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Availability of phosphorus for maize in crop-livestock integration system[1]

Disponibilidade de fósforo para o milho em sistema de integração lavoura-pecuária

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ABSTRACT - The objectives of this study were to assess the concentrations of available phosphorus (P) extracted using the Mehlich-1 (M1), ion exchange resin (IER) and modified Morgan (MM) methods after 36 months of experimentation with the application of phosphates in a crop-livestock integration system (CLIS). Moreover, this study aimed to assess which extraction method provides the best correlation of P concentrations with the maize crop attributes. The treatments consisted of applying annual doses of P_2O_5 on the soil surface of the total area during the sowing of winter forage crops in the form of triple superphosphate (TSP), rock phosphate (RP) or magnesium thermophosphate (MTP). After 36 months of experimentation, soil samples were collected from the 0-5, 5-10, 10-15 and 15-20 cm layers to assess the concentrations of available P. The dry matter yield (DMY), the grain yield (GY), the accumulation of phosphorus in the dry matter (P-DM) and the export of phosphorus (P-Ex) were quantified in maize. The IER method extracted greater amounts of available P, followed by the M1 and MM extractions. The treatments that included the application of TSP and either the M1 or IER extractions had similar efficacies when correlated with the DMY, GY, P-DM and P-Ex attributes of maize. The P extracted by the IER was most correlated with P-DM and P-Ex for the water-insoluble P sources (RP and MTP). The MM solution was ineffective at predicting the availability of P for maize grown in a soil with that had received varying doses and sources of P applications in a CLIS.

Key words: *Zea mays* L. Mehlich-1. Ion exchange resin. Modified Morgan.

RESUMO - Os objetivos deste trabalho foram determinar as concentrações de P disponível extraído pelos métodos de Mehlich-1 (M1), Resina trocadora de íons (RTI) e Morgan Modificado (MM), após 36 meses de experimentação com aplicação de fosfatos em um sistema de integração lavoura-pecuária (SILP). Ainda, verificar qual método de extração proporciona melhor correlação com atributos da cultura do milho. O delineamento empregado foi em esquema fatorial incompleto, com quatro repetições. Foram aplicadas doses de P_2O_5, na superfície do solo, por ocasião da semeadura da forrageira de inverno, nas formas de superfosfato triplo (SFT), fosfato natural reativo (FNR) e termofosfato magnesiano (TFM). Após 36 meses, procederam-se coletas de solos das camadas de 0-5; 5-10; 10-15; 15-20 cm, visando determinar as concentrações de P disponível. Na cultura do milho foram quantificados o rendimento de massa seca (RMS), o rendimento de grãos (RG), o acúmulo de fósforo na massa seca (P-MS) e a exportação de fósforo (P-Ex). A RTI extraiu maiores quantidades de P disponível, seguida do extrator de M1 e do MM. Nos tratamentos que receberam aplicação de SFT, os extratores de M1 e RTI obtiveram eficiência semelhante quando correlacionados com os atributos de RMS, RG, P-MS e P-Ex do milho. Para as fontes insolúveis em água (FNR e TFM), o P extraído pela RTI melhor se correlacionou com o P-MS e o P-Ex. A solução de MM foi ineficiente para prever a biodisponibilidade de P para o milho, cultivado em solo que recebeu a aplicação de doses e fontes de P, em SILP.

Palavras-chave: *Zea mays* L. Mehlich-1. Resina trocadora de íons. Morgan modificado.

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INTRODUCTION

Crop-livestock integration systems (CLIS) combined with no-tillage (NT) have been increasingly used in farms of southern Brazil (BALBINOT JUNIOR *et al*., 2009). The management of soil fertility is a key practice to achieve high yields in production systems because Brazilian soils are highly weathered and low in phosphorus (P) (NOVAIS; SMYTH, 1999).

In that context, supplying P has been an essential practice in Brazil, and the source of the phosphate used has resulted in different crop responses (RESENDE *et al*., 2006; SANTOS; GATIBONI; KAMINSKI, 2008). The phosphates most commonly used in Brazil include (i) water-soluble phosphates - superphosphates; (ii) water-insoluble phosphates and phosphates with high solubility in citric acid (20 g L^{-1}) - thermophosphates; and (iii) water-insoluble phosphates and phosphates with low solubility in citric acid (20 g L^{-1}) - rock phosphates (RAIJ, 2011). The methods of P application have also been diverse, and the most commonly used in grain production are (i) row placement, (ii) soil surface and (iii) band placement (SANTOS; GATIBONI; KAMINSKI, 2008). However, anticipated broadcast applications of phosphate fertilizers in NT systems have been shown to provide high grain yields of soybean and maize (BERTOLINI *et al*., 2008; BOHAC; CÂMARA; SEGATELLI, 2007), making it an interesting alternative for CLIS.

Soil chemical analysis is the primary tool for safe recommendations for phosphate fertilization (PAZ-FERREIRO; VÁZQUEZ; ABREU, 2005). However, the efficacy of using extractions to evaluate P bioavailability has been questioned because it may often fail to represent the actual fertility conditions of P in many soil and management situations (SILVA; RAIJ, 1999). Most Brazilian soil chemical analysis laboratories use the Mehlich-1 (M1) extraction to assess soil P, and this is also the official method used by the State of Paraná (PAVAN *et al*., 1992). However, that method has been one of the least recommended for clayey soils, especially upon application of water-insoluble phosphates (BORTOLON; GIANELLO; KOVAR, 2011).

The ion exchange resin (IER) extraction method is officially used in the state of São Paulo and has been shown to be more effective in assessing soil P availability than the other methods commonly used in Brazil (RAIJ; QUAGGIO; SILVA, 1986; SANTOS; KLIEMANN, 2005). On the other hand, one of the critiques of the IER method has been the low practicality of the method, complicating its large-scale use (MOREIRA; MALAVOLTA, 2001).

Therefore, it is interesting to study other P extraction methods, for example, the modified Morgan (MM) solution, suggested by Magdoff *et al*. (1999). That method has been used in the United States and has high extraction capacity for P linked to the organic fraction of the soil, which is significant in soils under NT (RHEINHEIMER; ANGHINONI, 2003). The MM solution would most likely not overestimate the P derived from water-insoluble phosphates because the pH of the solution is similar to that of the rhizosphere.

The efficacy of different extraction methods in assessing P bioavailability under NT has not been adequately studied and is not well understood for integrated production systems. The objectives of this study were to: (i) assess the concentrations of soil P extracted using the M1, IER and MM methods in different soil layers in CLIS, fertilized with different sources and doses of P_2O_5 at the sowing of the winter annual forage crops; and (ii) assess which extraction method provided the best correlation (for each source used) with maize attributes.

