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Full Length Research Paper

Phosphorus Mobilization in Acid Soil: The Role of Humic Compounds and Phosphate-Solubilizing Bacteria

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This study aims at elucidating the combined effects of humic compounds and phosphate-solubilizing bacteria (*Pseudomonas putida*) to improve characteristics of ultisol and to increase the yields of soybean conducted in a glasshouse. The humic compounds are extracted from rice-straw compost, the phosphate-solubilizing bacteria obtained from Bogor Agricultural University, and the soil (*Typic paleudult*) collected from Kentrong Banten, Indonesia. The results have shown that application of humic compounds combined with inoculation of phosphate-solubilizing bacteria increases pH and available P, and decreases exchangeable Al of an ultisol. The improved soil characteristic, however, does not lead to the significant differences in the uptake of macronutrients by plant.

Key words: Acid soil, exchangeable aluminium, humic compounds, *Pseudomonas putida*, soybean.

INTRODUCTION

Most organic or agricultural wastes are applied to soil in the form of compost containing humic compounds. Humic compounds are the production of biologically mature, stable and chemically complex organic compounds other than carbon dioxide, water and minerals that are released during decomposition of organic matter (Plaza et al., 2005). In soils, humic compounds are recognized to exert a number of essential physical, chemical and biological functions to sustain soil fertility and to protect soil from degradation. Humic compounds consist of humic acid, fulvic acid and humin fraction. These materials are polyelectrolyte of structurally heterogeneous composition, various molecular weights and consist of aromatic and aliphatic structures associated with carboxyl, alcoholic and phenolic hydroxyl, carbonyl, amine, amide and other functional groups (Fernandez et al., 2007). Therefore, humic compounds can control the biological availability, physicochemical behavior, and environmental fate of macro- and micronutrients, toxic metal ions, and xenobiotic organic cations such as pesticides or organic and inorganic pollutants (Angin et al., 2008). Previous studies have suggested that humic compounds may control the high solubility of Al in acid soils (Andre et al., 2008; Cornard et al., 2006; Yu et al., 2008). In acid soils, dissolved Al can reach a certain concentration level, which is toxic to plants and microorganisms due to both its negative influence on root growth (Delhaize et al., 2007) and ability to block the nutrients uptake (Dolling et al., 1991), especially Mg (Tan and Keltjens, 1995). A series of study on application of humic acids from sewage sludge, compost, peat and commercial leonardite to an acid soil significantly increase barley yield (Ayuso et al., 1997).

The use of phosphate-solubilizing bacteria as inoculants simultaneously increases P uptake by the plant and crop yield. Strains from the genera *Pseudomonas, Bacillus* and *Rhizobium* are among the most powerful phosphate solubilizers (Rodriguez and Fraga, 1999). The principal

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Table 1. Composition of medium for phosphate solubilizers (Pikovskays's medium).

Components	Amounts (g l^{-1})
Glucose	10
$Ca_3(PO_4)_2$	5
$(NH_4)_2SO_4$	0.5
NaCl	0.2
$MgSO_4.7H_2O$	0.1
KCl	0.2
Yeast extract	0.5
$MnSO_4.H_2O$	0.002
$FeSO_4.7H_2O$	0.002
pH	7.0

mechanism for mineral phosphate solubilization is the production of organic acids such as acetic, succinct, prop ionic, butyric, formic, oxalic, and citric acids (Perez et al., 2007; Premono, 1994). Acid phosphatase plays a major role in the mineralization of organic phosphorous in soil (Gyaneshwar et al., 1998; Kim et al., 1998). Phosphate-solubilizing bacteria added to media or soil significantly increases acid phosphatase activity at day 7 and maintained this value until the end of 21 days, about 900 µg p-nitrophenyl phosphate day^{-1} (Premono, 1994). The aforementioned processes finally increase phosphorus contents, both $NaHCO_3$-extractable P, water-extractable P (Wan and Wong, 2004). Hence, phosphate-solubilizing bacteria could effectively be used as biofertilizers to enhance yield of crops in phosphate-deficient acid soils. The phosphate-solubilizing bacteria display the ability to solubilise dicalcium phosphate, tricalcium phosphate and hydroxyapatite even ferric and aluminium phosphates. Gyaneshwar et al. (1998) have found two phosphate-solubilizing bacteria that could solubilise both rock phosphate and di-calcium phosphate in unbuffered. This paper reports the combined effect of humic compounds and a phosphate-solubilizing bacterium (*Pseudomonas putida*) in improving P availability in an Ultisol to increase yield of soybean.

