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Comparison of methods for extracting available phosphorus from soils of the semi-arid[1]

Comparação de métodos de extração de fósforo disponível em solos do semiárido

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ABSTRACT - Mehlich-1 is the most used method in Brazil for extracting available phosphorus (P) from the soil. Given the heterogeneity of soils in the semi-arid region of the Northeast, and the need for an analytical protocol for P extraction, the Mehlich-1, Olsen and ion-exchange resin methods were compared in samples of ten representative soils taken from the semi-arid region between the valley of the River Piranhas-Açu (Rio Grande do Norte) and the valley of the River Jaguaribe (Ceará), where the soil pH ranges from 4.1 to 8.0. The soil samples received four doses of P based on the phosphate buffering capacity (PBC) of the soils, and were incubated for 30 days. The treatments were divided into randomised blocks, in a 10 × 4 factorial arrangement, represented by ten soils and four doses of phosphorus, each with three replications. Each experimental unit comprised one plastic pot containing 3.0 dm^3 of soil, where two maize plants were grown for 31 days in a greenhouse. The P extracted by each method was correlated with the P accumulated in the aerial part of the plants. The mixed-resin and Olsen methods showed the best correlation with the P accumulated in the plants, irrespective of the pH or PBC of the soil. Neutralisation of the acidity of Mehlich-1 was significant in alkaline soils with high levels of $CaCO_3$ equivalent. Mehlich-1 was suitable for strongly acidic soils with a low PBC, however, the use of this extractor in pH neutral to alkaline soils with a high Ca^{2+} content can overestimate P availability when the soils are rich in P-Ca, or underestimate the availability in soils rich in $CaCO_3$.

Key words: Alkaline soils. Mehlich-1. Olsen. Ion-exchange resin.

RESUMO - O método Mehlich-1 é o mais utilizado no Brasil para extração do fósforo (P) disponível no solo. Diante da heterogeneidade de solos na região semiárida nordestina e necessidade de um protocolo de análise para extração de P, os métodos Mehlich-1, Olsen e resina trocadora de íons foram comparados em amostras de dez solos representativos da região semiárida compreendida entre os vales dos rios Piranhas-Açu (RN) e Jaguaribe (CE), com pH do solo variando de 4,1 a 8,0. As amostras de solos receberam quatro doses de P de acordo com a capacidade tampão de fosfato (CTF) dos solos e foram incubadas durante 30 dias. Os tratamentos foram distribuídos em blocos casualizados, com arranjo fatorial 10 × 4, sendo estes representados por dez solos e quatro doses de fósforo, com três repetições cada. Cada unidade experimental consistiu de um vaso plástico contendo 3,0 dm^3 de solo, onde foram cultivadas duas plantas de milho durante 31 dias em casa de vegetação. O P extraído pelos métodos foi correlacionado com o P acumulado na parte aérea das plantas de milho. A resina mista e o Olsen apresentaram as melhores correlações com o P acumulado nas plantas, independentemente do pH e da CTF dos solos. A neutralização da acidez do Mehlich-1 foi expressiva nos solos alcalinos com alto teor de $CaCO_3$ equivalente. O Mehlich-1 foi adequado para solos fortemente ácidos e baixa CTF, porém o uso desse extrator em solos de pH neutro a alcalinos com alto teor de Ca^{2+} pode superestimar a disponibilidade de P em solos ricos em P-Ca ou subestimar em solos ricos em $CaCO_3$.

Palavras-chaves: Solos alcalinos. Mehlich-1. Olsen. Resina trocadora de íons.

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INTRODUCTION

A great diversity of soils is found in the semi-arid region of the northeast of Brazil, which range from little to highly developed. In acidic soils, P is mainly found fixed to compounds of Fe (P-Fe) and Al (P-Al). In alkaline soils, most of the P precipitates with Ca (P-Ca), and becomes less available (SOUZA JÚNIOR *et al*., 2012). As the various methods extract different P fractions from the soil, it is difficult to choose a suitable method for regions of heterogeneous soils.

Mehlich-1 (0.05 mol L^{-1} HCl and 0.0125 mol L^{-1} H_2SO_4) is the most used P extractor in Brazil, showing good predictive capacity in acidic soils ($pH < 6.0$), soils with low CEC, and soils poor in, or with no, Ca-bound forms of P (VALADARES *et al*., 2017). However, these characteristics are not present in most agricultural soils in the semi-arid region of the Northeast, such as those derived from limestone in the Chapada do Apodi, where soils with a neutral to alkaline pH and a high Ca content are common (BRITO *et al*., 2017).

In clayey soils and those with a greater $CaCO_3$ content, the amount of P extracted by Mehlich-1 is reduced. Under these conditions, the initial pH of 1.2 is neutralised during the extraction process and the value raised towards that of the soil. This makes the extractor lose its extraction capacity or underestimate the available P (NOVAIS *et al*., 2015). On the other hand, Mehlich-1 overestimates the available P in soils that are naturally rich in P-Ca or fertilised with rock phosphate, as it extracts far more P from these soils than is available to the plants (SOUZA; PEGORARO; REIS, 2017).

The few studies have shown that this extractor may not be suitable as an indicator of P availability, as it can over- or underestimate the available P in soils with a high pH and high Ca content (CALHEIROS *et al*., 2012; SANTOS *et al*., 2016). These variations can result in incorrect fertiliser recommendations. It is therefore necessary to evaluate other methods that would consider the complete range of soils in the region.

The Olsen and ion-exchange resin methods have potential in semi-arid soils. An alkaline solution of $NaHCO_3$, proposed by Olsen *et al*. (1954) for calcareous soils, proved to be suitable for acidic and alkaline soils in the state of Pernambuco (ROCHA *et al*., 2005). Still, this extractor does not solubilise the P bound to the Ca from rock phosphate as happens with Mehlich-1 (HARTMANN *et al*., 2019). According to Raij *et al*. (2001), extraction with ion-exchange resin is superior to all other methods due to its efficiency and applicability to a wide range of soils. This method was suitable for 12 soils in the state of Paraíba, ranging from acid to alkaline (FARIAS *et al*., 2009), however, analytical difficulties prevent its use in many routine laboratories in Brazil.

Given the heterogeneity of soils in the semi-arid region of the Northeast, there is a need for an analytical protocol for P extraction. Accordingly, the aim of this study was to compare the Mehlich-1, Olsen and ion-exchange resin methods in acidic to neutral/alkaline soils. Neutralisation of the acidity of Mehlich-1 and its relationship with the soil characteristics were also evaluated.

MATERIAL AND METHODS

Samples were collected from the surface layer (0 – 30 cm) of 10 representative soils of the semi-arid region in the Chapada do Apodi, located between the valley of the River Piranhas-Açu, in Rio Grande do Norte (RN), and the valley of the River Jaguaribe, in Ceará (CE), mainly used with irrigated agriculture (Figure 1). The sample collection sites were covered by native vegetation and had no history of fertiliser use. One of the soils is derived from sandstone (RQ), two derived from sediments of the Barreiras Group (LVA and PVA1), another from alluvial sediments (RY), and six soils are derived from limestone (CX1, CX2, CX3, PVA2, MD and VX).

Soil samples were sent to the Soil Fertility Laboratory of the Federal Rural University of the Semi-arid, RN, Brazil, where they were air-dried in the shade, broken up, and passed through a 4.0-mm mesh sieve. A subsample of each sample of soil was passed through a 2.0-mm sieve (ADFE) to carry out a physical and chemical analysis as per Teixeira *et al*. (2017) (Table 1). The remaining phosphorus (P-rem) was evaluated after agitating 5 cm^3 ADFE with 50 mL of a 10 mmol L^{-1} $CaCl_2$ solution containing 60 mg L^{-1} P for 5 minutes, followed by decanting for 16 hours (ALVAREZ V. *et al*., 2000). The free P in the solution was determined using the method of Braga and Defelipo (1974).

The experiment was conducted in a greenhouse at the Centre for Agrarian Sciences of UFERSA from February to March 2019. The treatments were randomly distributed in randomised blocks, in a 10 × 4 factorial arrangement of 10 soils and 4 doses of phosphorus, with three replications. The 120 experimental units consisted of plastic pots containing 3.0 dm^3 of soil, in which two Priorizi M274 Morumbi hybrid maize plants (*Zea mays* L.) were grown.