MATERIAL AND METHODS

The experiment was started in April 2009 in the municipality of Castro-PR (latitude: 24°51’49” S, longitude: 49°56’61” W and average altitude: 1020 m), located in the First Paraná Plateau. The area had been under a NT system for eight years at the time the experiment was implemented, and the succession of crops included soybean and maize in the summer and black oats, annual ryegrass and wheat in the winter. The soil was a clayey Typic Distrudept, and its chemical and granulometric attributes are outlined in Table 1.

The mineralogical analysis showed a soil with simple mineralogy, predominantly consisting of quartz, kaolinite and gibbsite and, subordinately, hematite and goethite. The minerals kaolinite and gibbsite were dominant in the clay fraction, and iron oxides (mainly hematite and goethite) were less significant.

The experimental design used for the study consisted of randomized blocks in an incomplete factorial scheme (3x3+1) with four replicates and ten treatments, namely: *Treatment 1* (*T1*) without P application (absolute control); *T2*, *T3* and *T4* consisting of the application of 60, 120 and 180 kg ha^{-1} of total P_2O_5, respectively, in the form of TSP (granulated) with 460, 380 and 130 g kg^{-1} of total P_2O_5, water-soluble P_2O_5 and calcium oxide (CaO), respectively; *T5*, *T6* and *T7* consisting of the application of 60, 120 and 180 kg ha^{-1} of total P_2O_5, respectively, in the form of RP (Arad) with 330, 100 and 370 g kg^{-1} of total P_2O_5, 20 g L^{-1} P_2O_5 soluble in citric acid and CaO, respectively; and *T8*, *T9* and *T10* consisting of the application of 60, 120 and 180 kg ha^{-1} of total P_2O_5, respectively, in the form MTP containing 180, 165, 180, 70 and 100 g kg^{-1} of total P_2O_5, 20 g L^{-1} P_2O_5 soluble in citric acid, CaO, magnesium oxide (MgO) and silicate (SiO_2), respectively.

The amounts of each source were calculated based on the total P_2O_5 content of the fertilizers and applied annually on the soil surface when the sowing of the winter forage crops. Each plot had a total area of 425 m^2 (17 x 25 m). Excluding the 2.0 m guard rows, each plot had a usable area of 273 m^2.

The crop succession employed for the CLIS for the years studied (from April/2009 to April/2012) was as follows: (i) black oat (2009) and maize (2009/10); (ii) annual ryegrass (2010) and soybean (2010/11); and (iii) black oat (2011) and maize (2011/12). In the autumn-winter period, the area was divided into four paddocks of equal size (5525 m²) to adopt the rotational grazing method. Fifteen Canchim, Brangus and Senepol-breed calves with a mean live weight of 300 kg were used in all years. The animals remained in each paddock from four to five days when the crop was black oat and from six to seven days when the crop was annual ryegrass.

Maize (2011/12) was regarded as an indicator plant for P bioavailability in this study. Soil sampling was performed 36 months into the experiment (April/2012), in this case, following the maize harvest. Twelve simple samples were collected (using a probe) to form a composite sample per plot for each soil layer assessed (0-5, 5-10, 10-15 and 15-20 cm), using a soil auger. The available P concentrations were also estimated for the 0-10 cm layer (based on the weighted average between the 0-5 and 5-10 cm layers) and the 0-20 cm layer (based on the weighted average between the 0-5, 5-10, 10-15 and 15-20 cm layers).

Maize (2011/12) samples were collected to quantify the dry matter yield (DMY), grain yield (GY), P accumulation in dry matter (P-DM) and P exportation by grains (P-Ex) upon harvesting. When the maize plants reached the developmental stage R3, 1.0 m of plants were collected per plot and then separated into subsamples of leaves, stalks and cobs to quantify the DMY and P-DM. Maize was harvested and threshed to assess the grain yield (GY) after physiological maturity (R6 stage). Five central rows of 4.0 m were harvested in each plot, for a total harvested area of 17.0 m². Grain subsamples (100 g) were collected to quantify the P concentration in grains (P-Ex).

Soil samples were broken apart, ground and sieved through a 2.0 mm mesh sieve following drying in a forced air convection oven at 40 °C for 48 hours, after which they were termed oven-dried soil (ODS). The ODS samples were prepared for the assessment of available P using the following methods: (i) available P was extracted using Mehlich-1 solution (P-M1): [0.05 mol L^{-1} hydrochloric acid (HCl) + 0.025 mol L^{-1} sulfuric acid (H_2SO_4)], following Pavan *et al.* (1992); (ii) available P was extracted using the ion exchange resin method (P-IER): [Amberlite IRA-410 and Amberlite IRA-120], following Raij, Quaggio, and Silva (1986); and (iii) available P was

Table 1 - Results of chemical and granulometric soil analyses in different soil layers of the experimental area at the time of experiment implementation in April/2009

Layer	pH[1]	H + Al	Al^{3+}	Ca^{2+}	Mg^{2+}	K^{+}	CEC[2]	V[3]
-----cm-----		mmol$_c$ dm$^{-3$}						------%-----
0-5	5.3	70.7	0.0	57.3	37.0	5.0	170.0	58.4
5-10	4.7	89.9	0.4	30.4	21.9	3.0	145.2	38.0
10-15	4.5	103.9	0.6	20.1	18.0	3.0	145.0	28.0
15-20	4.5	104.0	0.6	16.9	16.9	3.0	140.8	26.1
Mean	4.8	92.1	0.4	31.2	23.5	3.5	150.3	38.0

	P[4]	S-SO_4^{2-}	TOC[5]	TN[6]	Clay	Silt	Sand
	mg dm^{-3}		g dm^{-3}		g kg^{-1}		
0-5	9.0	10.4	33.8	2.9	570	252	178
5-10	4.6	11.4	30.5	1,8	610	218	172
10-15	2.0	13.4	27.8	1.7	620	212	168
15-20	1.0	16.1	26.2	1.4	620	217	163
Mean	4.2	12.8	29.6	2.0	605	225	170

[1] pH in 0.01 mol L^{-1} calcium chloride solution. [2] CEC: cation exchange capacity at pH 7.0. [3] V: base saturation. [4] P available by Mehlich-1 solution. [5] TOC: total organic carbon. [6] TN: total nitrogen

extracted using the modified Morgan solution (P-MM): [ammonium hydroxide (NH_4OH) + 1.25 mol L^{-1} acetic acid (CH_3COOH) at pH 4.8], following McIntosh (1969) and Magdoff *et al*. (1999).