MATERIALS AND METHODS

The used soil is air-dry (<2 mm) top soil (0 to 30 cm depth) collected from Kentrong, Banten, Indonesia. The soil is classified as a typic paleudult and has the following characteristics: pH 4.2; 2.4% organic C; 0.27% total N; 100 mg total P kg^{-1}; 1 mg available P kg^{-1}, cation exchange capacity 29.2 cmol kg^{-1}; 0.7, 2.4, 3.7 and 1.3 cmol kg^{-1} of Na^+, K^+, Ca^{2+} and Mg^{2+}, respectively in ammonium acetate pH 7; and 6.7 cmol exchangeable Al kg^{-1}. Humic compounds extracted from rice straw compost are added to the soil and are incubated for 30 days. The compost is previously prepared by composting rice straw naturally in the field (no added chemicals and decomposers). The humic compounds in the compost are extracted by squeezing and diluting the compost in water. The amount of humic compounds obtained from this simple water squeezing and dilution method is 5.63 mg kg^{-1} containing 13,000 me COOH $100g^{-1}$ humic compounds. A strain of phosphate-solubilizing bacterium, that is *P. putida* 27.4B (Premono, 1994) is selected for this study. Prior to application, the effectiveness of bacterium is tested in plates containing Pikosvaya media (Table 1). The bacterial density is measured using a spectrophotometer (Spectronic 21D) at a wavelength of 620 nm. Each combination of five levels of humic compounds and three levels of *P. putida* population are mixed with 5 kg of soil in a 10 kg plastic pot. The five levels of humic compounds are Ho = no added humic compounds (control), H1 = 0.1% humic compounds added before planting, H2 = 0.1% humic compounds continuously supplied during plant growth, H3 = 0.2% humic compounds continuously supplied during plant growth, and H4 = 0.01% humic compounds continuously supplied during plant growth. The three levels of *P. putida* inoculation are P0 = no inoculation, P1 = inoculation of 10^7 CFU g^{-1} soil, and P3 = inoculation of 10^9CFU g^{-1} soil. Each pot receives basal fertilisers consisting of 35 kg N (urea) ha^{-1}; 36 kg P_2O_5 (superphosphate) ha^{-1}; 45 kg K_2O (KCl) ha^{-1}; and 1,100 kg rock phosphate ha^{-1}. The moisture content of the soil in each pot is adjusted 85% of its approximate water holding capacity.

Two seeds of soybean cultivar Wilis from The Tuber Crop Research Institute of Malang are planted in each pot. The fifteen treatments (five levels of humic compound concentration and three levels of *P. putida* population) are arranged in a randomized factorial block design with three replicates. All pots are placed outdoor with plant borders grown around the plots. Soybean is harvested in 30 days after planting and the harvested materials (shoots and roots) are dried at 60°C for 72 h, weighed, ground and analyzed for N, P and K contents. A treatment of 0.1% humic compounds continuously supplied during plant growth with no addition of rock phosphate (H2-BFA) is also performed for evaluating effectiveness of the rock phosphate.