The liming requirement for the soils (RQ, LVA and PVA1) was calculated using the base saturation method (Ve = 50%) (RAIJ *et al*., 1997), and by neutralising the Al^{3+} and increasing the levels of Ca^{2+} and Mg^{2+} (mt = 15%; X = 2 $cmol_c$ dm^{-3}) (RIBEIRO; GUIMARÃES; ALVAREZ V., 1999). The $CaCO_3$ suspension (p.a.) added to each soil corresponded to the arithmetic mean of the values for liming requirement estimated by the two methods. Thus, the RQ soil received 0.862 g $CaCO_3$ per pot, the LVA soil received 1.921 g $CaCO_3$ per pot, and the PV1 soil 3.104 g $CaCO_3$ per pot.

Amounts of 3.3 dm³ of each soil were placed in plastic bags. $CaCO_3$ (p.a.) was dissolved in the volume of water needed to reach 50% of total soil porosity, and applied to 100% of the volume of soil. The mixture was incubated for 14 days with the bags left unsealed. The samples were then air-dried, broken up, and passed through a 4-mm sieve.

Figure 1 - Spatial distribution of the collection sites of representative soils from the semi-arid region located between the valley of the River Piranhas-Açu, in Rio Grande do Norte, and the valley of the River Jaguaribe, in Ceará

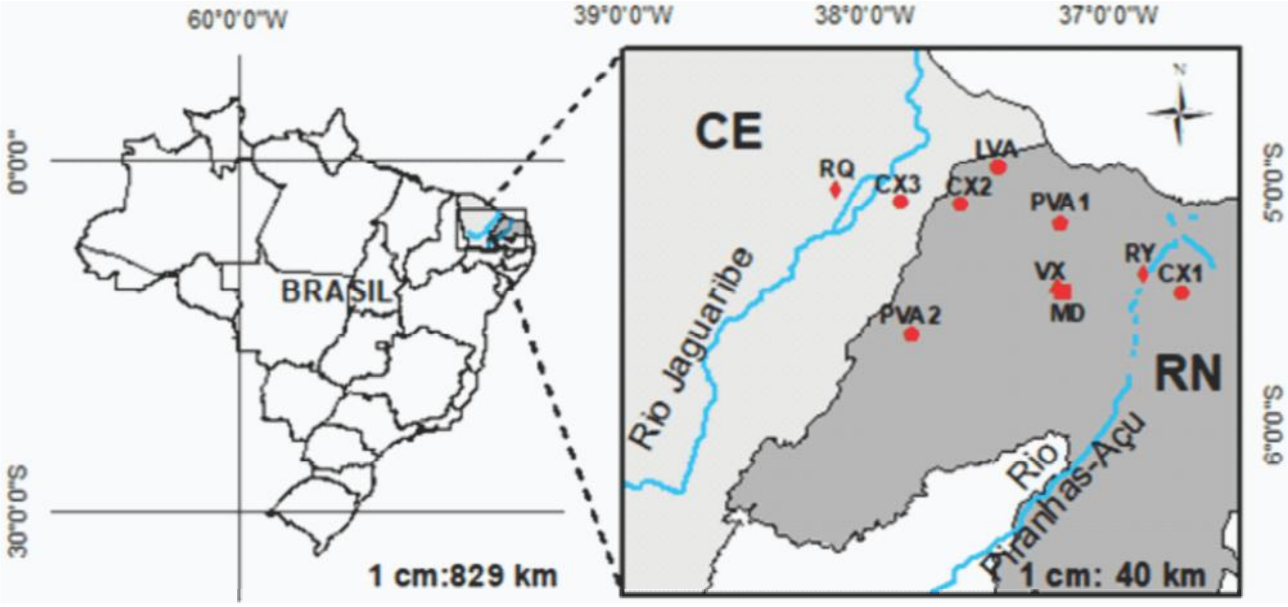


Table 1 - Physical and chemical characteristics of the ten soils used in the experiment(1)

Characteristic	Soil(2)									
	RQ	LVA	PVA1	RY	CX1	CX2	PVA2	MD	CX3	VX
Sand (g kg^{-1})	920	940	850	640	680	660	520	290	380	290
Silt (g kg-1)	40	10	20	22	70	70	90	160	220	320
Clay (g kg^{-1})	40	50	130	140	250	270	390	550	400	390
Bulk density (kg dm^{-3})	1.64	1.59	1.57	1.35	1.39	1.42	1.38	1.20	1.37	1.28
Freundlich K(3)	0.032	0.052	0.061	0.040	0.154	0.144	0.153	0.293	0.406	0.226
MPSC(4) (mg g^{-1})	0.050	0.087	0.101	0.077	0.383	0.436	0.454	0.880	0.884	0.774
P-rem(5) (mg L^{-1})	57.9	58.8	52.1	49.1	22.7	33.0	25.9	12.1	11.6	18.1
Fe_2O_3(6) (g kg^{-1})	1.86	2.14	12.69	45.23	23.01	47.07	44.12	72.86	121.39	50.03
Crystalline Fe(7) (g kg^{-1})	0.07	0.49	7.17	1.81	8.79	17.57	23.42	20.76	40.46	7.21
Amorphous Fe(8) (g kg^{-1})	0.12	0.32	0.80	2.03	1.25	0.47	1.22	2.73	1.81	1.68
Equiv. $CaCO_3$ (g kg^{-1})	1.11	1.01	1.59	8.22	6.76	4.23	15.34	27.53	9.49	254.56
pH	5.6	4.6	4.1	6.5	6.7	6.6	7.5	7.6	6.7	8.0
P-Ca (mg dm-3)(9)	2.82	2.45	2.69	179.25	2.87	4.88	7.79	15.69	16.46	-
Org. Mat. (g kg^{-1})	5.1	2.4	7.5	6.2	9.1	9.4	5.5	14.4	11.0	7.0
P-Mehlich-1 (mg dm^{-3})	5.0	3.3	4.2	60.7	2.0	4.5	3.7	3.1	4.8	1.6
K^+ (mg dm^{-3})	53.3	16.7	35.7	137.3	184.3	144.8	233.9	185.8	149.5	64.0
Na^+ (mg dm^{-3})	8.5	7.6	20.1	133.1	20.9	54.0	40.0	47.1	116.3	76.2

Continuation Table 1

Ca^{2+} ($cmol_C\ dm^{-3}$)	0.89	0.24	0.74	6.94	12.23	6.04	9.33	40.33	11.50	35.73
Mg^{2+} ($cmol_C\ dm^{-3}$)	0.73	0.25	0.29	3.10	1.68	1.17	3.03	4.77	1.29	5.60
Al^{3+} ($cmol_C\ dm^{-3}$)	0.10	0.24	0.61	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(H+Al) ($cmol_C\ dm^{-3}$)	1.71	1.23	4.02	1.19	1.81	1.58	0.13	0.15	1.97	0.00
SB ($cmol_C\ dm^{-3}$)	1.79	0.56	1.20	10.97	14.47	7.81	13.13	45.78	13.68	41.83
t ($cmol_C\ dm^{-3}$)	1.89	0.80	1.81	10.97	14.47	7.81	13.13	45.78	13.68	41.83
CEC ($cmol_C\ dm^{-3}$)	3.50	1.79	5.22	12.16	16.28	9.39	13.26	45.93	15.65	41.83
V (%)	51	31	23	90	89	83	99	100	87	100

(1) Chemical and physical analysis carried out as per Teixeira *et al.* (2017). (2) Chemical and physical analysis carried out as per Teixeira *et al.* (2017). (2) RQ = Quartzarenic Neosol (Russas, CE); LVA = Red-Yellow Latosol (Mossoró, RN); PVA1 = Red-Yellow Argisol (Mossoró, RN); RY = Fluvic Neosol (Carnaubais, RN); CX1 = Haplic Cambisol (Afonso Bezerra, RN); CX2 = Haplic Cambisol (Baraúna, RN); PVA2 = Red-Yellow Argisol (Apodi, RN); MD = Rendzic Chernosol (Mossoró, RN); CX3 = Haplic Cambisol (Quixeré, CE); VX = Haplic Vertisol (Mossoró, RN). (3) 'k'Freundlich isotherm constant. (4) MPSC = Maximum phosphate sorption capacity. (5) P-rem = remaining phosphorus (ALVAREZ V. *et al*., 2000). (6) Iron extracted by ADFE sulphuric attack. (7) Difference between the iron extracted with citrate-bicarbonate-dithionite and the amorphous iron extracted with ammonium oxalate. (8) Amorphous iron extracted with ammonium oxalate. (9) Inorganic phosphorus bound to calcium. Source: Paiva (2019)

Based on the values for P-rem, a measurement that has a high correlation with the PBC, the soils were divided into three groups: low, medium and high PBC. The RQ, LVA and PVA1 soils, with P-rem ranging from 50 to 60 mg L^{-1} and pH from 4.1 to 5.6, made up the group with a low PBC; the RY, CX1, CX2 and PVA2 soils, with P-rem ranging from 20 to 49 mg L^{-1} and pH from 6.5 to 7.5, comprised the group with medium PBC; and the MD, CX3 and VX soils, with P-rem less than 20 mg L^{-1} and pH ranging from 6.7 to 8.0, made up the group of soils with a high PBC.