To quantify the DMY, the plant samples were dried in the forced air convection oven at 60 ºC until reaching a constant mass. The grains were weighed, and the GY was expressed at 130 g kg^{-1} moisture. Subsequently, all subsamples were ground in a Wiley-type mill equipped with a 1.0 mm mesh screen and stored until the nitric-perchloric digestion and the quantification of P concentrations, following the methods proposed by Malavolta, Vitti, and Oliveira (1997). The accumulation of P-DM and P-Ex was calculated by multiplying the P concentration in the subsample by the DMY and GY, respectively. All P concentration measurements (of the soil and plant extracts) were performed using molecular absorption spectrophotometry.

The soil concentrations of P assessed using the different extraction methods (M1, IER and MM) were subjected to univariate statistical analysis in randomized blocks and an incomplete factorial scheme. A Tukey's test (P=0.05) was applied when F was significant (P<0.05) to compare the effects of the sources and doses of P among the treatments. The effects of the variables were adjusted using regression models for linear or quadratic orthogonal polynomials. The following were considered replicates in the absence of any interaction: (i) for doses - the blocks (four) and the means of the sources (TSP, RP and MTP) and (ii) for sources - the blocks (four) and the means of doses (0, 60, 120 and 180 kg ha^{-1} of total P_2O_5).

Pearson product-moment correlation coefficients (r-values) between the soil (concentrations of P for each extraction in the different soil layers) and maize (DMY, GY, P-DM and P-Ex) variables were used to assess the efficacy of the extraction methods for each P source used (TSP, RP and MTP). All statistical analyses were performed using the SAS program (SAS, 2010).

RESULTS AND DISCUSSION

Soil phosphorus concentrations according to different extraction methods

An interaction occurred between sources and doses of P in the 0-5, 0-10 and 0-20 cm layers for the concentrations of P extracted using the Mehlich-1 solution (P-M1) (Table 2). No interaction or dose effect was found in the other layers (5-10, 10-15, 15-20 cm), although there was an effect of P doses on P availability (Table 2).

The concentrations of P-M1 increased linearly in the 0-5, 0-10 and 0-20 cm layers when TSP, RP or MTP was applied (Figure 1). The increase in those layers was greater with higher doses of RP. For TSP and MTP, the increases were noticeably similar to each other and increased with the dose. The doses of P_2O_5 had no effect on P-M1 availability in the other layers (5-10, 10-15 and 15-20 cm) (Table 2).

The concentrations of P-M1 following the application of RP were noticeably higher than for the other P sources (TSP and MTP) in all layers assessed (Figure 2). Thus, the Mehlich-1 extraction method had high extraction ability for P bound to calcium (Ca) (SILVA; RAIJ, 1999), which overestimated the concentrations of available P (RESENDE *et al*., 2006). Furthermore, the solution pH (approximately 2.0) has been shown to be inadequate for solubilizing iron (Fe) and aluminum (Al) phosphates and favorable for extracting Ca phosphates, even in non-labile forms (RAIJ, 2011; SILVA; RAIJ, 1999). Similar results were found in previous studies (BRASIL; MURAOKA, 1997; SANTOS; KLIEMANN, 2005; SILVA *et al*., 1999), suggesting that the Mehlich-1 extraction method would not be adequate to extract P from soils treated with RP.

However, the M1 extraction was sensitive to increasing P doses and to sources with high solubility (in water for TSP and in citric acid for MTP), indicating that the application of TSP and MTP would not hinder the interpretation of available P using the official method of the Paraná State, despite the deficiencies of the extraction method (particularly with respect to the gradient of clay in the soil) (BORTOLON; GIANELLO; KOVAR, 2011; SILVA *et al*., 1999). Kaminski, Pessoa, and Rheinheimer (1997) found that increasing doses of MTP and TSP increased the concentrations of P-M1, similarly to the present study. Steniner *et al*. (2012) found similar results for soils from Paraná.

Higher concentrations of P-M1 in the 0-5 and 0-10 cm layers (Table 3) may result from: (i) the topsoil application of fertilizer, without incorporation; (ii) the higher concentrations of total organic carbon occurring in these layers (Table 1), which causes a decrease in the specific adsorption of orthophosphate ($H_2PO_4^-$) (GUPPY *et al*., 2005); (iii) the presence of livestock, which provide a high concentration of feces to the system, increasing the accumulation of organic matter (OM) in the topsoil layers, 0 to 10 cm (BARKER, 2012; SOUZA *et al*., 2008); and (iv) defoliation because of grazing greatly changing the architecture of the root system of the forage species (CARVALHO *et al*., 2010), increasing the release of organic acids that compete with P for adsorption sites (PAVINATO; ROSOLEM, 2008) in the topsoil layers.

Table 2 - *F* values for available phosphorus for the different extraction methods in two different soil layers after 36 experimental months with annual application on the soil surface of sources (S) and doses (D) (when sowing the winter forage crops) in crop-livestock integration system

Layer	Variation	P-M1^1	P-IER^2	P-MM^3
0-5 cm	Source	46.79**	1.00^{NS}	5.73**
	Dose	132.26**	27.00**	10.86**
	S *vs* D	11.90**	1.05^{NS}	6.24**
5-10 cm	Source	8.14**	0.46^{NS}	1.24^{NS}
	Dose	2.77^{NS}	1.85^{NS}	2.60^{NS}
	S *vs* D	1.08^{NS}	1.11^{NS}	0.85^{NS}
10-15 cm	Source	5.21**	1.04^{NS}	0.00^4
	Dose	2.64^{NS}	0.24^{NS}	
	S *vs* D	2.24^{NS}	0.25^{NS}	
15-20 cm	Source	12.55**	0.39^{NS}	0.00^4
	Dose	2.81^{NS}	2.56^{NS}	
	S *vs* D	2.14^{NS}	0.94^{NS}	
0-10 cm	Source	47.38**	0.42^{NS}	2.26^{NS}
	Dose	94.45**	5.93**	2.25^{NS}
	S *vs* D	7.95**	0.37^{NS}	2.09^{NS}
0-20 cm	Source	43.05**	0.22^{NS}	4.44**
	Dose	87.30**	4.76**	5.36**
	S *vs* D	7.26**	0.07^{NS}	5.15**