RESULTS

Soil exchangeable Al and P availability

The application of humic compounds combined with inoculation of *P. putida* significantly increases the soil pH (Figure 1) and increases of soil pH, as explained in Table 2. The highest pH value is observed for the H3 treatment (0.2% humic compounds continuously supplied during plant growth). The addition 0.1% humic compounds before planting (H1 treatment) results in a higher pH increase than that of 0.1% humic compounds supplied during plant growth. Except for H1 and H3 treatments, the inoculation of *P. putida* on various concentrations of humic compounds increases the soil pH. The addition of rock phosphate also increase the soil pH. The addition of 0.1% humic compounds combined with inoculation of *P. putida* at 10^9 CFU g^{-1} (H2P2 treatment) results in a higher soil pH than that of the H2-BFA treatment (no added rock phosphate) (Figure 1). The pH increase furthermore affects the dissolution of Al or exchangeable Al that decreases in line with the increasing concentration of humic compounds added. However, as shown in Figure 2, the soil remains to have a relatively high content of exchangeable Al that is not safe for the growth of plants sensitive to Al toxicity. The aluminum is not essential nutrient to plant. Their cation form, Al^{+3}, is toxic to many plants at micromolar concentrations and can limit the growth of plant (Ma et al., 2001). Aluminum-induced cell death is observed in root-tip cells of barley (*Hordeum vulgare*) (Pan et al., 2001). The germination of barley is treated with Al shows a decrease in root growth (in the root tips and loss of cell viability) and a considerable increase in hydrogen peroxide production, as observed in Al-treated germinated seeds in comparison to that of control non-treated seeds (Tamás et al., 2004). In addition, the roots of soybean put in Al solution for 20 h (concentration 20 and 50 µM) shows the growth of roots decreases (Llugany et al., 1995).

The decrease of exchangeable Al content in no added rock phosphate treatment (H2-BFA treatment) is 68.2%, whereas that of the H2P2 treatment is 60.9%. The percentage of exchangeable Al content in the soil varies from 85.5% for the H3P2 treatment to 2.1% for the H0P1 treatment (Table 3). As shown in Figure 3, the soil pH is inversely correlated (r = -0.729) with exchangeable Al content. The combination of humic compounds and *P. putida* significantly increases the amount of dissolved P although its concentration does not reach the amount of P required by most of plants (Figure 4). As observed, the nutrients status after harvesting shows that P-available is very low ranging from 0.698 to 3.859 ppm or at average of 2.212 ppm. This finding supports the observation by PTT (1983) showing the medium P-available of 16 to 25 ppm and the optimum of P-available by Sawyer et al. (2011) 15 to 20 ppm. The improvement of the soil acid characteristics such as increasing pH, CEC, and P-available, and decreasing exchangeable Al does not affect the growth and yield of soybean. The addition of 0.2% humic compounds combined with inculcation of *P. putida* at 10^9 CFU g^{-1} (H3P2 treatment) results in the highest increase of dissolved P (4.175 ppm). The increase of dissolved P in all treatments is higher than that of the H2-BFA treatment (no-added rock phosphate) (Figure 5).

Growth and yield of soybean

As discussed earlier, the addition of humic compounds combined with *P. putida* increases pH and P-available, and decreases exchangeable Al. However, the changes in soil characteristics do not significantly rise the amounts of N, P, and K taken up by soybean. The nutrients uptake and plant dry weight are weakly correlated with the amount of dissolved P (Figure 7). This is in line with ratio of shoot:root that tends to decrease with the increasing concentration of humic compounds added (Figure 8). The addition of humic compounds combined with *P. putida* does not significantly increase the dry weight of soybean seed (Figure 9).