The doses of P applied to each soil were defined based on the P-rem (ALVAREZ V. *et al*., 2000), developed to obtain P response curves in the greenhouse. Each soil received four doses of P corresponding to levels 0, 1, 2 and 4, with level 4 corresponding to the highest applied dose. As such, the soils in the group with a low PBC received doses of 0.0, 32.5, 65.0 and 130.0 mg dm^{-3} P, the soils with medium PBC received doses of 0.0, 45.0, 90.0 and 180 mg dm^{-3} P, and the soils with a high PBC received doses of 0.0, 57.5, 115.0 and 230.0 mg dm^{-3} P. The source of P, K_2HPO_4 (p.a.), was dissolved in distilled water and applied to 100% of the volume of soil. The samples with P were incubated for 30 days in unsealed bags with the soil moisture maintained at 50% of the porosity of each soil.

Following the incubation period, the samples were again air-dried, broken up using a 4 mm sieve, and transferred to plastic pots in the greenhouse. Before planting the maize, a subsample of 0.3 dm^3 of soil was collected from each bag to determine the available P content by the Mehlich-1, Olsen and ion-exchange resin extractors.

One day before planting, all the soil samples in the pots were fertilised with a nutrient solution. The doses and sources of the applied nutrients were in accordance with Farias *et al.* (2009): 50 mg dm^{-3} N ($(NH_4)_2SO_4$), 50 mg dm^{-3} K (KCl); 4 mg dm^{-3} Mn ($MnCl_2.4H_2O$); 4 mg dm^{-3} Zn ($ZnSO_4.7H_2O$); 1.5 mg dm^{-3} Cu ($CuSO_4.5H_2O$); 0.5 mg dm^{-3} B (H_3BO_3), and 0.15 mg dm^{-3} Mo ($(NH_4)6Mo_7O_{24}.4H_2O$).

Seven maize seeds were sown in each pot. Five days after emergence, the plants were thinned to leave two plants per pot. During cultivation, top dressing fertiliser was applied, corresponding to 5 mg dm^{-3} Fe ($FeCl_3.6H_2O$) dissolved in a diluted solution of EDTA) with additional doses of N and S: 50 mg dm^{-3} N ($CO(NH_2)_2$) during the first week, 22.7 mg dm^{-3} S ($(NH_4)2SO_4$) and 30.2 mg dm^{-3} N ($CO(NH_2)_2$) during the second week, and 50 mg dm^{-3} of N ($(NH_4)2SO_4$) during the third week. The pots were weighed daily, and deionised water was added to maintain the soil moisture at 50% of the total porosity of each soil.

After 31 days of growth, the aerial part of the maize plants was cut to 1 centimetre above the surface of the soil, washed in running water, rinsed in deionised water and then stored in perforated paper bags to facilitate drying, which was initially carried out for one day in the greenhouse; the samples were then placed in a forced air circulation oven at 68 °C to constant weight.

The dry matter weight of the aerial part of the plants was determined from the dry samples. The samples were ground and sieved (< 1.7 mm) in a Wiley mill and stored in plastic jars. Subsequently, 0.4 g from each sample was transferred to a teflon tube and then digested with 65% p.a. HNO_3 in a wet digestion system using a microwave oven (SILVA, 2009). After digestion, the extract was filtered on slow-speed quantitative filter paper, and the P concentration measured by colorimetry (BRAGA; DEFELIPO, 1974). Based on the dry matter weight and the P content of the plant, the amount of P accumulated in the aerial part of both plants was calculated for each pot, as per Souza, Pegoraro and Reis (2017).

After the incubation period of the soil samples with the P doses, a subsample of 0.3 dm^3 of soil was taken from each pot to extract the available P by the Mehlich-1 (TEXEIRA *et al*., 2017), Olsen (SCHOENAU; O'HALLORAN, 2006) and ion-exchange resin (RAIJ *et al*., 2001) methods. The analysis using the resin method was carried out at the Routine Soil laboratory of the Instituto Agronômico de Campinas (IAC). The other analyses were carried out at the Soil Fertility Laboratory of UFERSA. The concentration of available P in the extracts obtained by the Mehlich-1 and Olsen methods was determined as described in Braga and Defelipo (1974).

For the extraction with Mehlich-1, the samples were agitated for 5 minutes at a speed of 120 rpm (SANTOS *et al*., 2015). For neutralisation of the acidity of Mehlich-1, the pH of the soil-extract suspension was measured with a glass electrode immediately after the 5-minute extraction time, and again after the 16-hour decantation period, as specified by Novais *et al*. (2015).

To extract P by the Olsen method ($NaHCO^3$ 0.5 mol L^{-1} at pH 8.5), a soil to solution ratio of 1:20 was used (2.5 cm^3 soil to 50 mL extractor). To eliminate the effects of humic substances in the extracts, 0.4 mL of an activated charcoal suspension, prepared at a ratio of 1:6 (1 g charcoal plus 6 mL distilled water) was added to each Erlenmeyer flask. The samples were then agitated for 30 minutes at 120 rpm in an orbital shaking incubator at 25 °C.

After agitation, the extract was filtered using medium-speed quantitative filter paper and acidified with 0.25 mol L^{-1} H_2SO_4, using a 0.25% (m/v) p-nitrophenol indicator until the colour of the solution was reduced ($pH < 5.0$). The P content of the filtrates was then determined. Before being used in the analysis, the activated carbon was washed in 6 mol L^{-1} HCl, followed by repeated washing with distilled water. A blank sample with and without activated carbon was analysed to show that P was removed from the carbon.

The results were submitted to analysis of variance and regression analysis. To obtain the P recovery rate of the extractors, linear regression equations were fitted to the available P content determined by the three extractors as a function of the applied doses of P. A correlation analysis (Pearson) at a significance level of 1% and 5% was carried out between the P recovery rates and the soil characteristics related to PBC.

To assess the predictive capacity of the extractors regarding soil P availability to the plants, simple linear regression equations were fitted to the P accumulated in the aerial part of the plants as a function of the available P content determined by the three extractors (ALVAREZ V., 1996). The method that presented R^2 values > 0.70 was considered suitable.

RESULTS AND DISCUSSION

The amount of extracted P varied between the extraction methods. For all the soils and levels of P, the mean values for available P obtained by Mehlich-1 ranged from 9.35 to 60.14 mg dm^{-3}, with 5.87 to 81.59 mg dm^{-3} for the ion-exchange resin, and 4.26 to 68.52 mg dm^{-3} for Olsen (Table 2). The greatest difference between the lowest and highest values was found for Mehlich-1 in the VX and RY soils respectively. In the VX soil, Mehlich-1 extracted 96% and 92% less than did the ion-exchange resin and Olsen. In contrast, this extractor recovered 71% and 82% more P from the RY soil, respectively, than did the ion-exchange resin and Olsen extractors.

For the zero dose, without the addition of fertiliser, the Olsen method had the lowest coefficient of variation (CV = 80%) and Mehlich-1 the highest (CV = 251%) (Table 2). Considering the mean values of the four doses of P, the mixed resin had the lowest (CV = 26%) and Mehlich-1 the largest variation between the doses (CV = 107%). Such variations between methods can be attributed to the different soils and extraction mechanisms, and the extraction capacity, influenced by the properties of the soil (SHAHRIARIPOUR *et al*., 2018).

The ion-exchange resin extractor showed the greatest extraction capacity, with an average of 0.4442 mg dm^{-3} P extracted for each mg dm^{-3} P applied, followed by Olsen and Mehlich-1 (Table 3), demonstrating that the transfer of orthophosphate ions to the ion-exchange resin was more effective than the chemical mechanisms in releasing P. The variation in P recovery rates was low for ion-exchange resin (CV = 25%), and high for Olsen (CV = 72 %) and Mehlich-1 (CV = 88%) (Table 3). As such, the high CV of the chemical methods of extraction means that their extraction capacity is influenced by the properties of the soil, altering the effectiveness of the extractors.

