 13, 14, 15, and 16) because of the presence of these nutrients in chicken litter biochar. This observation is consistent with that of Ch'ng et al. (2014a).

![Figure 10](image1.png)

Figure 10. Effects of treatments on soil exchangeable Fe$^{3+}$ at 30, 60 and 90 DAI. Means between columns with different letter(s) indicate significant difference between treatments by Tukey's HSD test at $P \leq 0.05$. Bars represent the mean values $\pm$ SE.

![Figure 11](image2.png)

Figure 11. Effects of treatments on soil CEC at 30, 60 and 90 DAI. Means between columns with different letter(s) indicate significant difference between treatments by Tukey's HSD test at $P \leq 0.05$. Bars represent the mean values $\pm$ SE.

![Figure 12](image3.png)

Figure 12. Effects of treatments on soil total N at 30, 60 and 90 DAI. Means between columns with different letter(s) indicate significant difference between treatments by Tukey’s HSD test at $P \leq 0.05$. Bars represent the mean values $\pm$ SE.
Figure 13. Effects of treatments on soil exchangeable K after 30, 60 and 90 DAI. Means between columns with different letter(s) indicate significant difference between treatments by Tukey’s HSD test at $P \leq 0.05$. Bars represent the mean values ± SE.

Figure 14. Effects of treatments on soil exchangeable Ca at 30, 60 and 90 DAI. Means between columns with different letter(s) indicate significant difference between treatments by Tukey’s HSD test at $P \leq 0.05$. Bars represent the mean values ± SE.

Figure 15: Effects of treatments on soil exchangeable Mg at 30, 60 and 90 DAI. Means between columns with different letter(s) indicate significant difference between treatments by Tukey’s HSD test at $P \leq 0.05$. Bars represent the mean values ± SE.
The use of 75% and 50% chicken litter biochar of 5 t ha⁻¹ with 25% TSP showed greater interaction and higher release of soil total P, available P, and water soluble P because these rates were able to reduce Al and Fe ions in the soil. This study also showed that significant amounts of Al and Fe were fixed within the first 30 days of incubation. The soil total P, available P, and water soluble P increased with increasing incubation period because of continued decomposition of the chicken litter biochar. The significant fixation of Al and Fe by biochar within the first 30 days of incubation suggests that biochar can be used in the tropical acid soil to unlock P for short terms crops such as maize and some vegetables. 75% and 50% of 5 t ha⁻¹ biochar with 25% TSP can be used to increase P availability for the cultivation of crops such as maize on tropical acid soils with high P fixing capacity.

Acknowledgements

The authors would like to thank Ministry of Higher Education, Malaysia for financial assistance, Fundamental Research Grant Scheme (FRGS), and Universiti Putra Malaysia for providing research facilities.

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