DISCUSSION

The higher pH increase due to addition of 0.1% humic compounds before planting (H1 treatment) compared to that of 0.1% humic compounds during plant growth indicates that the addition of humic compounds only temporary changes soil pH. The application of humic compounds combined with inoculation of *P. putida* companied by rock phosphate addition results in a higher pH increase compared to that of with no rock phosphate addition. This seems to be related to bases contained in the rock phosphate. The difference of the decrease in exchangeable Al concentration in no added rock phosphate treatment from that in added rock phosphate treatments could possibly be due to the roles played by *P. putida* in releasing organic acids such as acetic, succinic, propionic, butyric, formic, oxalic, and citric acids (Premono, 1994), as well as phosphatase and phytase enzymes (Rodriguez and Fraga, 1999). These organic acids would then decrease the pH and chelate dissolved Al. In acid soils, dissolved Al could strongly buffer soil pH through hydrolysis reaction (Lindsay, 1979). The higher decrease in exchangeable Al concentration in humic compounds added treatments compared to that of no added humic compounds treatment is thought to be due to soil pH increase and chelation of exchangeable Al by functional groups, especially COOH, contained in the humic compounds. The functional groups can dissociate their protons at pH of less than 6.53 or at intrinsic constants, $\alpha 1 = 0.109$, so they have negative charges that can bind cations such as Al^{3+}. The higher increase in dissolved P in all treatments compared to that of no

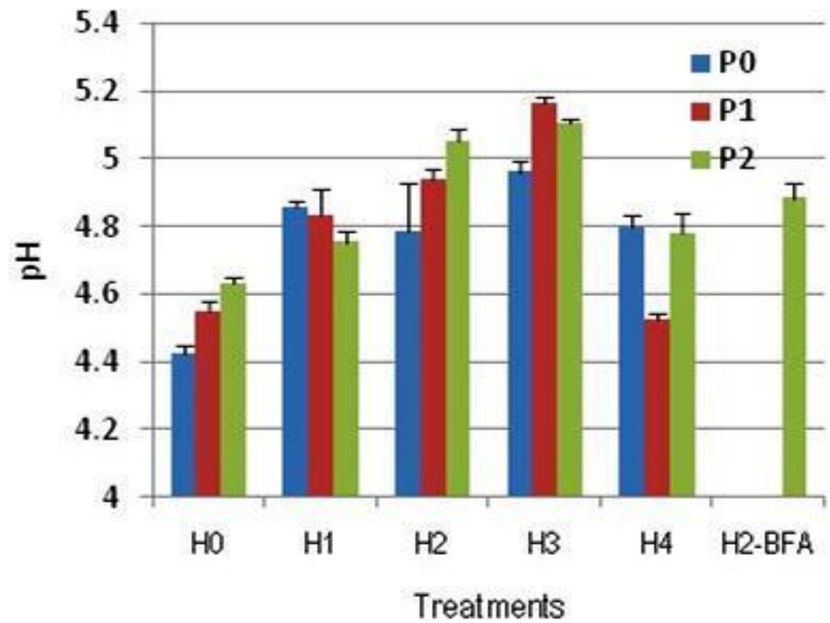

Figure 1. Combined effect of humic compounds and inoculation of *P. putida* on soil pH. Ho = no humic compounds added (control), H1 = 0.1% humic compounds added before planting, H2 = 0.1% humic compounds continuously supplied during plant growth, H3 = 0.2% humic compounds continuously supplied during plant growth, H4 = 0.01% humic compounds continuously supplied during plant growth, H2-BFA = 0.1% humic compounds continuously supplied during plant growth.

Table 2. Combined effect of humic compounds and inoculation of *P. putida* on increased of soil pH to no humic compounds added (control).

Treatment	Humic compounds added	Increasing of soil pH to no humic compounds added (control)		
		Inoculation *P. putida* (CFU mL^{-1})		
		P0 = no	P1 = 10^7	P2 = 10^9
H1	0.1% humic compounds added before planting.	0.43	0.28	0.12
H2	0.1% humic compounds continuously supplied during plant growth.	0.36	0.39	0.42
H3	0.2% humic compounds continuously supplied during plant growth.	0.53	0.61	0.47
H4	0.01% humic compounds continuously supplied during plant growth.	0.37	-0.02	0.15

Table 3. Combined effect of humic compounds and inoculation of *P. putida* on soil exchangeable Al.