The pH of the soils influenced the amounts of P extracted by the methods, especially by the Mehlich-1 extractor. This effect was also observed by Rosan, Silva and Maluf (2018), in which soil pH was the characteristic that most influenced the P extracted by Mehlich-1 in a clayey sandy soil treated with rates of humic acid. Manghabati *et al.* (2018), comparing P extractors in acidic and alkaline soils, showed that soil pH strongly influenced the results obtained with each method, and that this characteristic should be considered when extracting P. These studies show pH to be one of the characteristics for explaining P availability in the soil.

Table 2 - Available P content by the Mehlich-1, ion-exchange resin and Olsen extractors as a function of the P doses applied to each soil

(1)Soil	Level of P(2)				Mean
	0	1	2	4	
	------------------------------ P-Mehlich-1 (mg dm^{-3}) ------------------------------				
RQ	5.52	31.57	60.30	111.35	52.19
LVA	2.23	26.07	53.22	108.41	47.48
PVA1	2.94	16.09	26.53	57.60	25.79
RY	76.13	102.25	114.66	163.17	114.05
CX1	0.80	4.32	13.14	42.54	15.20
CX2	1.91	9.36	19.27	55.68	21.56
PVA2	1.34	4.23	9.35	28.05	10.74
MD	1.06	3.57	7.29	15.56	6.87
CX3	1.41	4.74	6.17	16.23	7.14
VX	0.19	0.40	1.27	2.80	1.17
Mean	9.35	20.26	31.12	60.14	30.22
CV (%)	251	151	114	86	107
	------------------------------ P-Resin (mg dm^{-3}) ------------------------------				
RQ	2.78	19.41	45.51	80.75	37.11
LVA	3.70	20.60	47.92	81.57	38.45
PVA1	3.41	10.43	19.42	44.34	19.40
RY	21.77	46.51	68.02	104.44	60.19
CX1	3.31	13.84	42.35	94.09	38.40
CX2	4.62	11.01	35.25	77.91	32.20
PVA2	3.49	16.39	38.75	83.94	35.64
MD	4.56	23.35	47.27	92.77	41.99
CX3	6.51	15.54	37.80	80.56	35.10
VX	4.51	15.42	40.04	75.53	33.88
Mean	5.87	19.25	42.23	81.59	37.23
CV (%)	97	54	29	19	26
	------------------------------ P-Olsen (mg dm^{-3}) ------------------------------				
RQ	3.98	27.09	57.70	106.64	48.85
LVA	2.31	28.45	62.98	139.34	58.27
PVA1	4.75	12.45	22.46	44.66	21.08
RY	13.62	27.86	42.82	78.49	40.70
CX1	2.83	10.57	16.94	49.51	19.96
CX2	3.82	12.15	18.32	47.82	20.53
PVA2	2.16	8.55	18.46	51.91	20.27
MD	2.71	14.49	29.89	72.88	29.99
CX3	4.21	8.95	16.57	39.61	17.34
VX	2.24	11.15	28.62	54.32	24.08
Mean	4.26	16.17	31.48	68.52	30.11
CV (%)	80	51	55	47	45

(1) According to the Brazilian System of Soil Classification (EMBRAPA, 2006): RQ = Quartzarenic Neosol (Russas, CE); LVA = Red-Yellow Latosol (Mossoró, RN); PVA1 = Red-Yellow Argisol (Mossoró, RN); RY = Fluvic Neosol (Carnaubais, RN); CX1 = Haplic Cambisol (Afonso Bezerra, RN); CX2 = Haplic Cambisol (Baraúna, RN); PVA2 = Red-Yellow Argisol (Apodi, RN); MD = Rendzic Chernosol (Mossoró, RN); CX3 = Haplic Cambisol (Quixeré, CE); VX = Haplic Vertisol (Mossoró, RN). (2) Level 4.0 corresponded to the maximum dose of P applied. For the RQ, LVA and PVA1 soils, doses of 0.0, 32.5, 65.0 and 130.0 mg dm^{-3} P were applied. For the RY, CX1, CX2 and PVA2 soils, doses of 0.0, 45.0, 90.0 and 180.0 mg dm^{-3} P were applied. For the MD, CX3 and VX soils, the applied doses were 0.0, 57.5, 115.0 and 230.0 mg dm^{-3} P

Table 3 - Mean extraction pH for Mehlich-1 and linear regression coefficients of the equations for the P recovered by the Mehlich-1, ion-exchange resin and Olsen extractors, and P accumulated in the plants, as a dependent variable of the P doses applied

Soil[1]	Mean extraction pH Mehlich-1[2]		P recovery rate of the extractors			P recovery rate of the plants
	Start	End	Mehlich-1	Resin	Olsen	
			------------ (mg dm^{-3}) (mg dm^{-3})$^{-1}$ -------------			(mg pot^{-1}) (mg dm^{-3})$^{-1}$
RQ	1.21	1.22	0.8168	0.6107	0.7985	0.5721
LVA	1.24	1.24	0.8220	0.6104	1.0685	0.6308
PVA1	1.15	1.24	0.4185	0.3194	0.3110	0.2882
RY	1.32	1.34	0.4721	0.4547	0.3621	0.3115
CX1	1.32	1.39	0.2396	0.5235	0.2610	0.1796
CX2	1.25	1.33	0.3041	0.4261	0.2448	0.1412
PVA2	1.42	1.51	0.1522	0.4575	0.2825	0.0922
MD	1.55	2.08	0.0642	0.3877	0.3097	0.1304
CX3	1.34	1.46	0.0637	0.3332	0.1574	0.0771
VX	5.72	6.51	0.0119	0.3190	0.2327	0.1123
Mean	1.75	1.93	0.3365	0.4442	0.4028	0.2535
CV(%)	80	84	88	25	72	79

(1) According to the Brazilian System of Soil Classification (EMBRAPA, 2006): RQ = Quartzarenic Neosol; LVA = Red-Yellow Latosol; PVA1 = Red-Yellow Argisol; RY = Fluvic Neosol; CX1 = Haplic Cambisol; CX2 = Haplic Cambisol; PVA2 = Red-Yellow Argisol; MD = Rendzic Chernosol; CX3 = Haplic Cambisol; VX = Haplic Vertisol. The extraction pH was measured immediately after the addition of the Mehlich-1 extractor to the soil sample, and 16 hours after decanting the soil:extractant mixture before taking the P reading. Average of three replications

Acid neutralisation in Mehlich-1 extracts increased with the rise in pH occurring in soils with a pH > 6.0. In these soils, the 1.2 pH of the Mehlich-1 solution rose towards the pH of the soil after reacting with the soil during the 5-minute extraction process, ranging from 1.33 to 6.51 (Table 3), and was more expressive in alkaline calcareous soils (pH: 7.6 to 8.0; $CaCO_3$ equivalent: 27.53 to 254.56 g kg^{-1}), where the pH of the solution rose by 5 pH units, resulting in poor P extraction in the VX soil. Similarly, Penn *et al*. (2018), studying the impact of soil pH on Mehlich-3 extraction, showed that with Mehlich-3, extraction is influenced by changes in the form of P.

Similar behaviour was seen by Novais *et al.* (2015) in 20 acidic soils with a marked variation in PBC, where there was no significant neutralisation of the acidity of Mehlich-1. Dari *et al*. (2019), studying the influence of pH and $CaCO_3$ content on the extraction capacity of P extractors in alkaline calcareous soils in the state of Idaho in the USA, showed that pH alone could not explain Bray-1, an acidic extractor like Mehlich-1, underestimating the available P, as a high soil pH does not necessarily mean that the soil will have a high $CaCO_3$ content. When relating pH to $CaCO_3$ content, they found that Bray-1 was the only extractor with values for available P equal to zero in soils with a pH > 7.5 and $CaCO_3$ content > 6.2 g kg^{-1}.

Thus, the levels of $CaCO_3$ equivalent in the soil should be considered when explaining low P extraction by acid extractors in calcareous soils. To this effect, Rodriguez (2017) proposed a simple gravimetric method to separate and quantify carbonates in calcareous soils. According to that author, calcite and calcite-domilite carbonates are highly reactive and can neutralise the acidity of the extractant; this does not happen with dolomite carbonates, which show low reactivity to the acid.