Three phosphorus sources were studied: triple superphosphate, rock phosphate and magnesium thermophosphate. Four doses were studied: 0, 60, 120 and 180 kg ha^{-1} of total P_2O_5. $^{**}P < 0.01$; $^{*}P < 0.05$; and NS: non-significant. ^{1}P-M1: Phosphorus extracted using the Mehlich-1 solution. ^{2}P-IER: Phosphorus extracted using the ion exchange resin method. ^{3}Phosphorus extracted using the modified Morgan solution. ^{4}Values below the detection threshold (> 0.1 mg dm^{-3} phosphorus) of the modified Morgan method

The concentrations of P-M1 in the 0-10 cm layer that received the TSP, RP and MTP treatments, respectively, were, on average, 24, 29 and 24% higher than those found in the medium layers, 0-20 cm (Table 3). Both this vertical gradient of soil P and the absence of effect of the P_2O_5 doses on the 5-10, 10-15 and 15-20 cm layers (Table 2) result from the following factors: (i) the fertilizer was applied to the topsoil layer (broadcast application), without incorporation; (ii) the migration of P is very low in tropical soils, given the high adsorption of orthophosphate to Fe and Al oxides (NOVAIS; SMYTH, 1999); and (iii) the conservation management adopted in NT causes soil disturbance only in the sowing row (CALEGARI *et al.*, 2013; SANTOS; GATIBONI; KAMINSKI, 2008), favoring higher concentration of nutrients in the 0-10 cm layer (SCHLINDWEIN; GIANELLO, 2008).

No interaction occurred between the sources and doses of P for the availability of P extracted using the ion exchange resin (P-IER) method in any of the soil layers assessed (Table 2). There was a quadratic increase in the concentrations of P-IER with increasing P_2O_5 doses in the 0-5, 0-10 and 0-20 cm layers (Figure 3). The maximum concentrations of P-IER in the 0-5 cm (44.4 mg dm^{-3}), 0-10 cm (27.0 mg dm^{-3}) and 0-20 cm (19.0 mg dm^{-3}) layers would be reached at doses of 128, 126 and 126 kg ha^{-1} of total P_2O_5, respectively.

The doses of P_2O_5 had no effect on the availability of P-IER (Table 2), as found for P-M1 in the other layers (5-10, 10-15 and 15-20 cm) (Table 2). Furthermore, the source of P had no effect on the soil concentrations for P-IER (Figure 2). The IER method was not particularly affected by P residues bound to soil Ca (a pertinent fact when the soil receives RP) (Table 3), given the buffering effect of the suspension at a pH slightly less than neutral and the presence of bicarbonate ions (RAIJ, 2011). Similar results were also found by Bissani *et al.* (2002); Moreira and Malavolta (2001); Schlindwein and Gianello (2008); Silva and Raij (1999) and Silva *et al.* (1999). Higher concentrations of P-IER were found in

Figure 1 - Available phosphorus extracted using the Mehlich-1 solution (P-M1) after 36 months with annual application on the soil surface of doses (0, 60, 120 and 180 kg ha^{-1} of P_2O_5) and sources of phosphate [triple superphosphate (○); rock phosphate (□); and magnesium thermophosphate (∆)] in crop-livestock integration system. Points are means of four replicates. $^{**}P<0.01$ and $^{*}P<0.05$

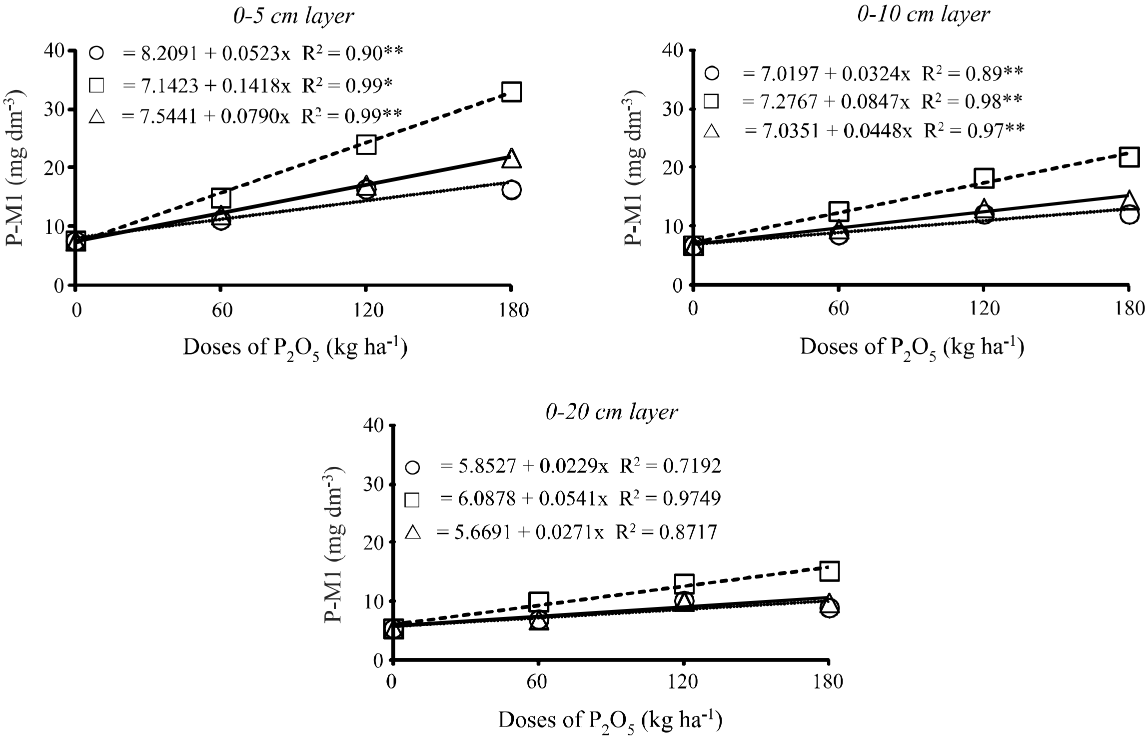


Figure 2 - Effect of varying P sources in varying soil layers on the availability of phosphorus extracted by the A) Mehlich-1 (P-M1), B) ion exchange resin (P-IER) and C) modified Morgan (P-MM) methods after 36 months with annual application on the soil surface of triple superphosphate (TSP), rock phosphate (RP) and magnesium thermophosphate (MTP). Within each layer, means followed by the same letter do not differ from each other according to the Tukey's test (P = 0.05)

