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Research Article

Transformation of aluminium fractions and phosphorus availability in acid soils as the result of microbes and ameliorant addition

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Abstract: Soil acidity and problems related to aluminium (Al) toxicity are usually limiting factors for soil use in agriculture. Problems with acid soils can be overcome by liming. Another potential way to overcome problems of acid soils is to utilize young coal enriched with sulfate-reducing bacteria (SRB) or Acidithiobacillus ferrooxidans. The purpose of this study was to assess the utilization of coal enriched with SRB or A. ferrooxidans as an alternative ameliorant to provide transformation of aluminium fractions and phosphorus availability in acid soils. There were two acid soils (Ultisols) studied with differences in the content of exchangeable Al. Al fraction was differentiated into exchangeable Al (Al-exch), crystalline Al (Al-dithionite), non-crystalline Al (Al-o), organic Al bound (Al-p), non-crystalline inorganic Al (Al-po), as low or medium complex with organic matter (Al-Cu), highly stabilized Al complex with organic matter (Al-pCu). The results showed that for acid soils from Jasinga West Java and Lebak Banten, coal or lime ameliorant, microbial A. ferrooxidans or sulfate-reducing bacteria (SRB) and coal or lime ameliorant enriched with A. ferrooxidans or sulfate-reducing bacteria (SRB) significantly affected the value of Al fraction. Coal or lime ameliorant enriched with A. ferrooxidans could reduce the Al fraction content in acid soils. Coal or lime ameliorant enriched with SRB could increase the availability of P in acid soil from Jasinga West Java with the distribution of Al-pCu > Al-dhitionite > Al-po > Al-exch > Al-Cu fraction. Coal or lime ameliorant enriched with SRB could increase the availability of P in acid soil from Lebak Banten with the distribution of Al-pCu > Al-po > Al-dhitionite > Al-Cu > Al-exch.

Keywords: acid sois, aluminium fractions, ameliorant, phosphorus, soil microbes

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Introduction

Soil acidification is a major problem in crop production throughout Indonesia. The main problem with acid soil is high aluminium (Al) solubility level which causes crop toxicity. Soil acidity is closely related to nutrient availability level, especially P, which will be fixed into Al-P and Fe-P in various acid soil (Wijanarko and Hanudin, 2010). Problems of acid soils are often overcome by limestone application. According to Iskandar (2012), acid to very acid soils can be neutralized using limestone for agricultural needs to increase soil pH and other nutrients availability, such as P and various micronutrients. As an alternative to limestone, ameliorant such as coal enriched with sulfate-reducing bacteria (SRB) such as *Acidithiobacillus ferrooxidans* is expected to improve the productivity of acid soils by reducing Al toxicity, besides enhancing soil chemical, physical, and biological conditions (Perez-Piqueres et al., 2006). Coal (lignite) has some

characteristic resemblance to active carbon, namely high porosity and natural cation exchanges (Havelcova et al., 2009). Lignite (brown coal) can produce humic acid, which can affect the nutrient release rate of soil minerals. The application of coal as a soil ameliorant has proved to increase the soil cation exchange capacity and phosphorus availability, besides reducing heavy metals availability. The application of coal to the soil can increase soil pH (Yazawa et al., 2000) and electrical conductivity in acid soils (Imbufe et al., besides reducing heavy 2004), metals bioavailability (Janos et al., 2010; Simmler et al., 2013) and increasing phosphorus absorption from fertilizer (Scheel et al., 2008). A humic substance derived from lignite is carboxylic and phenolic acids which can provide reactive sites for cation exchanges, phytotoxic element binding, soil buffer pH improvement, calcium penetration and retention in, as well as inducing nutrient transport to plant. Microorganism utilization to reduce the toxic effect in heavy metal contaminated soil began to catch the attention as it is more environmentally friendly. According to Kurniawan et al. (2015), A. ferrooxidans can perform the bioleaching process that is separated from Al metal. Besides Acidithiobacillus bacteria, sulfate-reducing bacteria can also be utilized to improve the chemical properties of acid soils. These bacteria can reduce sulfate production during sulphidic oxidation occurrence. This bacterial activity resulted in increased pH and decreased metal accumulation. Sulfate-reducing bacteria are capable of using sulfate, sulfite, or thiosulfate ions as an electron acceptor to gain energy in the metabolism process. Ions after receiving the electrons will be reduced to sulfides. The formation of hydrogen sulfide will also be very beneficial to the environment containing high dissolved metals, as these compounds are highly reactive and will soon react with metals, forming stable metalsulfide, therefore dissolved metal will precipitate. This study aimed to assess the utilization of coal ameliorant enriched with sulfate-reducing bacteria (SRB) or A. ferrooxidans as an alternative ameliorant besides limestone in the transformation of aluminium fractions and phosphorus availability in acid soils.