	Humic compounds added	Exchangeable Al (%)		
		Inoculation *P. putida* (CFU mL^{-1})		
		P0 = no	P1 = 10^7	P2 = 10^9
H0	no humic compounds added (control).		2.1	29.8
H1	0.1% humic compounds added before planting.	55.5	70.8	62.2
H2	0.1% humic compounds continuously supplied during plant growth.	26.3	47.0	60.9
H3	0.2% humic compounds continuously supplied during plant growth.	77.8	77.7	85.5
H4	0.01% humic compounds continuously supplied during plant growth.	12.1	2.4	32.4
H2-BFA	0.1% humic compounds continuously supplied during plant growth.	68.2		

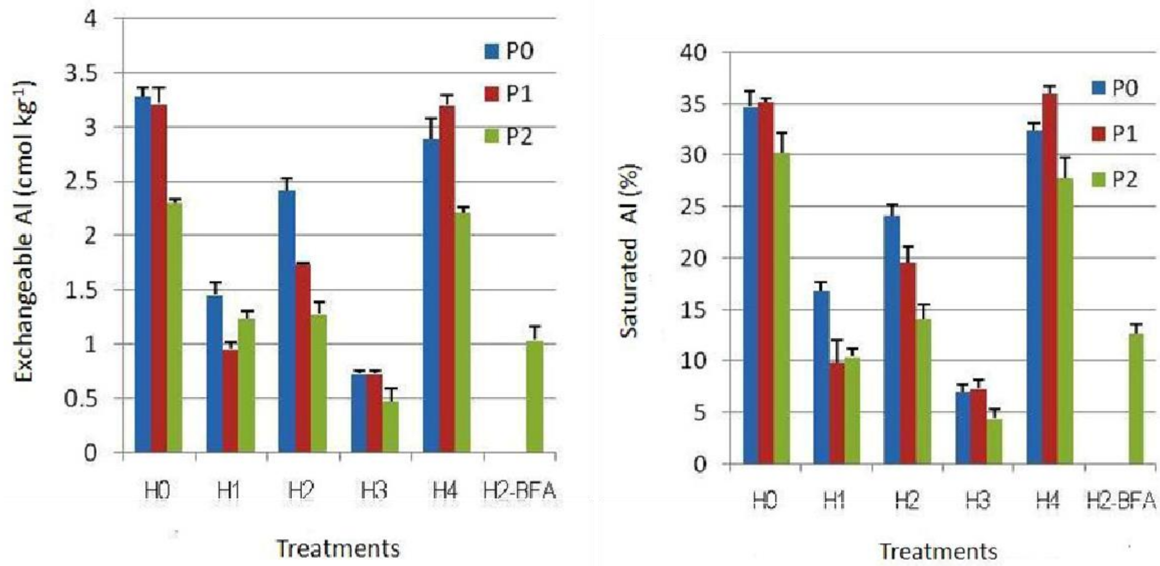

Figure 2. Combined effect of humic compounds and inoculation of *P. putida* on soil exchangeable Al and saturated Al. Ho = no humic compounds added (control), H1 = 0.1% humic compounds added before planting, H2 = 0.1% humic compounds continuously supplied during plant growth, H3 = 0.2% humic compounds continuously supplied during plant growth, H4= 0.01% humic compounds continuously supplied during plant growth, H2-BFA = 0.1% humic compounds continuously supplied during plant growth.