The available P increased linearly as a function of the applied P dose (Figures 2, 3 and 4). In the group of soils with a low PBC (RQ, LVA and PVA1), which includes the three acidic soils and P-rem values greater than 50 mg L^{-1}, the three extractors showed almost the same extraction capacity (Figure 2), although the ion-exchange resin extracted slightly less.

This is possibly related to the more soluble forms of P that in low pH soils are predominantly Fe and Al phosphate minerals (PENN *et al*., 2018; SOUZA JÚNIOR *et al*., 2012), as well as to the lower P sorption of sandy soils (SANTOS *et al*., 2016). In these soils, there was no neutralisation of the acidity of Mehlich-1, as the pH of the soil extract remained practically unchanged during the extraction process (Table 3), confirming the suitability of Mehlich-1 for acidic and low CEC soils.

The amount of P recovered by the extractors in neutral soils is less than in acidic soils (Table 3). This may be related to the P shifting to less soluble forms. At pH 6.0 – 6.5, the forms of P change from Fe and Al phosphate minerals to P

Figure 2 – Available soil P content recovered by the Mehlich-1, ion-exchange resin and Olsen extractors, as a function of the P doses applied to the RQ, LVA and PVA1 soils

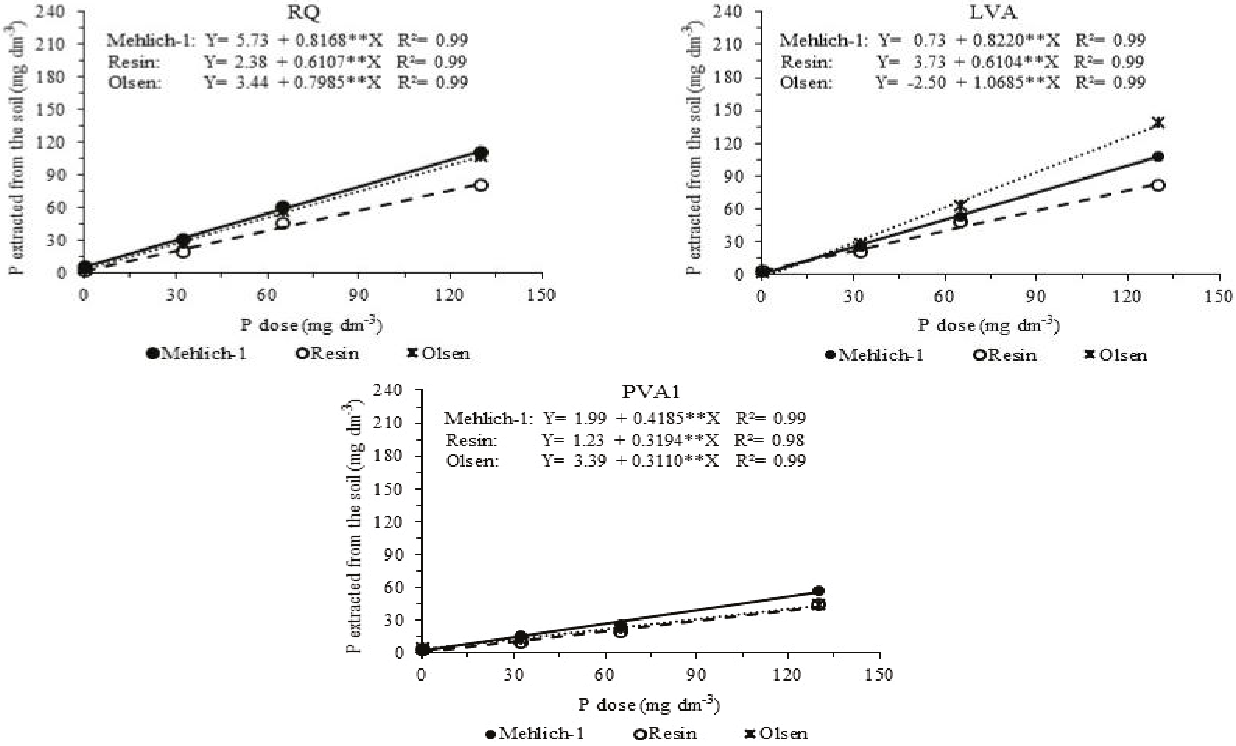


Figure 3 - Available soil P content recovered by the Mehlich-1, ion-exchange resin and Olsen extractors, as a function of the P doses applied to the RY, CX1, CX2 and PVA2 soils

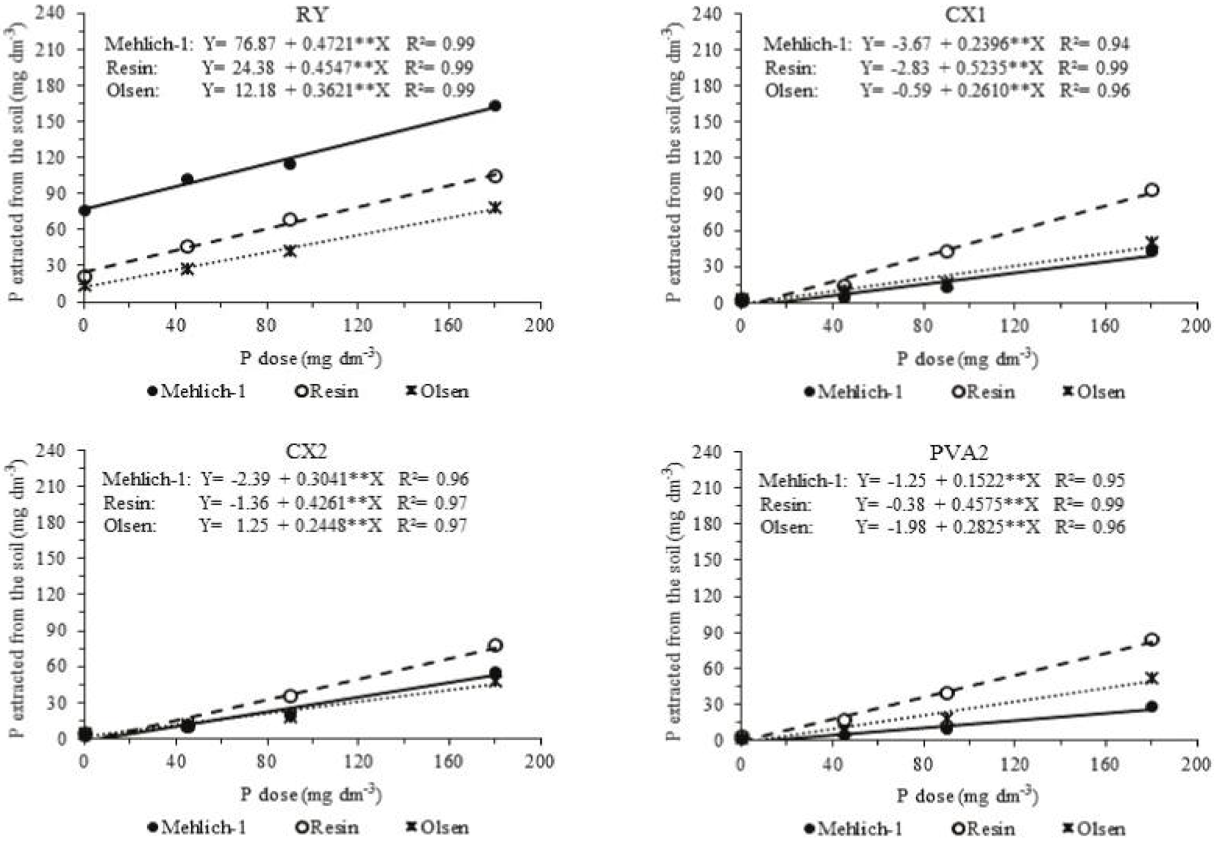
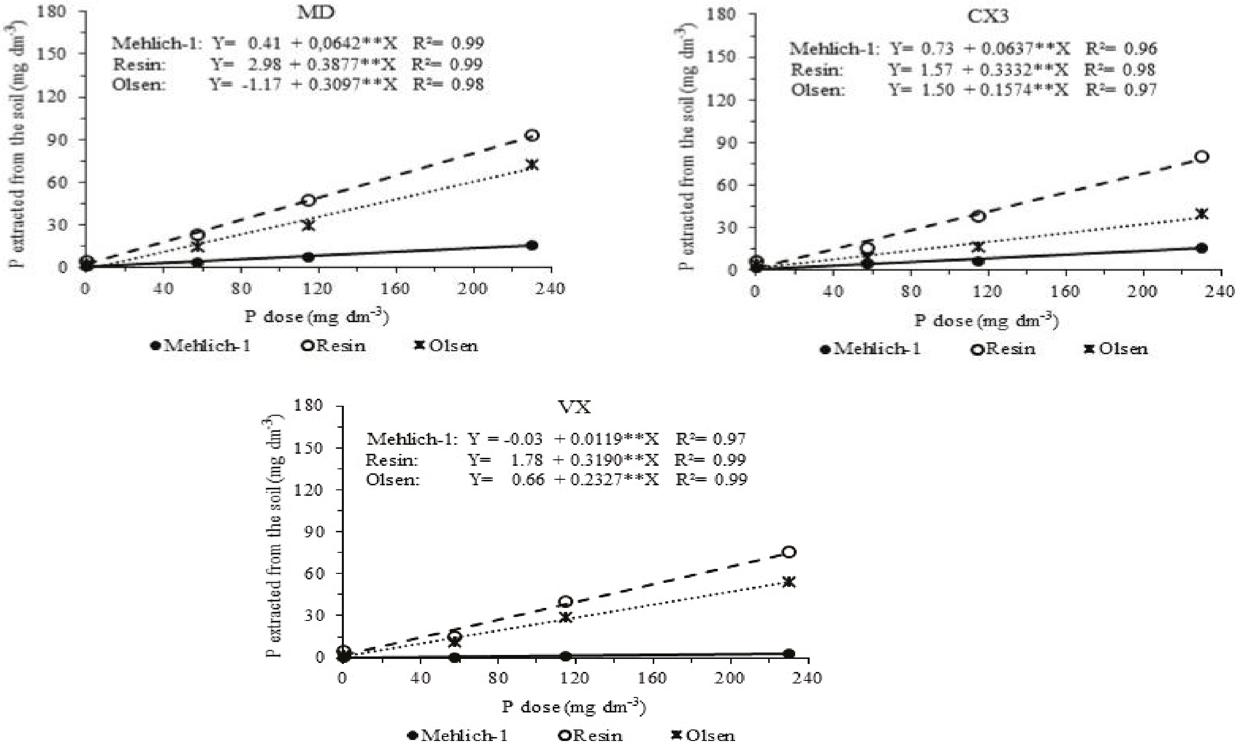