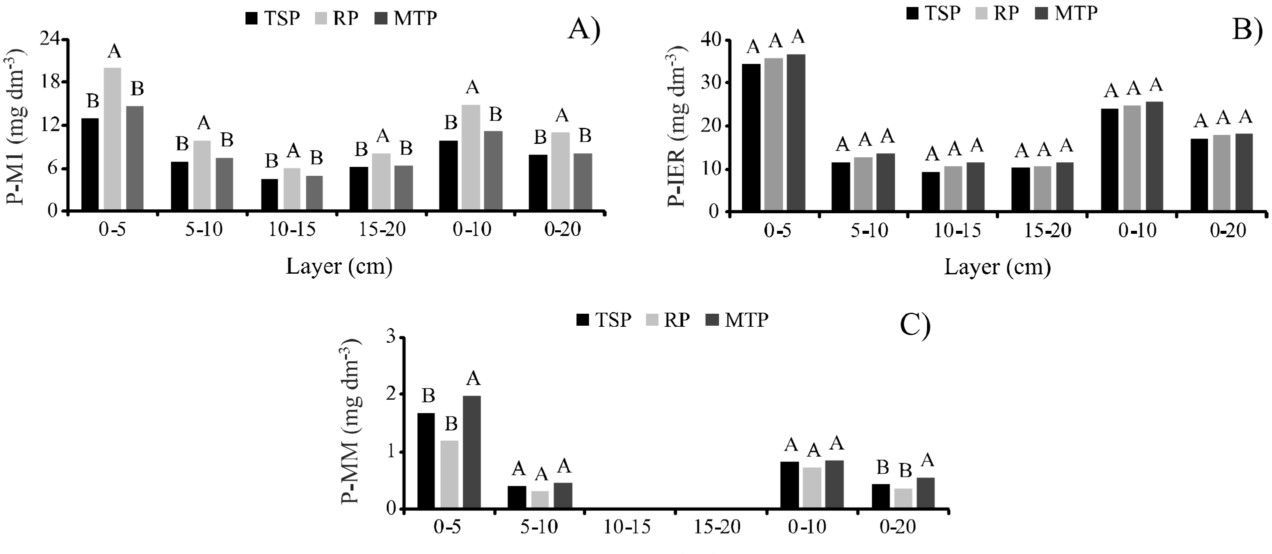


Table 3 - Minimum (min), maximum (max) and mean concentrations (mg dm^{-3}) of available phosphorus from the different extraction methods in different soil layers after 36 experimental months with annual application on the soil surface of sources and doses of phosphorus (when sowing the winter forage crops) in crop-livestock integration system

Layer	Triple superphosphate			Rock phosphate			Magnesium thermophosphate		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
---cm---	--mg dm^{-3} ---								
Available phosphorus by Mehlich-1 solution (P-M1)									
0-5	8.30	21.57	14.94	11.48	34.56	23.02	11.83	23.93	17.88
5-10	4.76	9.53	7.15	7.11	13.50	10.31	4.96	11.73	8.35
10-15	3.02	10.42	6.72	4.27	9.21	6.74	3.92	8.84	6.38
15-20	5.27	8.25	6.76	6.04	10.85	8.45	3.09	10.52	6.81
0-10	6.77	15.05	10.91	9.36	23.90	16.63	7.41	15.92	11.67
0-20	4.92	11.73	8.33	8.07	15.87	11.97	6.12	11.68	8.90
Available phosphorus by ion exchange resin method (P-IER)									
0-5	29.68	54.05	41.87	24.60	54.38	39.49	23.98	53.71	38.85
5-10	6.43	16.64	11.54	8.82	22.50	15.66	7.17	23.07	15.12
10-15	5.74	12.70	9.22	7.36	15.16	11.26	6.39	13.17	9.78
15-20	7.39	16.62	12.01	6.95	15.35	11.15	6.43	23.92	15.18
0-10	18.72	31.36	25.04	22.44	36.19	29.32	16.41	35.04	25.73
0-20	12.89	23.51	18.20	11.99	24.95	18.47	12.40	27.44	19.92
Available phosphorus by modified Morgan solution (P-MM)									
0-5	1.58	4.33	2.96	0.80	1.66	1.23	1.05	5.39	3.22
5-10	0.15	0.77	0.46	0.15	0.54	0.35	0.15	0.84	0.50
10-15	0.00[1]	0.00[1]	0.00[1]	0.00[1]	0.00[1]	0.00[1]	0.00[1]	0.00[1]	0.00[1]
15-20	0.00[1]	0.00[1]	0.00[1]	0.00[1]	0.00[1]	0.00[1]	0.00[1]	0.00[1]	0.00[1]
0-10	0.43	2.55	1.49	0.27	1.03	0.65	0.27	2.77	1.52
0-20	0.20	1.24	0.72	0.14	0.52	0.33	0.24	1.39	0.82

Three phosphorus sources were studied: triple superphosphate, rock phosphate and magnesium thermophosphate. Four doses were studied: 0, 60, 120 and 180 kg ha^{-1} of total P_2O_5. [1] Values below the detection threshold (> 0.1 mg dm^{-3} phosphorus) of the modified Morgan method

the 0-5 and 0-10 cm layers (Figure 3), as also noted for P-M1 (Figure 1). However, the concentrations of P-IER in the 0-10 cm layer for the TSP, RP and MTP treatments, respectively, were, on average, 27, 37 and 23% higher than in the 0-20 cm layer (Table 3).

P extraction using the modified Morgan solution (P-MM) enabled the assessment of P concentrations in only the 0-5, 5-10, 0-10 and 0-20-cm layers (Tables 2 and 3). The concentrations of extracted P in the other layers (10-15 and 15-20 cm) were below the detection threshold of the method (> 0.1 mg dm^{-3}) and were thus considered zero values (0.00) when calculating the weighted average for the 0-20 cm layer (Tables 2 and 3).

An interaction occurred between sources and doses of P for P-MM availability in the 0-5 and 0-20-cm layers (Table 2 and Figure 4). There was no treatment effect on P-MM availability in the other layers (5-10 and 0-10 cm) or the concentrations were below the detection threshold (10-15 and 15-20 cm) (Table 2).

The concentrations of P-MM linearly increased with increasing doses of TSP and MTP in the 0-5 and 0-20 cm layers, although this extraction method was unable to detect the increase in soil P derived from RP (Figure 4). However, the concentrations of P extracted in the treatments receiving MTP were higher than those receiving TSP (Figure 2). The MM solution consists of a diluted organic acid, buffered at pH 4.8, which may have favored the solubilization of MTP because of its high solubility in citric acid, despite being water insoluble (RAIJ, 2011). The mean concentrations of

P-MM in the 0-5 cm layer did not differ between the treatments receiving TSP and RP, and this similarity also occurred in the 0-20 cm layer (Figure 2).