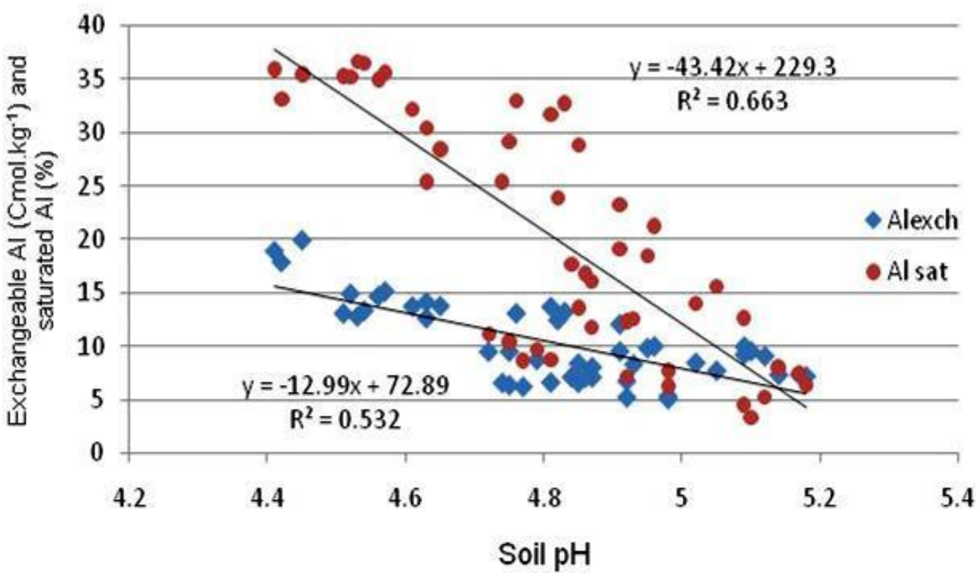

Figure 3. Correlation between pH and soil exchangeable Al (Al_{exch}) and soil saturated Al (Al_{sat}).

added rock phosphate treatment (H2-BFA) indicates that combination of humic compounds and *P. putida* increases available P from rock phosphate (Figure 5). It means that the increase in soil pH will be followed by the decrease in exchangeable Al and conversely, followed by the increase in dissolved P.

When the values of pH and exchangeable Al are linked to the dissolved P values, it is observed that the correlation of pH and exchangeable Al is stronger (r = 0.832) than that of pH and P-dissolved (r = 0.594). This

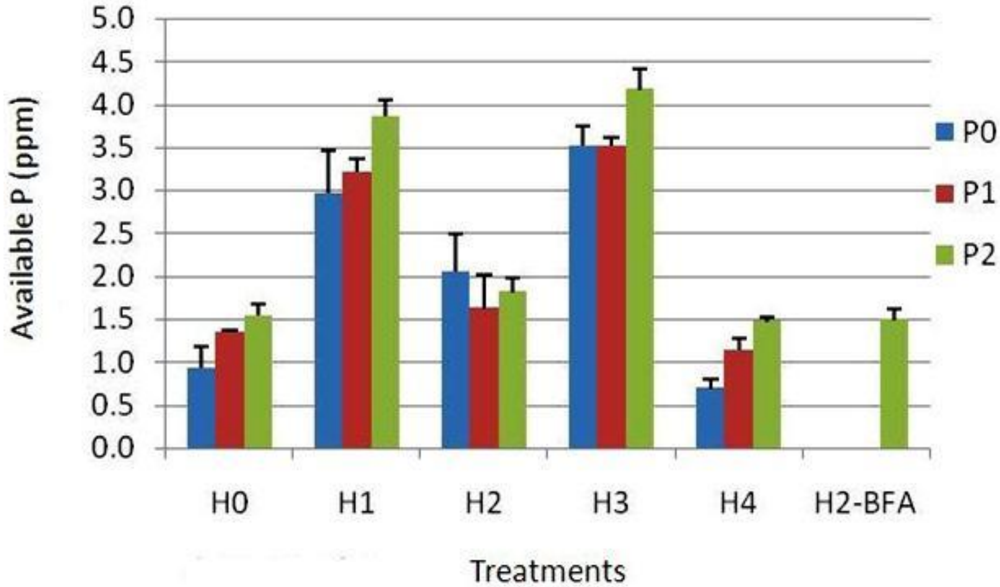

Figure 4. Combined effect of humic compounds and inoculation of *P. putida* on soil P availability. Ho = no humic compounds added (control), H1 = 0.1% humic compounds added before planting, H2 = 0.1% humic compounds continuously supplied during plant growth, H3 = 0.2% humic compounds continuously supplied during plant growth, H4 = 0.01% humic compounds continuously supplied during plant growth, H2-BFA = 0.1% humic compounds continuously supplied during plant growth.