Figure 4 - Available soil P content recovered by the Mehlich-1, ion-exchange resin and Olsen extractors, as a function of the P doses applied to the MD, CX3 and VX soils



adsorbed on the surface of Fe and Al oxides and hydroxides plus the formation of P-Ca (PENN *et al*., 2018; SOUZA; JÚNIOR *et al*., 2012).

In the group of soils of medium PBC (RY, CX1, CX2 and PVA2), formed by the soils with a neutral pH and P-rem values greater than 25 mg L^{-1} and less than 50 mg L^{-1}, the ion-exchange resin extracted the most P (Figure 2), except for the RY soil. In this soil, Mehlich-1 extracted the most P, particularly at zero dose (without the addition of fertiliser).

In the RY soil, the P-Ca fraction was naturally high compared to the other soils (Table 1). As natural P-Ca is highly soluble in acidic solutions, more P was extracted by Mehlich-1 than by the other extractors (VALADARES *et al*., 2017). On the other hand, the basic Olsen solution at pH 8.5, and the extraction process with the ion-exchange resin at a close to neutral pH, meant that the undesirable preferential extraction of P-Ca did not take place (HARTMANN *et al*., 2019; RAIJ; *et al*., 2001). The greater amount of P extracted by the Mehlich-1 method than by the Olsen method was also seen in other studies of soils with a high pH and Ca content in the semi-arid region of the Northeast (CALHEIROS *et al*., 2012; ROCHA *et al*., 2005).

The acidity of the Mehlich-1 extractor was partially neutralised in these soils, as the initial pH of 1.2 of the acidic solution rose to a range of 1.33 to 1.51 (Table 3). This can be attributed to the presence of $CaCO_3$ (0.4% to 0.9%) and to the clay content (14% and 40%) (Table 1) that reacted with the extractor, reducing its extraction capacity. Under such conditions, a measurement related to the PBC, such as P-rem, can stratify the slight neutralisation of Mehlich-1 (AVAREZ V. *et al*., 2000; NOVAIS *et al*., 2015).

In the alkaline and high-PBC soils (MD, CX3 and VX), the ion-exchange resin again extracted the most P from the soil, with Mehlich-1 extracting the least (Figure 4). With the rise in pH (> 7.0), P is predominantly in the form of low-solubility P-Ca (PENN *et al*., 2018; SOUZA JÚNIOR *et al*., 2012), but the P derived from the recent input of fertiliser may be adsorbed on the surfaces of the Fe oxides, calcite and clay minerals, forming more-soluble or more-available compounds (ANDERSSON *et al*., 2019).

Mehlich-1 did not overestimate the available P in these soils rich in P-Ca, but its extraction capacity was significantly reduced with the rise in pH. In the VX soil with a pH equal to 8.0 (alkaline calcareous), the extractor barely responded to the addition of the phosphate fertiliser, with the available-P data close to the X axis (Figure 4). The pH of the Mehlich-1 solution remained above the recommended 1.2 during P extraction in these soils, ranging from 1.46 to 6.51 (Table 3). This is due to the greater amount of $CaCO_3$ in these soils (Table 1), which neutralised most of the acidity of Mehlich-1, precluding the use of this extractor in these soils.

A similar result was found by Silveira, Araújo and Sampaio (2006), where Mehlich-1 extracted only 1% of the total P from two Vertisols in the semi-arid region of the northeast of Brazil, although they found high values for total P in these soils in the states of Paraíba and Pernambuco. Santos *et al*. (2016) expected an overestimation by Mehlich-1 in a Vertisol rich in P-Ca in Paraíba, however, these authors also found a low amount of extracted P.

The P recovery rates of the extractors were correlated with the characteristics of the soil related to PBC (Table 4). Considering the ten soils under study, the values for P-rem were positively correlated with the P recovery rates of the Mehlich-1 (r = 0.937) and Olsen (r = 0.743) extractors; however, they did not correlate with the P recovery rates of the ion-exchange resin (r = 0.577). This shows that Mehlich-1 was the most sensitive extractor to the PBC, and the ion-exchange resin the least sensitive.

Correlations between the available P content for each extractor and the P content in the aerial part of the maize plants

Initially, a linear regression was carried out between the P content in the plant and the available P determined in each soil by the three extractors. In this case, the coefficients of determination (R^2) for the Mehlich-1 and Olsen extractors were greater than 70%, while for the ion-exchange resin it was only 46% (Figure 5); however, the heterogeneity of the soils and the results showed the need to compare methods for groups of soils of similar properties, such as the PBC, to obtain clearer results (Figure 6, 7 and 8).

In acidic soils and soils with a low PBC, the three extractors had R^2 values ranging from 86% to 89% (Figure 6), with slight superiority of the ion-exchange resin. This similarity in the coefficients of determination between the methods shows an almost identical ability to predict P availability in these soils. The forms related to Al and Fe were the main sources of P available to the maize plants (Table 3) (CABEZA *et al*., 2017). Other

Figure 5 - Regression equations for P content in the aerial part of the plant as a dependent variable of the available P content in the soil, extracted by the Mehlich-1, ion-exchange resin and Olsen extractors, for the ten soils under study

