Humic acid-phosphate fertilizer interaction and extractable phosphorus in soils of contrasting texture[1]

Interação ácido húmico-fertilizantes fosfatados e fósforo extraível em solos de textura contrastante

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ABSTRACT - Extractable phosphorus (P) in soil depends on soil test employed, reactivity of fertilizer and interaction of P added to the soil properties, including organic matter. The aim of this paper was to assess the contents of P extracted from Red Latosol (LVd) and Quartzarenic Neosol (RQ) samples, incubated with 0; 5; 10; 50 or 100 mg kg^{-1} C-humic acid (C-HA) and 400 mg kg^{-1} of P, via Araxá phosphate rock (AP), Bayóvar reactive phosphate rock (BP) or single superphosphate (SS). Thus, it was adopted a five (C-HA concentrations) x three (P sources) factorial arrangement, using a randomized block design with three replications. The mixtures of soil, C-HA concentrations and P sources were incubated for 60 days; in sequence, the soil P was extracted by solutions of Bray-1, Mehlich-1 and Mehlich-3 and mixed ion exchange resin. In the LVd and RQ samples, the addition of C-HA reduced extractable phosphorus when the source was AP and BP. The levels of extractable P increased with the application of C-HA in RQ when the source was SS; the average concentration of 50 mg kg^{-1} C-AH provided the maximum extractable P by Bray-1, Mehlich-3 and mixed resin. The pH is the chemical attribute that most influence the extractable P content of the soil.

Key words: Humic substances. Extractors of soil P. Soil pH. Phosphate rocks.

RESUMO - O fósforo (P) extraído do solo depende do extrator empregado, reatividade do fertilizante fosfatado e interação do P adicionado com os constituintes do solo, dentre esses a matéria orgânica. Objetivou-se avaliar o P extraível em amostras de Latossolo Vermelho (LVd) e de Neossolo Quartzarênico (RQ), incubadas com 0; 5; 10; 50 ou 100 mg kg^{-1} de C-ácido húmico (C-AH) e 400 mg kg^{-1} de P, disponibilizadas pelas fontes fosfato natural de Araxá (FA), fosfato natural reativo de Bayóvar (FB) ou superfosfato simples (SS). Desse modo, os tratamentos foram constituídos por esquema fatorial cinco (concentrações de C-AH) x três (fontes de P), utilizando o delineamento em blocos casualizados, com três repetições. As misturas entre solo, C-AH e fonte de P foram incubadas por 60 dias e, posteriormente, o P do solo foi extraído pelas soluções de Bray-1, Mehlich-1 e Mehlich-3 e pelo extrator resina mista de troca iônica. No LVd e RQ, a adição de C-AH reduziu os teores extraíveis de P quando a fonte foi o FA e FB. Os teores extraíveis de P aumentaram com a aplicação de C-AH no RQ com o uso do SS, de modo que a concentração média de 50 mg kg^{-1} de C-AH foi a que propiciou o maior teor de P extraído por Bray-1, Mehlich-3 e resina mista. O pH é o atributo químico que mais influencia o teor de P extraível do solo.

Palavras-chave: Substâncias húmicas. Extratores de P do solo. pH do solo. Fosfatos naturais.

INTRODUCTION

The high specific adsorption of phosphorus (P), common in highly weathered Brazilian soils, reduces the ability of crops to uptake P from fertilizer (ROY *et al.*, 2016). Alternatives have been adopted to minimize adsorption and precipitation of P, including the use of humic acid (HA) (URRUTIA *et al.*, 2014). Humic acid can increase P availability in the soil through blocking P adsorption sites and developing a repulsive negative electrostatic field around them, as well as through complexation of Ca, Fe, and Al, preventing precipitation of phosphate (FU *et al.*, 2013; PERASSI; BORGNINO, 2014; URRUTIA *et al.*, 2014; WANG *et al.*, 2016). Furthermore, HA can form complexes of variable stability with P, intermediated by metallic cations that can be gradually solubilized, making P available to plants (GERKE, 2010; URRUTIA *et al.*, 2014).

Knowledge of the different sources of P and the use of technologies related to soil fertility and plant nutrition are necessary for increasing the efficiency of phosphate fertilization. In this respect, HA applied to soil can increase the solubilization of phosphates rocks (PRs) through complexation of cations, such as Ca and Fe present in these rocks, which can destructure the phosphate mineral and increase P solubility (PRAMANIK *et al.*, 2009; SINGH; AMBERGER, 1997). Furthermore, HA can minimize the precipitation and specific adsorption of P coming from sources with high water solubility, such as single superphosphate (SS), favoring P uptake by the plant.

The P extractable from the soil depends on the soil P test (extractant) employed, the source of P, and the type of soil (FREITAS *et al.*, 2013). The Mehlich-1 extractant overestimates the P concentrations in soils with high Ca^{2+} concentration or with a history of fertilization using PRs, due to the acidic character of the extractant, which solubilizes P-Ca; this does not occur with the less acidic Bray-1 and Mehlich-3 solutions (CORRÊA *et al.*, 2008). Nevertheless, all these solutions are subject to reduction in ability to extract P, especially in soils with higher clay content (BORTOLON; GIANELLO, 2008; NOVAIS *et al.*, 2015; SOUZA JÚNIOR *et al.*, 2012). In contrast, soil clay content does not affect P extraction by mixed ionic exchange resin (SIMÕES NETO *et al.*, 2009).