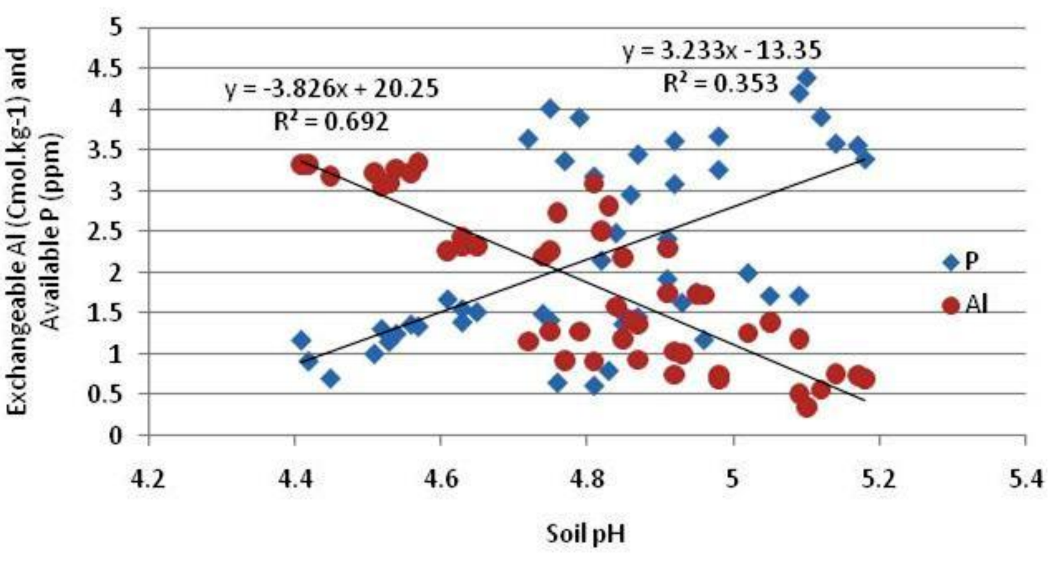

Figure 5. Correlation between pH and exchangeable Al and available P.

indicates that the process of dissolving P is affected by a complex of factors, rather than only by the precipitation of Al in acid soils. Although the addition of humic compounds combined with inoculation of *P. putida* significantly changes soil pH, exchangeable Al and P availability, the changes do not significantly increase the N, P and K uptakes by soybean (Figure 6), and even reduces shoot:root ratio. These corroborate the finding by