Materials and Method

Study area

This study was conducted in Chemical and Soil Fertility Laboratory, Soil Biotechnology Laboratory of the Department of Soil Science and Land Resource, Faculty of Agriculture, IPB University, and Laboratory of Soil Chemistry, Bogor Soil Research Institute. Two soil samples (Ultisols) used in this study were collected from Jasinga, Bogor West Java and Lebak, Banten, Indonesia, with the contents of exchangeable Al are 12.14 cmol(+)/kg and 7.53cmol(+)/kg respectively.

Experimental design

This study used a completely randomized design consisting of 9 treatments with three replications. The treatment arrangements were as follows Ct = without microbial and ameliorant, D = dolomite, C = coal, M1 = sulfate-reducing bacteria (SRB) code 8816 from Indonesian Center for Biodiversity and Biotechnology (ICBB) Bogor, M1D = sulfatereducing bacteria (SRB) code 8816 from Indonesian Center for Biodiversity and Biotechnology (ICBB) Bogor + dolomite, M1C =sulfate-reducing bacteria (SRB) code 8816 from Indonesian Center for Biodiversity and Biotechnology (ICBB) Bogor + coal, M2 = A. ferrooxidans code 8789 from Indonesian Center for Biodiversity and Biotechnology (ICBB) Bogor, M2D = A. ferrooxidans code 8789 from Indonesian Center for Biodiversity and Biotechnology (ICBB) Bogor + dolomite, and M2C = A. ferrooxidans code 8789 from Indonesian Center for Biodiversity and Biotechnology (ICBB) Bogor + coal. 500 g of soil samples were sterilized with fungicide nematicide insecticide (Basamid) and placed into a plastic bucket, then mixed well with limestone (11.95 g), coal (6.25 g), A.ferrooxidans (5 mL) and SRB (5 mL). The soil mixtures were incubated for 30 days and maintained under field capacity condition. Fractionation of Al compound was carried out via a simple extraction by shaking the samples with different extractants to remove the Al fractions bound to the soil as described by Alvarez et al. (2002) and Drabek et al. (2003). The exchangeable Al fraction (Al-exch) soil 5 g + KCl 1 M +1 h, crystalline Al (Al-dithionite) soil 1 g + 6 mL buffer DCB + 8h ,non-crystalline Al (Al-o) soil 1 g + 50 mL 0.2M ammonium oxalate + 4h,organic Al bound (Al-p) soil 1 g+ 100 mL Na₄P₂O₇ 0.1 M + 16h and as low or medium complex with organic matter (Al-Cu) soil 3 $g + 40 mL CuCl_2 0.5 M + 2h$. The Al-o from Al-p (Al-po) provides an estimate of the total Al formed as non-crystalline inorganic Al, and Al-Cu from Al-p (Al-pCu) provides highly stabilized Al complex with organic matter. Available P was determined with Bray 1. Al was determined in the extracts of each fraction by an Atomic Absorption Spectrometer (AAS).

Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) with 5% significant level

followed by the Least Significant Difference (LSD) test with 5% significant level.

Results and Discussion

Aluminium fraction

The statistical analysis results showed that for acid soil from Jasinga and Lebak, coal or lime ameliorant, *A.ferrooxidans* microbe or sulfatereducing bacteria (SRB) and lime or coal ameliorant enriched with *A. ferrooxidans* or sulfate-reducing bacteria (SRB) significantly affected the value of Al fraction. The average Al fraction is presented in Figure 1. Figure 1 shows that for acid soil from Jasinga and Lebak, the treatment of coal or lime enriched by *A. ferrooxidans* (M2D or M2C) was able to reduce the high Al fraction value compared to the control treatment.