Little is known regarding the efficiency of HA use in increasing the P that can be extracted from soils in tropical regions, nor its effect on the ability of different extractants to perform this extraction. Even so, HA has been applied in agriculture to increase the agronomic efficiency of phosphate fertilizers and P acquisition by plants. The hypotheses raised in this study are that the use of HA increases P extractable from the soil as a result of reducing adsorption and/or precipitation of P and increases solubilization of P-PRs; thus, it is possible to foresee that the magnitude of these effects depends on the concentration of HA and on the chemical attributes of the soil. The aim of this study was to evaluate the effect of humic acid carbon concentrations on the P contents extracted by the mixed resin extractant and by the Bray-1, Mehlich-1, and Mehlich-3 extractant solutions in soils of contrasting textures that were treated with Araxá phosphate rock, Bayóvar reactive phosphate rock, or single superphosphate. The degree of association among P contents extracted by the different soil tests and soil attributes was also investigated.

MATERIALS AND METHODS

Two experiments were conducted simultaneously in the Soil Organic Matter Laboratory (Laboratório de Estudo da Matéria Orgânica do Solo - LEMOS) in the Department of Soil Science/Universidade Federal de Lavras, MG, Brazil, one using samples of a dystrophic Red Latosol (LVd) and the other, samples of Quartzarenic Neosol (RQ). The main physical and chemical characteristics of the soils studied are shown in Table 1. The Brazilian System of Soil Classification separates soil classes in accordance with differential properties or characteristics. Thus, since they are different soil classes, each soil constituted an experiment because comparison of the results generated for the two soils is presumably already contrasting.

The soil samples were collected in Lavras, MG, Brazil, in the 0-0.2 m soil layer under native vegetation. The soils were incubated with $CaCO_3$ and $MgCO_3$ at a 3:1 ratio for 30 days for soil acidity correction and to raise base saturation to 60%, maintaining the samples at moisture content near 70% field capacity (FC).

In the two experiments, the treatments consisted of a 5×3 factorial arrangement through combination of five humic acid carbon concentrations (C-HA): 0, 5, 10, 50, and 100 mg kg^{-1}, with three P sources: Araxá phosphate rock (AP) (fluorapatite), Bayóvar reactive phosphate rock (BP) (francolite), and single superphosphate (SS). P was applied at the rate of 400 mg kg^{-1} based on its P soluble in 2% citric acid solution (CA) for the sources AP and BP, and 400 mg kg^{-1} based on the P soluble in neutral ammonium citrate (NAC) + water for the SS source. The treatments were arranged in a randomized block design in three blocks. The total P contents and the soluble fractions of P of the phosphate fertilizers (Table 2) were determined using the analytical protocols described in Silva (2009).

Table 1 - Physical and chemical characteristics of samples of dystrophic Red Latosol (LVd) and Quartzarenic Neosol (RQ), under natural conditions

Soil attribute	LVd	RQ
pH (H_2O)[1]	4.3	5.2
SOM (g kg^{-1})[2]	62	16
TC (g kg^{-1})[3]	46	9.0
P (mg dm^{-3})[4]	1.8	14
Rem-P (mg L^{-1})[5]	6.4	39
K (mg dm^{-3})[4]	51	39
Ca^{2+} (cmol$_c$ dm^{-3})[6]	0.6	0.4
Mg^{2+} (cmol$_c$ dm^{-3})[6]	0.2	0.1
Al^{3+} (cmol$_c$ dm^{-3})[6]	1.9	0.6
H+Al (cmol$_c$ dm^{-3})[7]	15.3	3.8
CEC at pH 7 (cmol$_c$ dm^{-3})[8]	16.2	4.3
V (%)[9]	6	13
m (%)[10]	68	53
Clay (g kg^{-1})	750	40
Silt (g kg^{-1})	110	30
Sand (g kg^{-1})	140	930

[1] pH in water 1:2.5 (v/v) [2] Soil organic matter Walkley-Black; [3] Total carbon by dry combustion (Elementar Vario EL analyzer); [4] Mehlich-1 extractant; [5] Remaining P [6] KCl extractant 1 mol L^{-1}; [7] SMP buffer; [8] Cation exchange capacity; [9] Base saturation; [10] Aluminum saturation

The HA used was from Sigma-Aldrich®, and its concentrations added to soils were calculated in relation to the C content present in the product, which had the following characteristics: pH in water 1:5 (v/v) 9.8, 376 g kg^{-1} C, 7.65 g kg^{-1} N, 0.28 g kg^{-1} P, 6.3 g kg^{-1} K, 5.9 g kg^{-1} Ca, 0.31 g kg^{-1} Mg, 4.44 g kg^{-1} S, 1.09 g kg^{-1} Fe, 39.9 mg kg^{-1} Cu, 14.8 mg kg^{-1} Mn, and 21.8 mg kg^{-1} Zn. The spectroscopic characteristics of the HA were obtained through use of the attenuated total reflectance of Fourier transform infrared spectroscopy (ATR-FTIR) technique, in a Brucker Vertex 70 v series equipment. The spectral bands were recorded in the mid-infrared region, from 4000 to 400 cm^{-1}, with resolution of 2 cm^{-1}. According to Stevenson (1994) and Silverstein, Webster and Kiemle (2007), the absorption bands from 3360 to 3180 cm^{-1}, with a peak at 3281 cm^{-1}, are characterized by OH groups (Figure 1). Aliphatic stretching of C-H can be attributed to the absorption bands of 2851 and 2919 cm^{-1}. The aromatic structure of C=C was identified at the 1558 cm^{-1} band and deformation of C-CH$_3$ groups at 1375 cm^{-1}. The absorption band at 1080 cm^{-1} represented the deformation of C-O in polysaccharides, and the bands of 1030 and 1009 cm^{-1} represented the stretching of CH-OH in polysaccharides. A ROCH=CH$_2$ grouping was identified at the 912 cm^{-1} band.