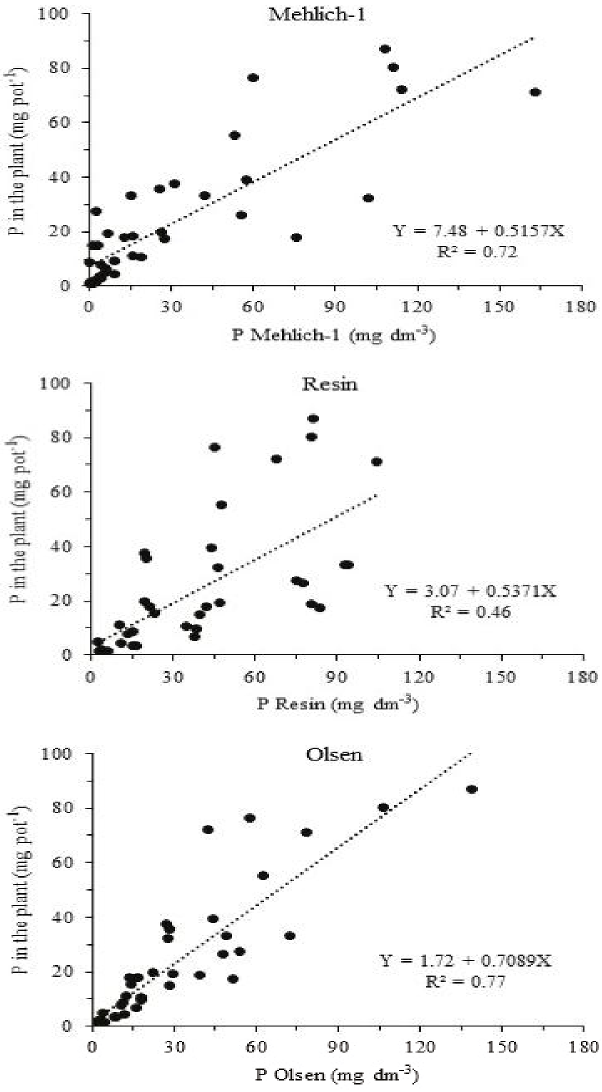


Table 4 - Simple linear correlation coefficients between P recovery rates for the Mehlich-1, ion-exchange resin, and Olsen extractors, and recovered by the plant, plus soil characteristics that reflect the P buffering capacity

Characteristic	P recovery rate			
	Mehlich-1	Resin	Olsen	Plant
MPSC⁽¹⁾	-0.871	-0.615	-0.613	-0.772
a⁽¹⁾	0.792	0.537	0.759	0.827
k⁽²⁾	-0.798	-0.590	-0.585	-0.717
P-rem⁽³⁾	0.937	0.577	0.743	0.878
Clay	-0.918	-0.616	-0.691	-0.852
P recovery rate by the plant	0.967	0.760	0.949	-

(1) Parameters of the Langmuir isotherm, where MPSC is the maximum phosphate sorption capacity and 'a' is the constant related to the adsorption energy. (2) Freundlich isotherm constant. (3) Remaining phosphorus (ALVAREZ V. *et al*., 2000)

studies (AMORIM *et al*., 2008; FARIAS *et al*., 2009) carried out in sandy acidic soils with low a PBC in the semi-arid region showed no differences between the extractors under test.

In the group of soils with a neutral pH (6.5 to 7.5) and medium PBC, the correlation between the extractors and the plant decreased relative to the group of soils with a low PBC, especially for the ion-exchange resin and Olsen extractors. In this case, the R^2 values were below 70% for the ion-exchange resin ($R^2 = 60\%$) and Olsen ($R^2 = 67\%$), with an R^2 of 84% for Mehlich-1 (Figure 7). However, in these soils with a pH > 6.0, the Mehlich-1 extractor was expected to have a poorer performance, as it was the most sensitive to the PBC, with a better performance for the

Figure 6 - Regression equations for P content in the aerial part of the plant as a dependent variable of the available P content in the soil, extracted by the Mehlich-1, ion-exchange resin and Olsen extractors, for the RQ, LVA and PVA1 soils

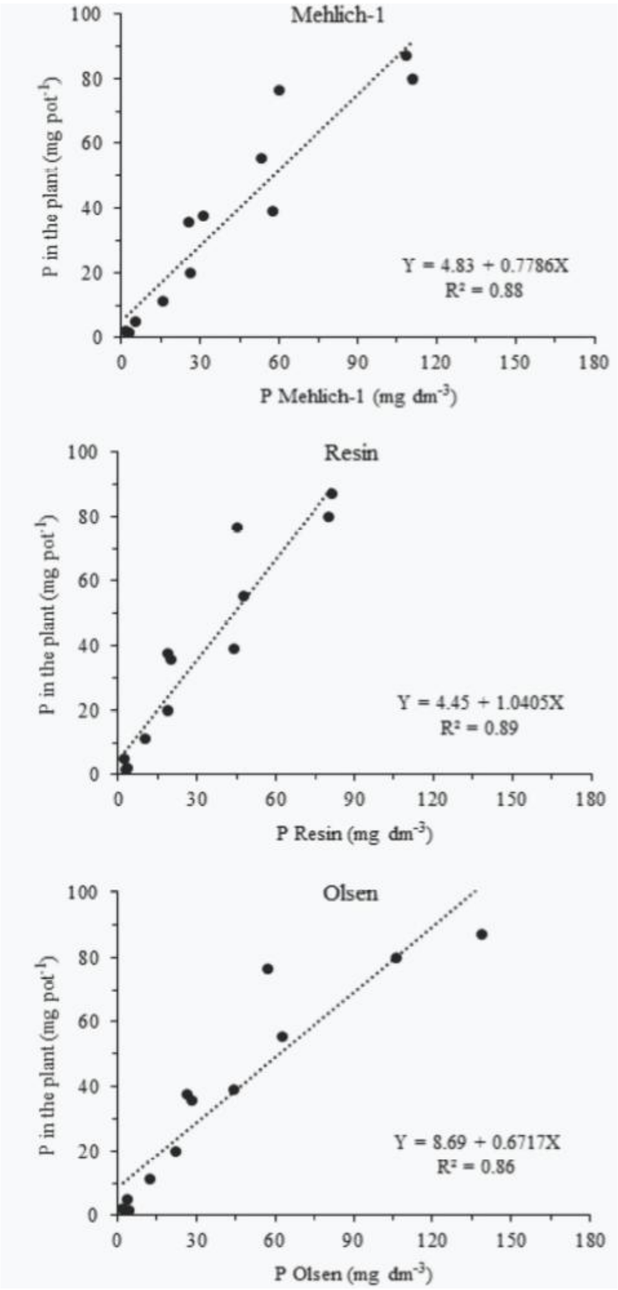


Figure 7 - Regression equations for P content in the aerial part of the plant as a dependent variable of the available P content in the soil, extracted by the Mehlich-1, ion-exchange resin and Olsen extractors, for the RY, CX1, CX2 and PVA2 soils. (In the three graphs, R^2 values marked with an asterisk (*) are for the same regression model, fitted without considering the data of the RY soil)