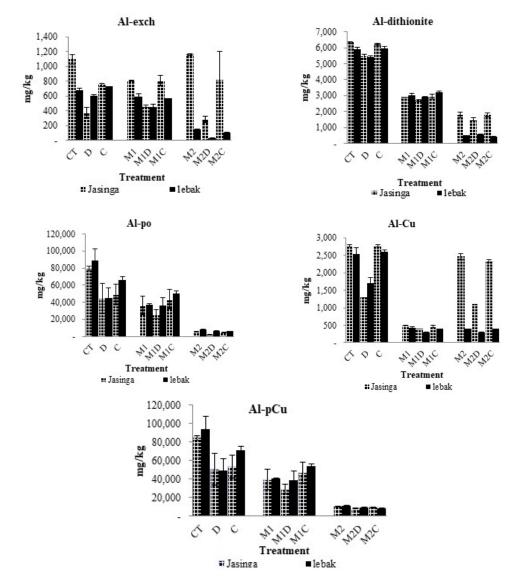


Figure 1. Distribution of Al fractions after microbes and soil ameliorant applied to acids soils from Jasinga and Lebak. Al-exch = exchangeable Al, Al-dithionite = crystalline Al, Al-po = non-crystalline inorganic Al, Al-Cu = low or medium Al complex with organic matter, Al-pCu = highly stabilized Al complex with organic matter, Ct = control without microbe and ameliorant, D = dolomite without microbe, C = coal without microbe, M1 = SRB without ameliorant, M1D = SRB + dolomite, M1C = SRB+coal, M2 = *A. ferrooxidans* without ameliorant, M2D = *A.ferrooxidans* + dolomite, and M2C = *A. ferrooxidans* + coal.

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According to Nogueirol et al. (2015), soil liming can decrease the level of Al in the soil. A.ferrooxidans bacteria can dissolve heavy metals and has long been used in bioleaching processes of copper and gold. Besides, A. ferrooxidans is capable of dissolving sulfide metals (SM) into sulfate (SO_4^2) and metal ions as metal sulfate compounds (MSO₄), thereby metals can be separated and reacquired by bioleaching process (Pradhan et al., 2008). A.ferrooxidans bacteria in metabolism produce organic and inorganic acids, as well as ligands, which successfully remediate metals in the soil. Organic ligand produced by these bacteria is citric acid or oxalic acid, forming a strong bond with metal ions; therefore, they can be extracted from the minerals (Ehrlich, 1992).

Sulfate-reducing bacteria are capable of using sulfate, sulfite, or thiosulfate ion as an electron acceptor to gain energy in the metabolism process. Ions after receiving electrons will be reduced to sulfide. The formation of hydrogen sulfide will also be very beneficial to the environment containing high dissolved metals, as these compounds are highly reactive, forming very stable metal-sulfide; therefore the dissolved metals in the soil will precipitate. Hydrogen sulfide produced in SRB activity is reactive, resulting in rapid reaction with metals forming a hard-soluble sulfide metal compound (insoluble-sulfides) (Hards and Hinggins, 2004). The activity of SRB causes the metal to precipitate, lowering the metal solubility level. The combination of bacteria and limestone gave good results as the application of limestone is very effective to improve soil pH and CEC. Increased CEC indicates induced negative charge amount on soil colloidal surfaces. Therefore, the application of limestone also affects the levels of alkaline exchangeable alkaline, in particular Ca and Mg, then increasing the soil retention capacity against heavy metals. According to Tisdale et al. (1985), when applied in the proper dose, liming gives positive influences, namely 1) Reducing the activity of H⁺ ions on the soil with pH < 4.5, therefore pH can be increased, 2) Elevating soil pH followed with the decrease of heavy metal solubility, and 3) increasing soil pH. This condition occurs due to the presence of hydroxyl ion cluster that binds acid cations (H and Al) in soil colloid to make them inactivated. The combination of bacteria and coal can lower the Al fraction because high organic content in coal does not only provide macronutrients but also makes nutrients available for plants. Besides, pathogenic a lignite is not potentially harmful to the soil.

The percentage distribution of Al fraction on each treatment experienced fluctuating rise value (Figure 2), whereas the interaction of the microbeenriched limestone treatment with *A.ferrooxidans* (M2D) showed the decreased concentration of Al fraction. The aluminium condition is strongly related to the organic material from the complex formed by metal and humic acid, which is unavailable for absorption and less toxic to plants (Vieira et al., 2008).

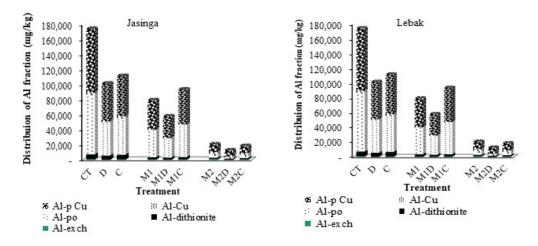


Figure 2. Distribution of Al fractions after microbes and soil ameliorant on acidic soil from Jasinga and Lebak. Al-exch = exchangeable Al, Al-dithionite = crystalline Al , Al-po = non-crystalline inorganic Al, Al-Cu = low or medium Al complex with organic matter, Al-p Cu = highly stabilized Al complex with organic matter, Ct = control without microbe and ameliorant, D = dolomite without microbe, C = coal without microbe, M1 = SRB without ameliorant, M1D = SRB + dolomite, M1C = SRB+coal, M2 = A. *ferrooxidans* without ameliorant, M2D = A.*ferrooxidans* + dolomite, and M2C = A. *Ferrooxidans* + coal.