After correction of acidity, each soil was mixed with the respective concentrations of C-HA and P sources. The experimental unit consisted of 180 g of the dry mixture (soil + treatment), which were placed in polyethylene cups and incubated for 60 days, with moisture maintained near 80% of FC. The pots were covered with plastic film and opened daily for 15 minutes to favor gas exchanges. After incubation, the samples were dried in open air and passed through a sieve with a 2 mm mesh for analyses of P extractable in mixed ion exchange resin; Mehlich-1 solution (0.05 mol L^{-1} HCl + 0.0125 mol L^{-1} H_2SO_4); Mehlich-3 solution (0.2 mol L^{-1} CH$_3$COOH + 0.25 mol L^{-1} NH$_4$NO$_3$ + 0.015 mol L^{-1} NH$_4$F + 0.013 mol L^{-1} HNO$_3$ + 0.001 mol L^{-1} EDTA), described in Silva (2009); and Bray-1 solution (0.025 mol L^{-1} HCl + 0.03 mol L^{-1} NH$_4$F) (BRAY; KURTZ, 1945). For all the extraction methods, P was determined by UV-visible spectrophotometry, following the procedure recommended by Braga and Defelipo (1974) for Mehlich-1. For the Mehlich-3 and Bray-1 solutions and mixed ionic exchange resin, the analytical protocols used were those described in Silva (2009).

In addition to extractable P, pH in water and electrical conductivity of the soil at the soil:water ratio of 1:2.5 (v/v) were determined. The exchangeable contents of Ca and Mg were extracted with 1 mol L^{-1} KCl solution and determined by atomic absorption spectrophotometry. Soil organic matter (SOM) was determined according to Silva (2009), with modifications, using sodium dichromate

Table 2 - Total P content, P soluble in neutral ammonium citrate (NAC) + water, P soluble in water and P soluble in citric acid (CA) from the P sources, and total P applied to soil samples

P source	Total P	P-NAC+water	P-water	P-CA	Total P applied to soil
	------------------------------------- % -------------------------------------				mg kg^{-1}
Araxá phosphate	11.8	0.87	0.07	1.75	2700
Bayóvar phosphate	12.7	-	-	5.68	890
Single superphosphate	8.3	7.9	5.24	6.11	422

Figure 1 - ATR-FTIR spectrum of the humic acid sample and main organic chemical groups recorded
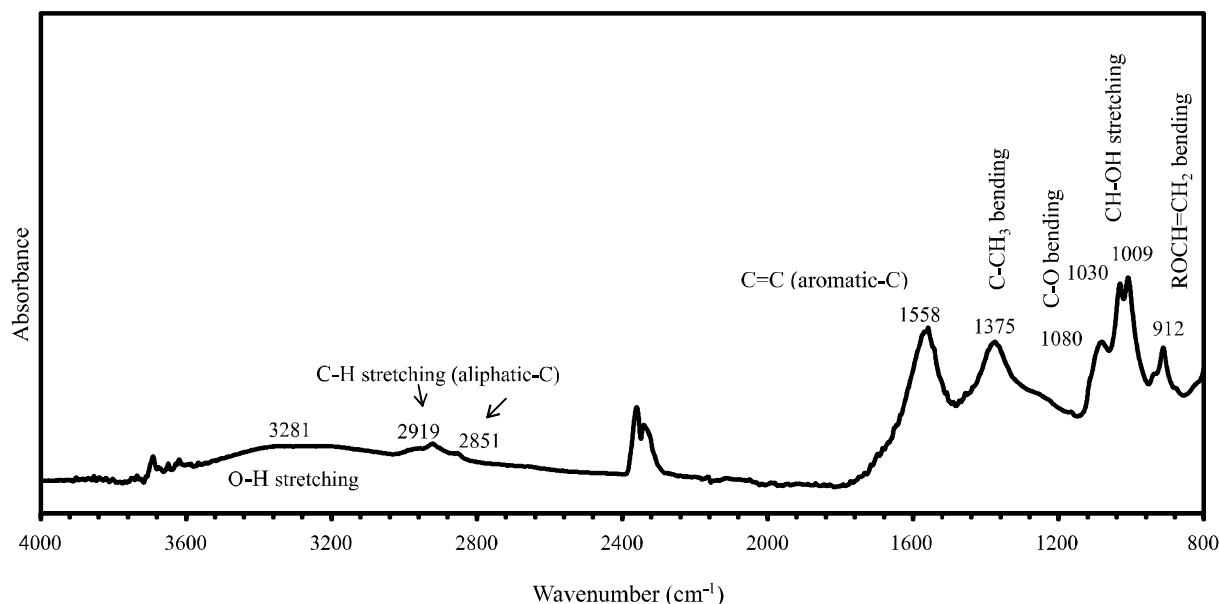
($Na_2Cr_2O_7$) as the oxidizing agent. The main chemical characteristics after incubation of the soil samples with the treatments are shown in Table 3.