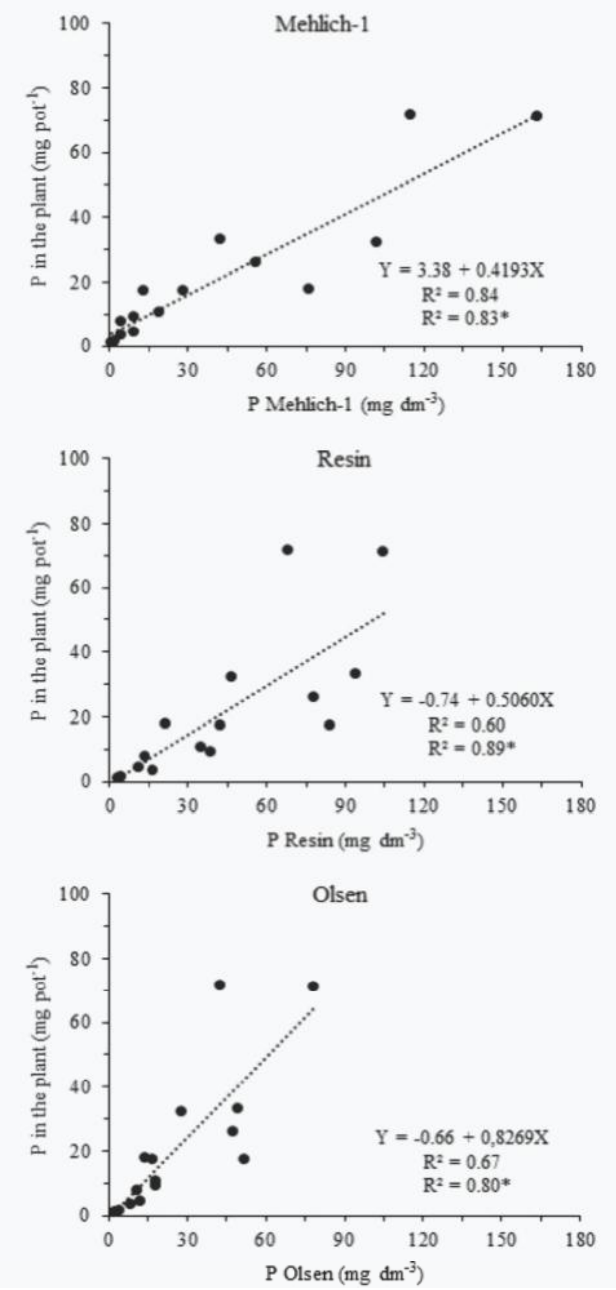
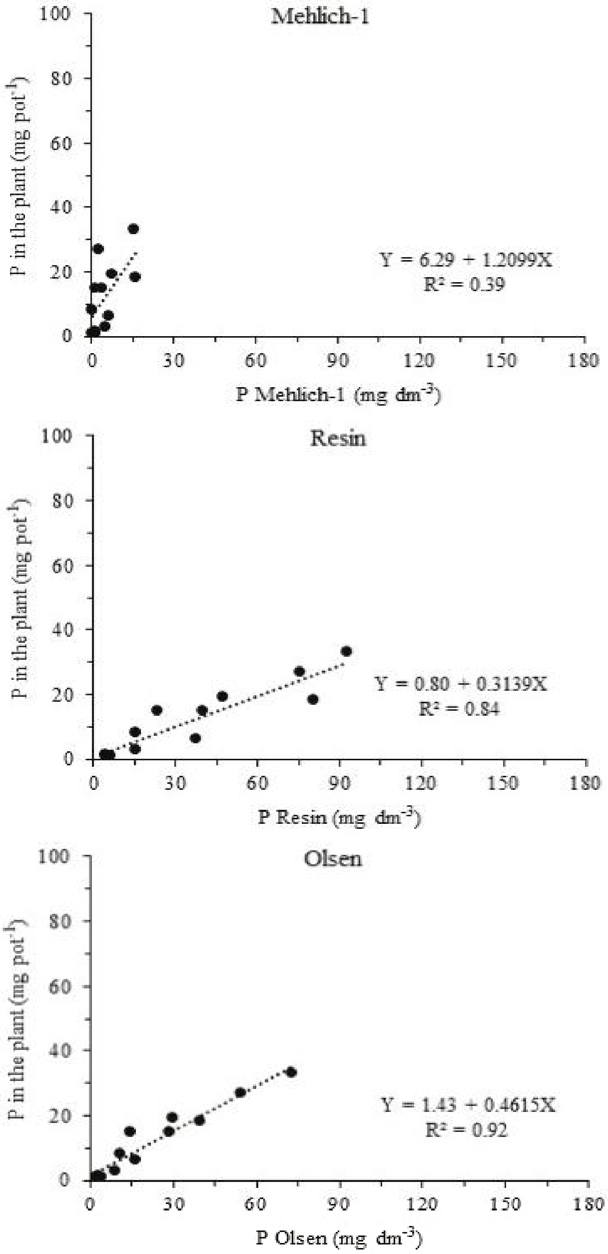


Figure 8 - Regression equations for P content in the aerial part of the plant as a dependent variable of the available P content in the soil, extracted by the Mehlich-1, ion-exchange resin and Olsen extractors, for the MD, CX3 and VX soils



ion-exchange resin and Olsen, which were less sensitive. As such, the points were more dispersed around the RY soil, showing that the low R^2 values for the ion-exchange resin and Olsen extractors were caused by this soil.

When the same regression model was fitted without considering the data of the RY soil, the R^2 values for the ion-exchange resin and Olsen extractors increased to 89% and 80% respectively, and that for Mehlich-1 to 83%, showing almost no change (Figure 7). The ion-exchange resin, therefore, also had the best performance in these soils. Furthermore, the regression analysis was not sensitive to the Mehlich-1 overestimation, since this acidic extractor removed a lot of P, while the maize plants do not have the same mechanism for acidifying the rhizosphere and, as a result, dissolving P-Ca in the short term (CABEZA *et al*., 2017).

In a study of fractionation in the soils of Paraíba, Souza Júnior *et al*. (2012) showed a low correlation of the P absorbed by maize plants with the form of P bound to Ca, and a high correlation with P bound to Al and Fe. In general, P is more available in soils with a pH of around 6.5 (PENN *et al*., 2018). Another hypothesis is that the greater amount of P accumulated in the plant (Table 3) was due to the maximum amount of P being available at a soil pH of 6.5, resulting in greater correlation with the Mehlich-1 extractor.

Souza, Pegoraro and Reis (2017), comparing the Mehlich-1, ion-exchange resin and Olsen extractors in two calcareous soils in the state of Minas Gerais, also found more P extracted by Mehlich-1 in a Fluvic Neosol with a pH of 6.5, relative to the other extractors. However, by means of multivariate cluster analysis, the P extracted by each method was correlated with the P absorbed by jatropha plants. By using this methodology, overestimation by Mehlich-1 in soils rich in P-Ca was confirmed.

In alkaline soils and soils with a high PBC (MD, CX3 and VX), Mehlich-1 showed a low correlation with the P extracted by the plant (R^2 = 39%), unlike the ion-exchange resin (R^2 = 84%) and Olsen (R^2 = 92%) extractors that showed a high correlation (Figure 8). The amount of P accumulated in the aerial part of maize plants decreases in these soils (Table 3), even when receiving a larger amount of phosphate fertiliser, indicating that most of the applied P was fixed in the soil. The most soluble phosphates and adsorbed species were in forms readily available to plants and not the precipitated forms, since the short-term dissolution of mineral P-Ca depends on strong acidification or long-term biological processes (ANDERSSON *et al*., 2019; CABEZA *et al*., 2017; HARTMANN *et al*., 2019).

With Olsen, the anions, especially HCO_3- in Ca^{2+} precipitation, were more efficient in these soils in extracting P from the solution than was the ion-exchange resin. According to Valadares *et al*. (2017), Ca^{2+}extraction by cation resin favours the solubilisation of P-Ca that is not readily available. What does not happen is that Olsen does not solubilise forms which are of poor solubility, such as tricalcium phosphate (HARTMANN *et al*., 2019). Souza, Pegorato and Reis (2017) showed that pure anionic resin is superior to Olsen in calcareous soils.

The low correlation of the P extracted by Mehlich-1 with the P absorbed by the plant confirms the available P being underestimated due to neutralisation of the acidity of the extractant, especially in the alkaline calcareous Vertisol. According to Guo, Yost and Jones (1996), carbonates do not react with the Olsen solution, but neutralise 50% of the acidity of the Mehlich-1 extractor. A better performance by Olsen compared to Mehlich-1 was also seen by Raiesi and Hosseinpur (2017), when comparing chemical extractors in 10 calcareous soils in Iran.

A similar work to the present study was developed by Farias *et al.* (2009) in 12 representative soils in the semi-arid region of the state of Paraíba, however, contradicting the present study, Mehlich-1 was superior to the ion-exchange resin, even in alkaline soils. Unlike in this study, the authors did not evaluate the neutralisation of Mehich-1 acidity, nor did they quantify the levels of $CaCO_3$ equivalent in the soils under study. The results of the present study therefore provide a greater understanding of how the pH and $CaCO_3$ content affect Mehlich-1.

Few studies were found on the performance of the Mehlich-1 and ion-exchange resin extractors in alkaline and calcareous soils, such as occur in the semi-arid region of the Northeast; this may show that Mehlich-1 is underused under these conditions. On the other hand, good performance by the Olsen extractor has also been reported in a wide range of soils and various locations around the world (MANGHABATI *et al.*, 2018; NAWARA *et al.*, 2017; SINGH *et al.*, 2020).

CONCLUSIONS

1. The ion-exchange resin and Olsen extractors showed the best correlations with the P accumulated in the plants, irrespective of the pH or PBC of the soil;
2. Neutralisation of the acidity of the Mehlich-1 extractor was significant in alkaline soils with high levels of $CaCO_3$ equivalent;
3. Mehlich-1 is suitable for strongly acidic soils and soils with a low PBC, however the use of this extractor in soils with a high pH and Ca^{2+} content may overestimate P availability in soils rich in P-Ca, or underestimate it in soils rich in $CaCO_3$;
4. Mehlich-1 is recommended for use in strongly acidic soils ($pH < 6.0$), soils with a low PBC (P-rem > 50 mg L^{-1}) and soils with a low carbonate content ($CaCO_3$ equivalent < 2.00 g kg^{-1}). It is recommended that Olsen be used in neutral or alkaline soils ($pH > 6.0$), soils with a medium to high PBC (P-rem < 50 mg L^{-1}) and soils with a $CaCO_3$ equivalent > 2.00 g kg^{-1}.

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