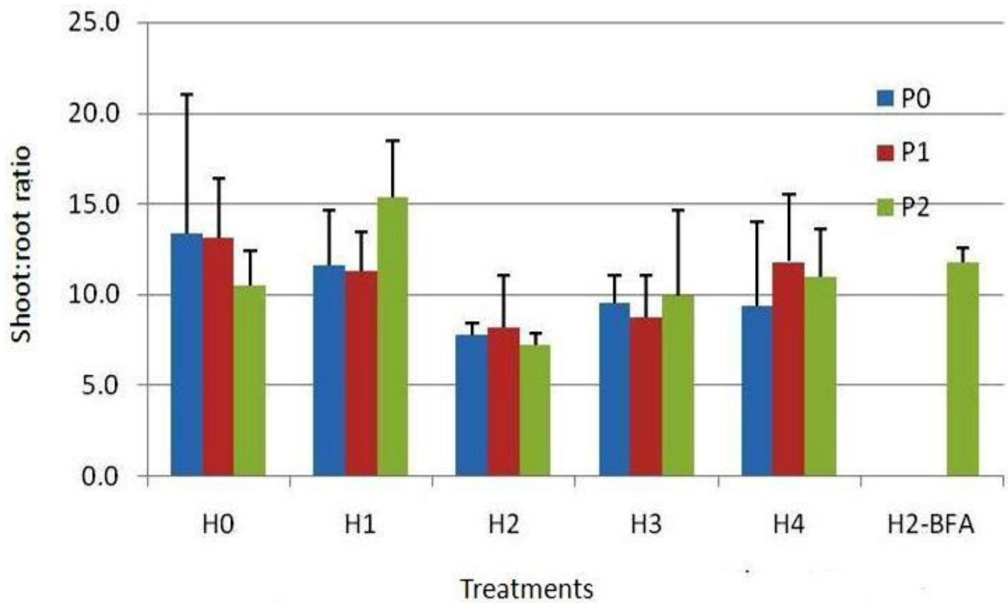

Figure 8. Combined effect of humic compounds and inoculation of *P. putida* on soybean shoot:root ratio. Ho = no humic compounds added (control), H1 = 0.1% humic compounds added before planting, H2 = 0.1% humic compounds continuously supplied during plant growth, H3 = 0.2% humic compounds continuously supplied during plant growth, H4 = 0.01% humic compounds continuously supplied during plant growth, H2-BFA = 0.1% humic compounds continuously supplied during plant growth.

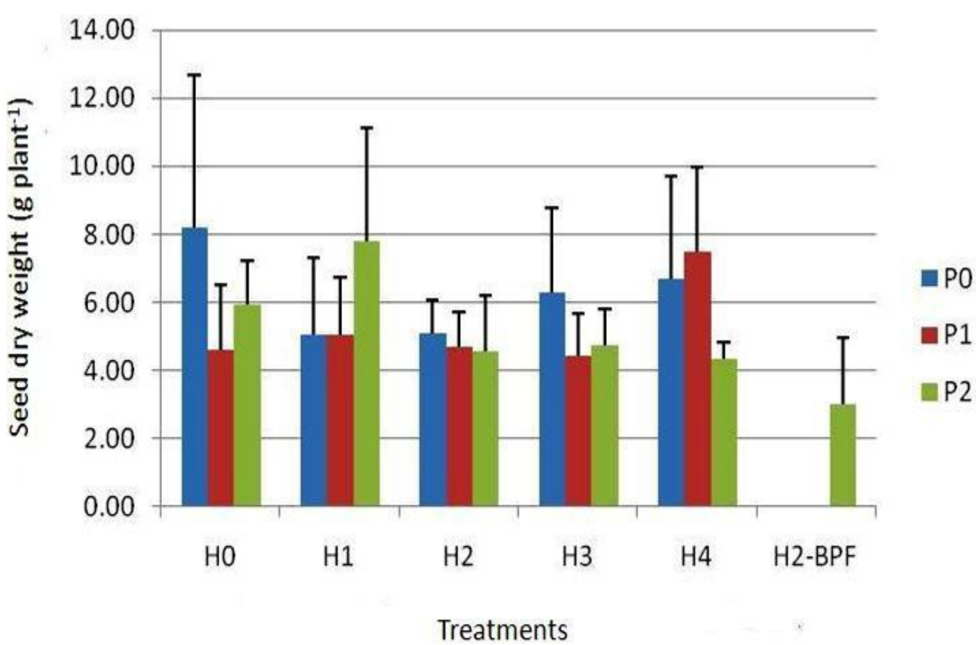

Figure 9. Combined effect of humic compounds and inoculation of *P. putida* on seed dry weight of soybean. Ho = no humic compounds added (control), H1 = 0.1% humic compounds added before planting, H2 = 0.1% humic compounds continuously supplied during plant growth, H3 = 0.2% humic compounds continuously supplied during plant growth, H4 = 0.01% humic compounds continuously supplied during plant growth, H2-BFA = 0.1% humic compounds continuously supplied during plant growth.

Kifuko et al. (2007) that the addition of humic compounds affected distribution of photosinthate to plant parts.

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