The data were subjected to analysis of variance using the computational program SISVAR 5.3 (FERREIRA, 2014) and, in sequence, to regression analysis, relating the concentrations of C-HA to the extractable P contents for each extractant. Normality was previously verified by the Shapiro-Wilk test in the R program (R CORE TEAM, 2014). The regression models were selected based on the significance of the equation parameters ($p < 0.05$), at the lowest value of the sum of squared errors, then choosing the equation with the highest fitted coefficient of determination (R^2).

Table 3 - Chemical characteristics of samples of dystrophic Red Latosol (LVd) and Quartzarenic Neosol (RQ) over humic acid carbon concentrations (C-HA) and P sources, after 60 days of incubation

	C-HA (mg kg⁻¹)														
	0	5	10	50	100	0	5	10	50	100	0	5	10	50	100
	--------Araxá phosphate rock-------					------Bayóvar phosphate rock------					--------Single superphosphate------				
								LVd							
Ca²⁺	5.1	5.3	4.8	4.9	5.1	4.4	4.4	4.5	4.5	4.4	6.8	8.3	8.4	8.6	8.3
Mg²⁺	1.7	1.7	1.5	1.7	1.8	1.4	1.4	1.4	1.4	1.4	1.7	1.6	1.5	1.6	1.6
SOM	60	55	51	55	54	47	54	57	53	56	60	53	53	54	52
pH	5.5	5.4	5.6	5.4	5.5	6.0	6.0	6.0	6.0	6.1	5.4	5.5	5.5	5.6	5.5
EC	0.5	0.5	0.5	0.6	0.7	0.6	0.5	0.5	0.5	0.5	1.1	1.1	1.1	1.0	1.1
								RQ							
Ca²⁺	2.1	1.8	1.9	1.9	1.7	2.0	2.0	1.8	2.0	2.0	5.3	5.8	5.7	5.8	5.9
Mg²⁺	0.6	0.5	0.7	0.7	0.6	0.6	0.5	0.5	0.5	0.5	0.7	0.8	0.7	0.8	0.7
SOM	9.0	11	10	10	11	9.0	10	10	10	11	11	10	10	11	11
pH	6.7	6.8	6.8	6.7	6.8	6.5	6.5	6.6	6.5	6.6	5.8	5.7	5.7	5.7	5.7
EC	0.1	0.1	0.1	0.1	0.1	0.3	0.3	0.3	0.3	0.3	0.9	0.9	0.9	0.9	0.9

Ca^{2+} and Mg^{2+} ($cmol_c\ dm^{-3}$); SOM: soil organic matter (g kg⁻¹); EC: electrical conductivity (dS m⁻¹)

Principal component analysis (PCA) was performed by the Stats version 3.4.0 package in the R program (R CORE TEAM, 2014) to check for the existence of multiple and linear relations between the extractable P contents for each extractant, C-HA, Ca^{2+}, Mg^{2+}, pH, and EC of the soils treated with each P source. Finally, the multiple linear regression procedure was adopted to determine the predictive variables of the P contents extractable for each extraction method and soil investigated, with the use of the *stepwise forward* procedure.

RESULTS AND DISCUSSION

In the dystrophic Red Latosol (LVd) and in the Quartzarenic Neosol (RQ) the P contents extracted by Bray-1, Mehlich-1, Mehlich-3, and mixed resin were affected by the interaction between humic acid carbon concentration (C-HA) and P source ($p < 0.05$) (Table 4).

In the LVd fertilized with Araxá phosphate rock (AP), the quadratic model best fit the reductions in P contents extractable by Bray-1 and Mehlich-1 over C-HA concentrations (Figure 2). The Bray-1 and Mehlich-1 extractant solutions exhibited the lowest extractable P contents, 19.8 and 1224 mg dm^{-3} of P, for the concentrations of 58.3 and 74.9 mg kg^{-1} of C-HA, respectively. For the P contents extractable by resin, the best fit was found with the decreasing exponential model, with reduction and tendency for stabilization of the P extracted as the C-HA concentration increased. In contrast, for Mehlich-3, no effect of the C-HA concentration on the P contents extracted was observed. When the Bayóvar reactive phosphate rock (BP) source was used (Figure 2), the quadratic model best fit the reductions in the P contents over the increasing addition of C-HA, when extracted by Bray-1, Mehlich-3, and resin. The concentrations of 58.7, 62.0, and 83.9 mg kg^{-1} of C-HA provided the lowest extractable P contents, 10.5, 109, and 79.2 mg dm^{-3} of P-BP for Bray-1, Mehlich-3, and resin, respectively. For the Mehlich-1 extractant solution, the P contents reduced in a linear manner as the C-HA concentration was increased.