The concentrations of P-MM were well below those found using the other extraction methods, particularly in the treatments with RP (Table 3). That

Figure 3 - Available phosphorus extracted using the ion exchange resin method (P-IER) after 36 months with annual application on the soil surface of doses (0, 60, 120 and 180 kg ha^{-1} of P_2O_5) and sources of phosphate [triple superphosphate (○); rock phosphate (□); and magnesium thermophosphate (Δ)] in crop-livestock integration system. Points are means of four replicates. ***P*<0.01

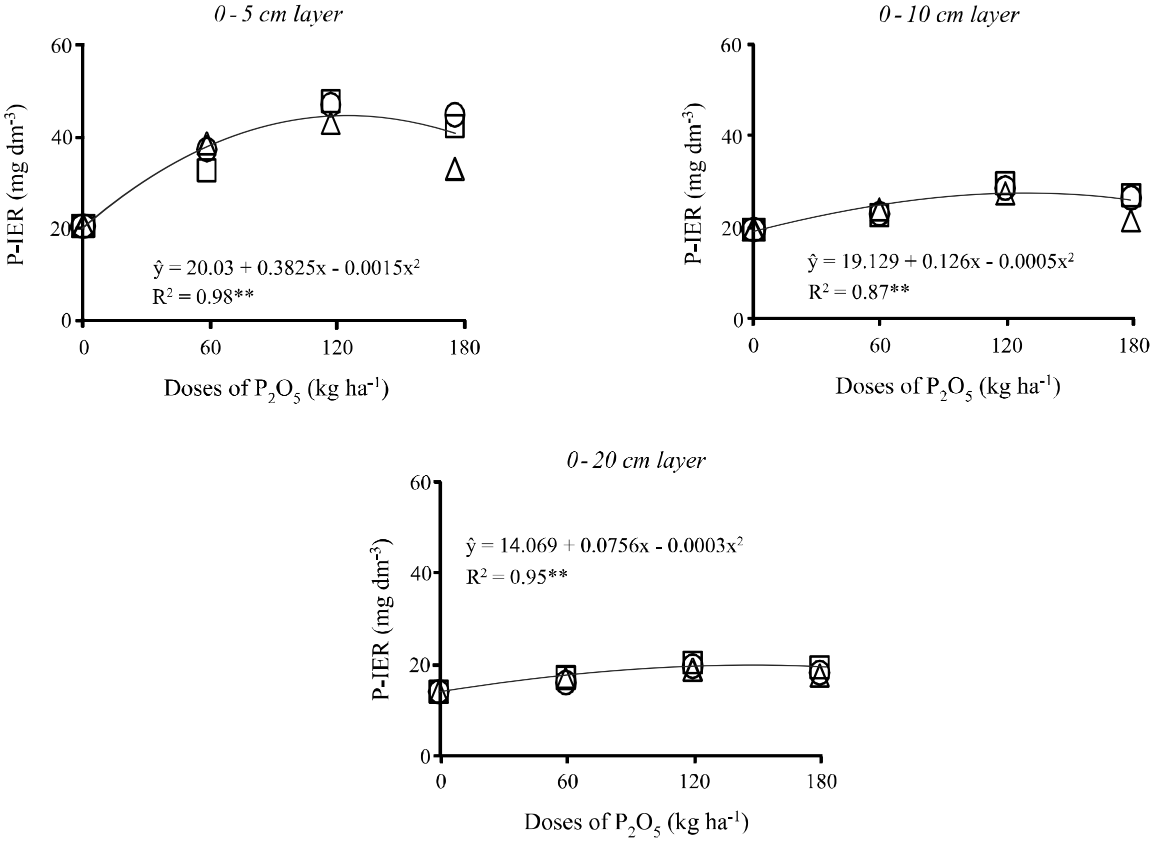
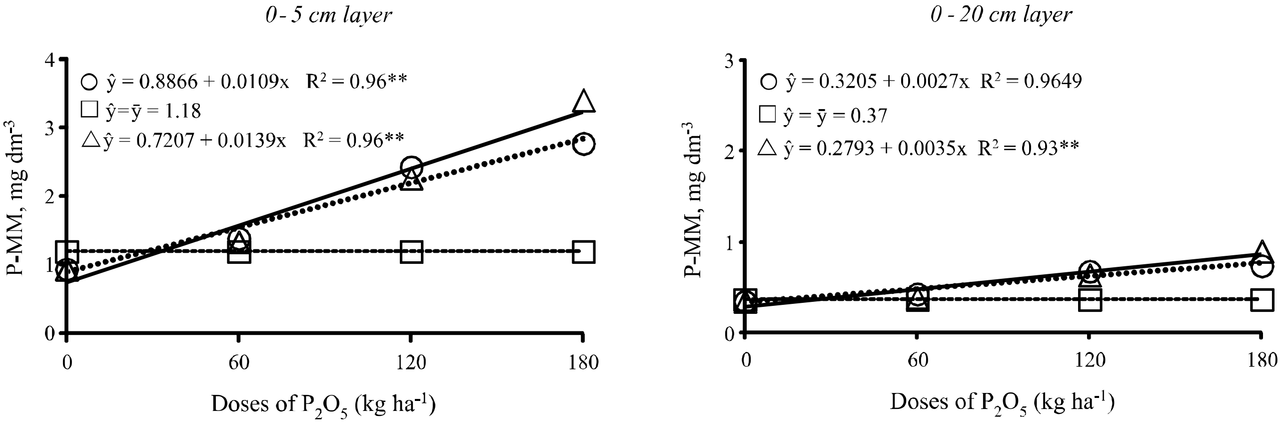


Figure 4 - Available phosphorus extracted using the modified Morgan method after 36 months with annual application on the soil surface of doses (0, 60, 120 and 180 kg ha^{-1} of P_2O_5) and sources of phosphate [triple superphosphate (○); rock phosphate (□); and magnesium thermophosphate (Δ)] in crop-livestock integration system. Points are means of four replicates. ***P*<0.01



distinction in the ability to extract P may be associated with the mode of action of the extractor and the interactions of P with Ca present in the phosphate sources. Most likely, acetic acid had a more moderate effect on apatite solubilization than did the other extractors (SILVA *et al*., 2008). Furthermore, Fe and Al phosphates were likely not solubilized given the solution pH (4.8), resulting in low concentrations of available P (CABALCETA; CORDERO, 1994).