A.ferrooxidans bacteria application with limestone significantly increased soil pH and Al organic bound, considered to be less toxic to plants than interchangeable Al. Most Al is classified as non-toxic form with organic bonds, such as aluminosilicate or deposits; thus Al toxicity may appear in plants when Al is dissolved in acid soils (Famoso et al., 2010).

Phosphorus availability

Statistical analysis results showed that coal or lime enriched with SRB (M1D or M1C) could increase the availability of P in acid soil (Figure 3). The increase in available P in the treatments added with coal or lime ameliorant enriched with BPS was caused by the release of P (Al-P, Fe-P or clay soil). According to the study of Sari et al. (2017), the increase in P-availability was not only due to the addition of P fertilizer but also due to the release of P (Al-P, Fe-P, or soil clays) absorbed by organic matter. Amelioration of the soil with coal increased the availability of P because the coal can produce humic acid which can affect the level of nutrient release from soil minerals. The use of humic compounds has been reported to reduce P uptake in soils that are high in aluminium and iron oxides (Hartono et al., 2013; Hanudin et al., 2014) The provision of coal as an ameliorant to the land has been proven to increase the capacity of cation exchange, increase the availability of phosphorus and reduce the availability of heavy metals. Application of coal to the soil can increase soil pH (Yazawa et al., 2000) and electrical conductivity in acid soils (Imbufe et al., 2004), reduce the bioavailability of heavy metals (Janos et al., 2010; Simmler et al., 2013) and increase phosphorus uptake from fertilizer (Scheel et al., 2008). Humic substances derived from coal are sources of carboxylic and phenolic acids which provide a reactive place for cation exchange, binding to phytotoxic elements, increasing soil buffering power, and increasing calcium retention in the soil and also increasing nutrient transportation to plants.

According to Malik et al. (2012), soil microbes play an important role in the transformation of soil P through absorption or release of microbial biomass, dissolving inorganic P, organic P formation and mineralization. Soil microbes also play a role in the process of organic P mineralization through the production of phosphatase enzymes such as acid and base phosphatase. Some of the common phosphatase enzymes found in soil are phosphomonoesterase, phosphodiesterase, and phosphoamidase. These enzymes are responsible for the hydrolysis of organic P into inorganic phosphate (H₂PO₄⁻, HPO₄²⁻) available to plants. Sulfate-reducing bacteria

(SRB) are bacteria which under certain conditions can reduce sulfate compounds to sulfides so the potential for controlling sulfates and metals.

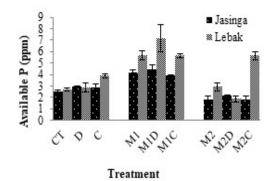


Figure 3. Available-P after microbes and soil ameliorant in acidic soil from Jasinga dan Lebak. Ct = control without microbe and ameliorant, D = dolomite without microbe, C = coal without microbe, M1 = SRB without ameliorant, M1D = SRB + dolomite, M1C = SRB+coal, M2 = A. ferrooxidans without ameliorant, M2D = A.ferrooxidans + dolomite, and M2C = A. Ferrooxidans + coal.

Al fraction and P availability

Available phosphorus is the amount of phosphate available in soil that can be taken up by plants. Figures 5 and 6 show that the highest available P content was found at coal or limestone ameliorant enriched sulfate-reducing bacteria with the distributive condition of Al-pCu >Al-dithionite > Al-po > Al-exch > Al-Cu fraction in acid soil from Jasinga. The highest available P in acid soil from Lebak was found at coal or limestone ameliorant enriched sulfate-reducing bacteria with the distributive condition of Al-pCu > Al-po > Aldithionite > Al-Cu> Al-exch. The increased available P on limestone treatment is thought as the result of increased soil reaction (pH), when the soil reaction happens in acidic to very acidic, the macronutrient availability, including phosphorus, can decrease. This is in line with Sutedjo (2002), who reported that the availability of macronutrients is quite optimal on neutral soil reactions (6.5-7.5), whereas soil pH range of less than 6 will rapidly decrease the availability of phosphorus, potassium, sulfur, calcium, magnesium, and molybdenum. Sulfate-reducing bacteria are the main degrading actor of organic matter in anaerobic sediment and play an important role in organic sulfur mineralization to produce soluble Fe and P.