In RQ, with the use of AP, an effect of the C-HA concentration on the extractable P contents was found only for the Mehlich-1 extractant, which exhibited a linear reduction in the P contents (Figure 2). Effects similar to the application of AP were found when BP was added to RQ, with a difference only in reduction of the P contents extracted by Mehlich-3 over the increasing addition of C-HA.

The application of phosphates rocks (PRs) to LVd and RQ tended to reduce the extractable P contents as C-HA was increased (Figure 2). In a more buffered environment, such as LVd, the application of HA, an humic substance alkaline fraction, may have contributed even more to the buffering of the pH of the soil at values

Table 4 - F value and coefficient of variation to extractable P by Bray-1, Mehlich-1, Mehlich-3 and mixed resin in samples of dystrophic Red Latosol (LVd) and Quartzarenic Neosol (RQ) treated with humic acid carbon concentrations and P sources

SV	df	Bray-1	Mehlich-1	Mehlich-3	Resin
--- LVd ---					
Block	2	0.74[ns]	1.75[ns]	1.85[ns]	1.52[ns]
P sources	2	2109.83***	1897.9***	71.06***	95.91***
[C-HA]	4	3.88*	16.34***	9.06***	17.42***
[C-HA]* P sources	8	2.71*	6.61***	6.82***	9.94***
Residues	28				
CV(%)		8.72	7.82	7.73	9.73
--- RQ ---					
Block	2	3.63*	0.60[ns]	0.76[ns]	0.42[ns]
P sources	2	302.80***	2861.03***	162.54***	1664.03***
[C-HA]	4	0.50[ns]	16.4***	8.06***	3.51*
[C-HA]* P sources	8	3.74***	7.04***	4.28***	2.30*
Residues	28				
CV(%)		2.59	6.14	7.09	7.93

SV – source of variation; df - degrees of freedom; [C-HA] - humic acid carbon concentrations; CV - coefficient of variation; ns - not significant; *,** and *** significant to $p < 0.05$, $p < 0.01$ and $p < 0.001$, by the F-test

Figure 2 - Extractable P by different analytical methods in samples of dystrophic Red Latosol and Quartzarenic Neosol treated with humic acid carbon concentrations and Araxá phosphate rock (AP), Bayóvar reactive phosphate rock (BP), or single superphosphate (SS). *, ** and *** significance of the mathematical equation parameters to $p<0.05$, $p<0.01$ and $p<0.001$, respectively. Error bars represent the standard error of the means
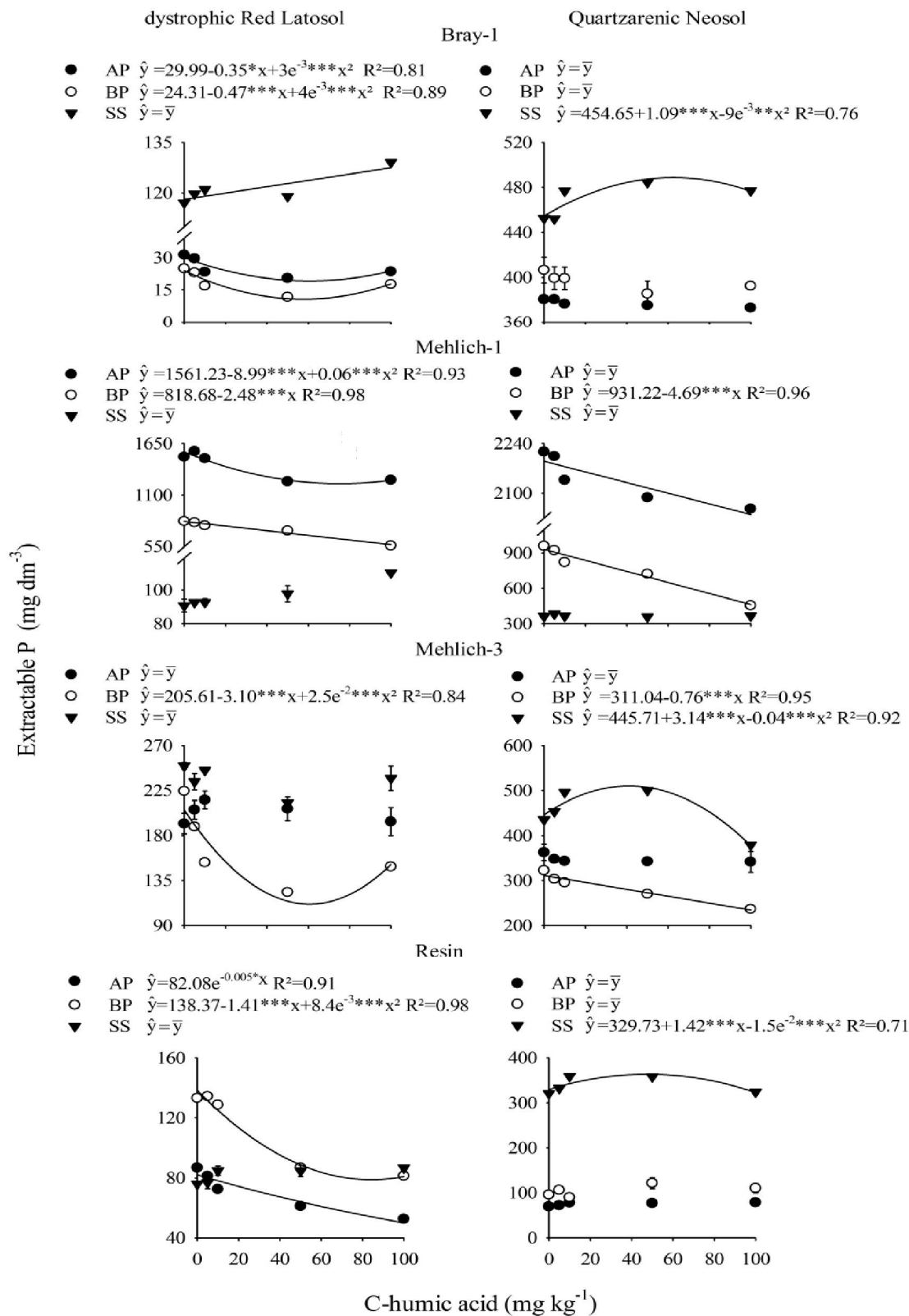
near 6 in the LVd and 7 in the RQ (Table 3). Maintenance of pH at values near neutral tends to maintain the integrity of the apatite, which probably prevented solubilization of P-PRs (CHIEN; PROCHNOW; MIKKELSEN, 2010; DIAS *et al.*, 2015), and increase in the extractable P contents with the application of C-HA to the soil. Chien, Prochnow and Mikkelsen (2010) reported, that among the soil attributes, pH has the greatest influence on the agronomic efficiency of PRs, their efficiency decreasing with an increase in pH. Thus, soil pH may be a more important factor for the solubilization of P-PRs than the presence of organic complexing agents, such as HA. In addition, the buffering of the pH promoted by applications of C-HA may also have reduced the extraction ability of the acid solutions, especially that of Mehlich-1, minimizing its characteristic of acidifying the medium in extraction (NOVAIS *et al.*, 2015). This result was observed in comparison with the soil without application of C-HA. However, the use of Mehlich-1 still overestimated the extractable P contents in the LVd and RQ with PRs compared to the contents observed with single superphosphate (SS) (Figure 2). The acidic character of Mehlich-1 solubilizes the P-Ca of PRs, overestimating the P contents (CORRÊA *et al.*, 2008; FREITAS *et al.*, 2013).