Similarly to P-M1 and P-IER, the concentrations of P-MM were higher in the topsoil layers (0-5 and 0-10 cm) (Table 3), indicating that soil surface fertilization combined with the higher concentrations of organic matter derived from CLIS resulted in greater P availability in the topsoil layers (down to 10 cm), which agreeing the findings of Souza *et al*. (2008).

In general, after 36 months of study, the extracted soil concentrations of available P were observed to be ranked in the following order: ion exchange resin > Mehlich-1 > modified Morgan (Table 3).

Correlations between extracted phosphorus and maize attributes

The effects of the sources and doses of phosphates on maize attributes (DMY, GY, P-DM and P-Ex) in CLIS were discussed previously. In the present study, those attributes were only used for analysis of their correlations (r-values) with available P. The results of the chemical analyses may not represent the nutrient concentrations available in the soil, but they must provide the best possible correlation (BORTOLON; GIANELLO; KOVAR, 2012). The r-values were grouped into five ranges for discussion in this study, following Gujarati (2006): (i) $0.00 < r < 0.19$: very weak; (ii) $0.20 < r < 0.39$: weak; (iii) $0.40 < r < 0.69$: moderate; (iv) $0.70 < r < 0.89$: strong; and (v) $0.90 < r < 1.00$: very strong.

The DMY, GY, P-DM and P-Ex maize attributes were positively correlated with the concentrations of P-M1 and P-IER in the 0-5, 0-10 and 0-20 cm layers for all three phosphate sources (TSP, RP and MTP) (Table 4).

No correlation occurred between the plant attributes (DMY, GY, P-DM and P-Ex) and P-M1 and P-IER in the other layers (5-10, 10-15 and 15-20 cm) (Table 4). The concentrations of P-MM were not correlated with the maize plant attributes (Table 4), given the poor ability of the modified Morgan solution to extract P from the soil (Table 3). Cabalceta and Cordero (1994) recorded values less than 5.0 mg dm^{-3} P-MM in four types of soils in Costa Rica and noted the inefficiency of the modified Morgan solution in assessing the available P in soils with high concentrations of Fe and Al oxides. The similar results of the present study indicate that the modified Morgan solution might not be effective in assessing P bioavailability for maize in clayey soils cultivated under CLIS.

The positive correlations between the concentrations of available P (P-M1 and P-IER) in the 0-5, 0-10 and 0-20 cm layers and the yield attributes (DMY and GY) were moderate, regardless of the P source (Table 4). These results were in contrast with a previous observation of the superiority of IER over M1 regarding those attributes (RAIJ, 2011; RAIJ; QUAGGIO; SILVA, 1986), but agreeing the findings of Moreira and Malavolta (2001) and Alleoni, Fernandes and Jordão (2012).

Strong correlations were found between P-DM and P-Ex and available P (P-M1 and P-IER) in treatments receiving TSP (Table 4). These results indicate the equivalence of the laboratory procedures for M1 and IER for the evaluation of available P in soils to which a highly water-soluble phosphorus source has been applied (SANTOS; KLIEMANN, 2005; SILVA *et al*., 1999).

The r-values for the correlations between P-IER and P-DM and between P-IER and P-Ex were similar to or greater than the values for the correlations of P-M1 with those maize attributes when RP and MTP were applied in increasing doses (Table 4). The better correlations between P-IER and P-DM or P-Ex of maize for those treatments result from: (i) the low saline concentration of the extraction medium of the method, favoring the solubilization of available P; (ii) the solution pH of approximately 7.0, a factor responsible for the extraction of the most available forms of P in the pH range relevant for plants; and (iii) the mixture of anionic and cationic resins reducing the ionic strength and the concentration of divalent and trivalent ions in the solution, increasing the activity of P in the solution and favoring the transfer of soil to the resin (RAIJ, 2011). Thus, the results of the present study agreeing the observations of Silva and Raij (1999) and Silva *et al*. (2008) in soils fertilized with water-insoluble P sources, in which the IER method was superior to M1 in predicting the bioavailability of P.

In treatments with RP and MTP, P-DM and P-Ex were strongly correlated with P-IER in the 0-5 and 0-20 cm layers and moderately in the 0-10 cm layer (Table 4), showing the superiority of IER for assessing the bioavailability of P, even in deeper layers (down to 20 cm), and agreeing the results of Schlindwein and Gianello (2008). The IER method has been used in the State of São Paulo to assess both available P and soil-exchangeable Ca, Mg and K. The IER method would be the most adequate assessment method for the extraction of P derived from water-insoluble sources, although it has not been routinely used throughout Brazil, given the preference for simpler methods (RAIJ, 2011).

Table 4 - Correlation coefficients between the concentrations of available phosphorus from the different extraction methods and different soil layers and maize attributes after 36 experimental months with annual application on the soil surface of sources and doses of phosphorus (when sowing the winter forage crops) in crop-livestock integration system