According to Baumgartner et al. (2006), SRB uses organic material as a carbon source (C). Carbon acts as an electron donor in metabolism and cell constituent material. The combination of bacteria and limestone gave good results as the application of limestone is very effective in improving soil pH and CEC. The increased CEC indicates elevated negative charge amount on soil colloidal surface. Thus, the limestone application also increased the level of exchangeable alkalines, especially Ca and Mg. The increase of soil pH due to application of limestone will increase the activity of sulfate-reducing bacteria. This occurs as SRB produces reactive H_2S that will immediately react with metals forming insoluble sulfide metal compounds (Hards and Higgins, 2004). Therefore, the metal will be precipitated, causing less metal solubility to be unharmful to the environment. Syahputra et al. (2015) stated that the phosphate deficiency in Ultisols 1 could be caused by soil phosphate content that is generally low, besides due to fixation of Al and Fe.

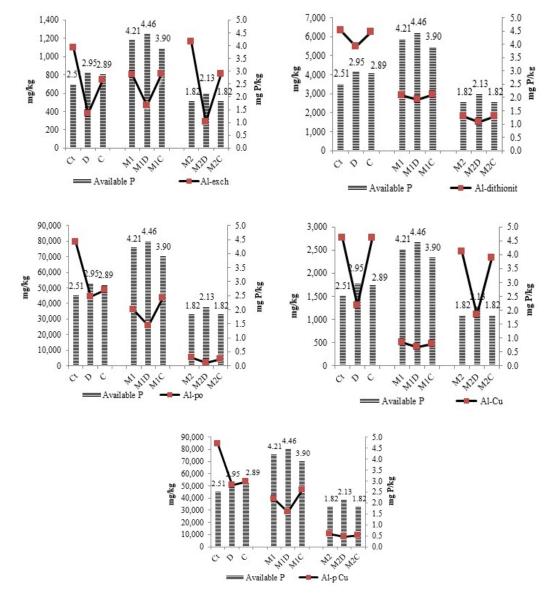


Figure 5. Al fraction and P availability in Jasinga soil. Ct: Control without microbe and ameliorant. Ct = control without microbe and ameliorant, D = dolomite without microbe, C = coal without microbe, M1 = SRB without ameliorant, M1D = SRB + dolomite, M1C = SRB+coal, M2 = A. ferrooxidans without ameliorant, M2D = A.ferrooxidans + dolomite, and M2C = A. Ferrooxidans + coal.

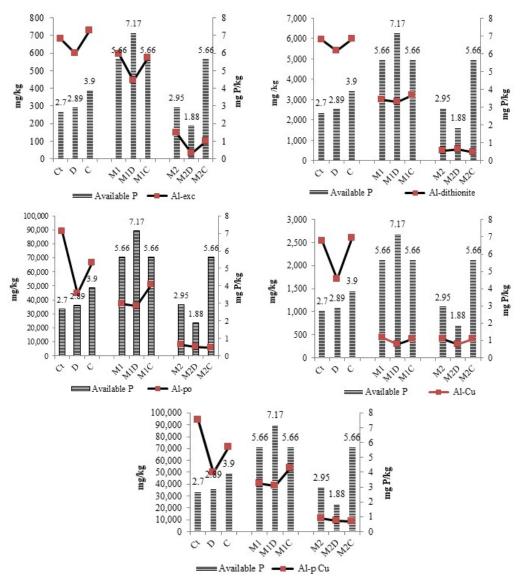


Figure 6. Al fraction and P availability in Lebak soil. Ct: Control without microbe and ameliorant. Ct = control without microbe and ameliorant, D = dolomite without microbe, C = coal without microbe, M1 = SRB without ameliorant, M1D = SRB + dolomite, M1C = SRB+coal, M2 = A. ferrooxidans without ameliorant, M2D = A.ferrooxidans + dolomite, and M2C = A. Ferrooxidans + coal.

Conclusion

The results showed that for acid soils from Jasinga West Java and Lebak Banten, coal or lime ameliorant enriched with *A. ferrooxidans* could reduce the Al fraction content in acid soils. Coal or Lime ameliorant enriched with SRB could increase the availability of P in acid soil from Jasinga West Java with the distribution of Al-pCu > Al-dhitionite> Al-po > Al-exch > Al-Cu fraction. Coal or lime ameliorant enriched with SRB could increase the availability of P in acid soil from Jasinga West Java with the distribution of Al-pCu > Al-dhitionite> Al-po > Al-exch > Al-Cu fraction. Coal or lime ameliorant enriched with SRB could increase the availability of P in acid soil from

Lebak Banten with the distribution of Al-pCu > Al-po > Al-dhitionite> Al-Cu> Al-exch. For future investigations, field tests are needed to draw final conclusions.

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