With the use of SS in LVd, the P contents extracted by Bray-1 had a direct and positive relation to the C-HA concentration, whereas, for the other extractants, the C-HA contents did not have an effect on the extractable P contents (Figure 2). The increase in the P content extracted by Bray-1 in the soil came to 8% with the application of 100 mg kg^{-1} of C-HA. Viégas, Novais and Schulthais (2010) found greater P recovery by Bray-1 than by Mehlich-1 and resin, due to the presence of fluoride in the composition of the Bray-1 extractant, which shows high affinity to Al, extracting the P-Al form more efficiently. This effect indicates that the main species of P in the LVd may be connected to Al. The Bray-1 extractant showed greater sensitivity in detecting the effects of C-HA. Thus, the C of the HA may in fact have reduced the specific adsorption and/or precipitation of P.

Humic acid has carboxylic groups that, when added to the soil, can compete with P adsorption sites and complex metallic cations of the solution, which results in an increase in the extractable P contents (FU *et al.*, 2013; URRUTIA *et al.*, 2014). In addition to these mechanisms, the HA adsorbed in the soil can generate a repulsive negative electrostatic field and, due to its high molecular weight, may also promote a physical barrier at the mineral surface, which reduces the P affinity to soil colloids (FU *et al.*, 2013; PERASSI; BORGNINO, 2014; WANG *et al.*, 2016; YAN *et al.*, 2016). Wang *et al.* (2016) furthermore found that HA reduces the specific surface area (SSA) of synthetic hematite and goethite, reducing adsorption capacity and P affinity with these minerals.

This effect was also observed by Yan *et al.* (2016), in which HA reduced the SSA of ferrihydrite to one fourth and the SSA of goethite to nearly half, which increased the P content in solution. Although these studies were performed on synthesized minerals in the laboratory, they assist in understanding the dynamics between HA and P in soils, above all those of tropical regions, since they have a higher proportion of iron oxides.

With the application of SS in the RQ, an increase was found in the P contents extracted by Bray-1, Mehlich-3, and resin up to the concentrations of 60, 40, and 47 mg kg^{-1} of C-HA, respectively, which represents an increase in the P contents of 8, 14, and 10 % for Bray-1, Mehlich-3, and resin respectively, followed by decline (Figure 2). In the RQ, not only the Bray-1 extractant but also the Mehlich-3 and the resin were sensitive to displaying the effects of application of C-HA over the P extracted from the soil. Yang *et al.* (2013), studying application of humic substances through fulvic acid (FA), also observed an increase in P availability in acid soil, which was correlated with the concentrations of FA applied and with the soil pH.

The RQ has a lower clay and organic matter (SOM) content and, therefore, lower charge density, both negatives and positives, in relation to the LVd, which may have increased HA reactivity in the soil. Thus, the C-HA may have blocked some adsorption sites or have reduced the formation of P precipitates, promoting the increase in extractable P up to a determined concentration of C-HA. As of this concentration, the HA may have its activity magnified in the RQ and promote an opposite effect, contributing to P adsorption in the soil through formation of HA-cation-P complexes, with reduction in P extractable content.