Layer	P-M1[1]			P-IER[2]			P-MM[3]		
---cm---	TSP[4]	RP[5]	MTP[6]	TSP	RP	MTP	TSP	RP	MTP
Dry matter yield of maize (DMY)									
0-5	0.66**[7]	0.52*	0.55*	0.69**	0.64**	0.65**	0.20^{NS}	0.14^{NS}	0.21^{NS}
5-10	0.18^{NS}	0.23^{NS}	0.18^{NS}	0.18^{NS}	0.24^{NS}	0.31^{NS}	0.01^{NS}	0.02^{NS}	0.11^{NS}
10-15	0.31^{NS}	0.19^{NS}	0.17^{NS}	0.34^{NS}	0.21^{NS}	0.29^{NS}	0.00[8]	0.00[8]	0.00[8]
15-20	0.36^{NS}	0.32^{NS}	0.24^{NS}	0.37^{NS}	0.33^{NS}	0.26^{NS}	0.00[8]	0.00[8]	0.00[8]
0-10	0.63**	0.51*	0.58*	0.64**	0.60**	0.67**	0.23^{NS}	0.20^{NS}	0.21^{NS}
0-20	0.62**	0.50*	0.53*	0.65**	0.65**	0.62**	0.18^{NS}	0.12^{NS}	0.20^{NS}
Grain yield of maize (GY)									
0-5	0.62**	0.50*	0.51*	0.65**	0.60**	0.59*	0.14^{NS}	0.12^{NS}	0.23^{NS}
5-10	0.12^{NS}	0.10^{NS}	0.17^{NS}	0.14^{NS}	0.20^{NS}	0.20^{NS}	0.04^{NS}	0.06^{NS}	0.18^{NS}
10-15	0.21^{NS}	0.21^{NS}	0.28^{NS}	0.22^{NS}	0.30^{NS}	0.30^{NS}	0.00[8]	0.00[8]	0.00[8]
15-20	0.18^{NS}	0.23^{NS}	0.19^{NS}	0.20^{NS}	0.25^{NS}	0.21^{NS}	0.00[8]	0.00[8]	0.00[8]
0-10	0.60**	0.52*	0.55*	0.61**	0.58*	0.58*	0.12^{NS}	0.08^{NS}	0.22^{NS}
0-20	0.63**	0.50*	0.52*	0.64**	0.59**	0.61**	0.10^{NS}	0.02^{NS}	0.15^{NS}
Phosphorus accumulation in dry matter of maize (P-DM)									
0-5	0.76**	0.62**	0.68**	0.79**	0.74**	0.75**	0.26^{NS}	0.18^{NS}	0.31^{NS}
5-10	0.19^{NS}	0.32^{NS}	0.16^{NS}	0.20^{NS}	0.35^{NS}	0.36^{NS}	0.05^{NS}	0.01^{NS}	0.17^{NS}
10-15	0.38^{NS}	0.20^{NS}	0.33^{NS}	0.38^{NS}	0.39^{NS}	0.42^{NS}	0.00[8]	0.00[8]	0.00[8]
15-20	0.40^{NS}	0.34^{NS}	0.42^{NS}	0.41^{NS}	0.55^{NS}	0.48^{NS}	0.00[8]	0.00[8]	0.00[8]
0-10	0.74**	0.63**	0.66**	0.75**	0.68**	0.69**	0.20^{NS}	0.12^{NS}	0.30^{NS}
0-20	0.73**	0.60**	0.64**	0.77**	0.72**	0.73**	0.23^{NS}	0.15^{NS}	0.25^{NS}
Phosphorus exportation by grains of maize (P-Ex)									
0-5	0.72**	0.61**	0.66**	0.76**	0.71**	0.73**	0.28^{NS}	0.20^{NS}	0.34^{NS}
5-10	0.14^{NS}	0.30^{NS}	0.23^{NS}	0.16^{NS}	0.33^{NS}	0.30^{NS}	0.23^{NS}	0.02^{NS}	0.32^{NS}
10-15	0.31^{NS}	0.18^{NS}	0.31^{NS}	0.32^{NS}	0.38^{NS}	0.37^{NS}	0.00[8]	0.00[8]	0.00[8]
15-20	0.18^{NS}	0.15^{NS}	0.22^{NS}	0.20^{NS}	0.35^{NS}	0.37^{NS}	0.00[8]	0.00[8]	0.00[8]
0-10	0.72**	0.62**	0.66**	0.73**	0.68**	0.69**	0.16^{NS}	0.15^{NS}	0.28^{NS}
0-20	0.71**	0.61**	0.62**	0.76**	0.70**	0.73**	0.17^{NS}	0.09^{NS}	0.27^{NS}

**$P < 0.01$; *$P < 0.05$; and NS: non-significant. [1]P-M1: Phosphorus extracted using the Mehlich-1 solution. [2]P-IER: Phosphorus extracted using the ion exchange resin method. [3]Phosphorus extracted using the modified Morgan solution. [4]TSP: Triple superphosphate. [5]RP: Rock phosphate. [6]MTP: Magnesium thermophosphate. [7]Corresponds to 16 observations. [8]Values below the detection threshold (> 0.1 mg dm^{-3} phosphorus) of the modified Morgan method

The r-values for the correlations between available P (P-M1 and P-IER) and P-DM and P-Ex were greater than for the correlations of available P with DMY and GY; 50% of the correlations between available P (P-M1 and P-IER) and P-DM and P-Ex were considered to be strong correlations (Table 3), and 100% of the correlations between available P (P-M1 and P-IER) and DMY and GY (Table 4) were considered moderate correlations. That difference in the strength of correlations indicates a greater precision of the extraction methods when they are correlated with the amounts of P in plants or grains (P-DM and P-Ex) than with crop yield attributes (SILVA *et al.*, 1999).

Of the 72 significant r-values (*P*<0.01 or *P*<0.05), 20 were classified as strong correlations and the others were in the moderate range (Table 4). The r-values for correlations of available P (P-M1 and P-IER) with maize attributes (P-DM and P-Ex) were better rated (strong correlations) for only four correlations because of the soil layer assessed (Table 4). Typically, the categorization of the r-values for the concentrations of P-M1 and P-IER was consistent whether the 0-5, 0-10 or 0-20 cm layer was examined. This indicated that fractionating the soil sampling into 0-10 cm layer, following the Comissão de Química e Fertilidade do Solo (CQFS, 2004), or 0-5 and 5-20 cm layers, following the Instituto Agronômico do Paraná (IAPAR, 1996), would not necessarily entail better correlations between P-M1 and P-IER and maize attributes following superficial application (total area) of phosphates (soluble and insoluble) in CLIS.

Therefore, the efficacy of the P extraction methods for assessment of bioavailability for maize plants after 36 months of study could be classified in the following order: (i) in treatments using TSP: Ion exchange resin ≅ Mehlich-1 > modified Morgan; (ii) in treatments applying RP: Ion exchange resin ≥ Mehlich-1 > modified Morgan; and (iii) in treatments using MTP: Ion exchange resin ≥ Mehlich-1 > modified Morgan (Table 4).

CONCLUSIONS

1. The concentrations of phosphorus (P) available in the soil after 36 months of study varied with the extraction method. The ion exchange resin (IER) extracted larger amounts of available P, followed by the Mehlich-1 (M1) and the modified Morgan (MM) methods;
2. The M1 and IER methods had similar efficiencies when correlated with the dry matter yield (DMY), grain yield (GY), accumulated phosphorus in dry matter (P-DM) and exported P by maize (P-Ex) on treatments in which triple superphosphate (TSP) was applied;
3. The P extracted by the IER was best correlated with P-MS and P-Ex for the water-insoluble sources (rock phosphate and magnesium thermophosphate);
4. The MM solution was ineffective in predicting the bioavailability of P for maize grown in a soil with high clay content and iron and aluminum oxides that had received the varying sources and doses of P applications used in this study in a CLIS.

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