Phosphorus adsorption in the soil can increase over HA concentration, depending on its activity, through associations among organic compounds, cation, and P, with variable reversibility of the complexes formed (GERKE, 2010; GUPPY *et al.*, 2005; URRUTIA *et al.*, 2014). These organometallic complexes can be intermediated by different cations, such as Ca, Fe, Al, Mg, Cu, Mn, and Zn, with stability of the bond dependent on the type of cation and the activity of the reagents of the complex formed (GUARDADO; URRUTIA; GARCÍA-MINA, 2007; GUPPY *et al.*, 2005; URRUTIA *et al.*, 2014). This effect can be represented in this study by the inflection of the curve of extractable P content, reflected in the decrease in the P contents with the increase in C-HA concentration (Figure 2). This organic complexation with greater stability of P may have been the main factor that prevented the linear increase in extractable P contents at the highest concentrations of

C-HA in the RQ when SS was used. In the short term, the reversibility of the complex is expected to be low, due to the greater availability of organic ligands in the medium over concentrations higher than 50 mg kg^{-1} of C-HA.

The extractable P contents in the RQ, in general, were greater than those in the LVd for the PRs (Figure 2), which shows the difference of P buffering capacity, directly related to the properties of these soils, such as clay content. Simões Neto *et al.* (2009), using the Mehlich-1 extractant for estimation of available P in soils growing sugarcane, observed greater P extraction in soils less buffered due to a low clay content, such as Espodosol and dystrophic Yellow Argisol. In contrast, the Mehlich-1 extractant underestimates the P contents in clayey soils due to consumption of SO_4^{2-} present in the extractant, which is adsorbed in soil mineral sites not yet occupied by P, causing Mehlich-1 to lose its extraction capacity (NOVAIS *et al.*, 2015; VIÉGAS; NOVAIS; SCHULTHAIS, 2010). Moreover, it can be observed that, for some extractants, the P contents for AP are higher than for BP, which is in agreement with the results of Freitas *et al.* (2013), who found higher P contents extracted in soils with AP than with the reactive phosphate rock.

Principal component analyses (PCA) allowed verification of the tendency or multivariate relation of the HA and of the P sources with the chemical attributes studied in each soil. It was found that the attributes studied explained 73.8% and 86.6% of the total variability of the data for the LVd and RQ, respectively (Figure 3). In the

LVd, the formation of two groups corresponding to the P sources used, PRs and SS, was observed, showing the difference in the response between the P sources. The variations observed with the SS were more correlated with the exchangeable Ca content and electrical conductivity (EC) and with the P content extracted from the soil by Bray-1. Thus, the Bray-1 extractant was the method most related to the variations of extractable P in the soil with SS, which confirms the sensitivity of this extractant in measuring the effect of the C-HA concentrations on the P content observed in the LVd (Figure 2). However, the P extracted by Mehlich-1 was more related to the PRs, especially to the use of AP; Mehlich-1 extracted the highest contents of P when that source was applied to the soil (Figure 2).

An additional observation in the RQ was the formation of two inversely correlated groups, represented by the PRs and SS, in a more prominent way than that observed in the LVd (Figure 3). In this soil, the P extracted by Mehlich-1 and the pH exhibited a positive correlation with the PRs and an inverse correlation with the exchangeable Ca contents. The P extracted by mixed resin, Bray-1, and Mehlich-3, exchangeable Ca and Mg, and EC were the variables most correlated with SS. This relationship between the soil P extractants and SS also confirms the patterns observed in the P extractable contents (Figure 2), which showed the greater sensitivity of these extraction methods in detecting the changes in the P contents over the addition of C-HA in the RQ.

Figure 3 - Principal component analyses between P contents extracted by Bray-1 (P-B1), Mehlich-1 (P-M1), Mehlich-3 (P-M3), Resin (P-res), exchangeable contents of Ca and Mg, electrical conductivity (EC), pH in water and humic acid carbon concentrations (C-HA) in samples of dystrophic Red Latosol (LVd) and Quartzarenic Neosol (RQ)
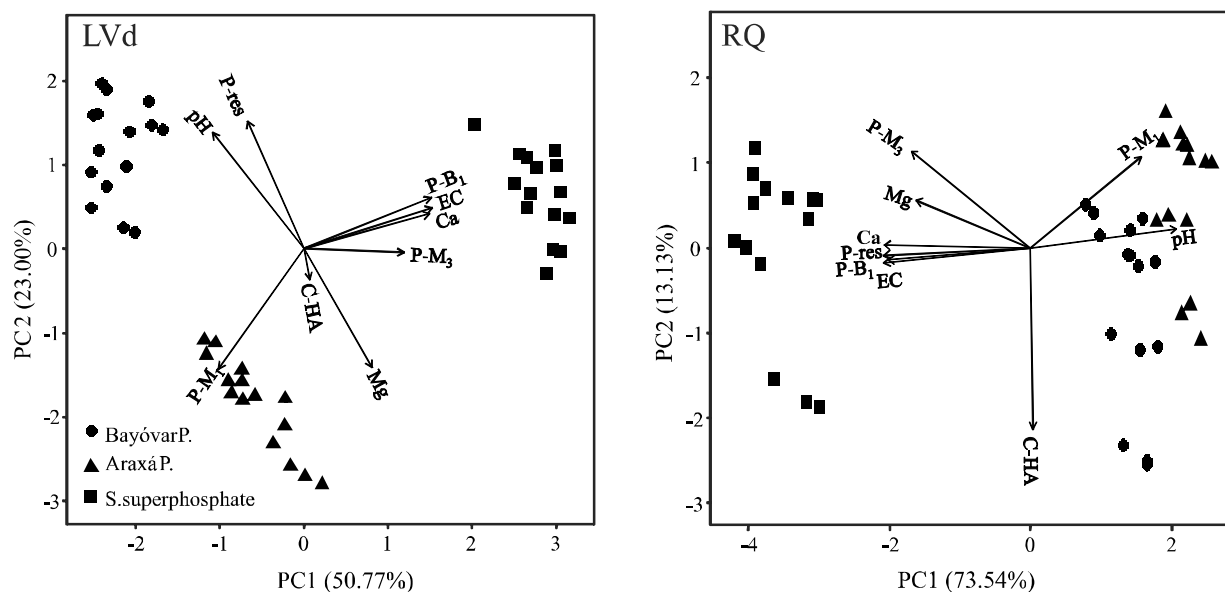
Multiple linear equations were estimated to investigate which variables studied, C-HA, EC, pH, and exchangeable Ca and Mg contents, can be highlighted for prediction of the extractable P contents, regardless of the P source (Table 5). After selection of the regression models, carried out for each method of P extraction from the soil, it could be observed that the PCA (Figure 3) was an analysis that indicated the predictive variables of the extractable P contents. It could be an analysis prior to selection of the models, and, moreover, indicate if there is collinearity or multicollinearity among the attributes.

In the multiple linear regression models selected for each soil P extractant, the presence of C-HA, EC, pH, and exchangeable Ca were found to be variables that predicted extractable P contents (Table 5). Thus, these variables explain the cumulative dependence of the P contents extractable by each method, especially the C-HA and pH variables, which were most related to the equations. The pH level is especially noteworthy, because this variable showed the highest angular coefficients in the models in which it was selected, indicating its importance to explain the extractable P contents. The multiple linear regression

models included a limited number of predictive variables of extractable P and the coefficient of determination (R^2) showed that the models fit well (Table 5). These characteristics facilitate understanding and the applicability of the equations (MINASNY; HARTEMINK, 2011). In a study conducted by Gimsing, Borgaard and Bang (2004), SOM, and especially pH, were the predictive variables that most explained soil P adsorption, which directly affects availability of the nutrient to plants.

In this study, the variables that most affected the P extracted through each analytical profile could be observed, which shows the particularities of each P extraction methods. Each extractant acts in a different manner for extraction of P from different soil compartments; the constituents of each extractant can chemically bind to different compounds (SIMÕES NETO *et al.*, 2009). According to the results, variations in the contents of P extracted by the different methods were regulated by multiple effects, with the dynamic between HA and P changed due to the reactivity or solubility of the phosphate fertilizer, C-HA concentration, pH, cations, and soil class.

Table 5 - Multiple linear regression and coefficient of determination (R^2) for the prediction of P contents in samples of the dystrophic Red Latosol (LVd) and Quartzarenic Neosol (RQ), for each extraction method

P-extractor	Soil	R^2
---	------ LVd ------	---
Bray-1	P (mg dm^{-3}) = -69.08 -0.09* C-HA +0.17* EC	0.96
Mehlich-1	P (mg dm^{-3}) = 9922.5 -1320.91* pH -2.24* EC	0.94
Mehlich-3	P (mg dm^{-3}) = 445.32 -0.28* C-HA -50.52* pH +0.07* EC	0.61
Resin	P (mg dm^{-3}) = -274.93 -0.28* C-HA +65.7* pH	0.65
---	------ RQ ------	---
Bray-1	P (mg dm^{-3}) = 987.1 -90,91* pH	0.89
Mehlich-1	P (mg dm^{-3}) = -31956.65 -3.13* C-HA +839.74* Ca^{2+} +4815.74* pH	0.95
Mehlich-3	P (mg dm^{-3}) = 265.52 -0.52* C-HA +35.77* Ca^{2+}	0.72
Resin	P (mg dm^{-3}) = 14.2 +0.35* EC	0.97

*Significant to $p<0.05$ by F-test. EC, electrical conductivity

CONCLUSIONS

1. The humic acid concentrations does not increase the P contents extracted from the dystrophic Red Latosol and the Quartzarenic Neosol treated with Araxá phosphate rock or Bayóvar reactive phosphate rock;

2. The use of humic acid with single superphosphate increases the extractable contents of P from the Red Latosol and from the Quartzarenic Neosol, especially

when the Bray-1 extractant is used. With the use of single superphosphate, the maximum content of P extracted by Bray-1, Mehlich-3, and resin in the Quartzarenic Neosol is reached at the mean concentration of 50 mg kg^{-1} of C-HA;

3. Soil pH is the chemical attribute that most affects the variations in the contents of P extractable from the Red Latosol and from the Quartzarenic Neosol, especially when the Mehlich-1 solution is used as soil P test.

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