Shiraz
University

Iran Agricultural Research (2015) **34**(**1**) 92-104

Development of an alternative to the Olsen test for determining corn plant-available phosphorus in calcareous soils

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ARTICLE INFO

***Article history*:**
Received **30December2013**
Accepted **25 January 2015**
Available online **12 July 2015**

***Keywords*:**
Calcareous Soils
Corn
Olsen test
Phosphorus

ABSTRACT-In this research, several chemical extractants and modified Olsen methods were studied in 25 surface-calcareous soils (0-30 cm) for determining available phosphorus (P) for corn (*Zea mays* L.) plant. In this experiment, the highest correlation was obtained between extracted P by Olsen and Colwell methods ($NaHCO_3$-extractable P) and dry weight of corn shoot ($r = 0.67$ and 0.75, $p<0.01$, respectively). Therefore, effects of varying effective conditions of extraction such as the period of shaking, the concentration and pH of $NaHCO_3$ solution on the amount of P extracted from the soil and its correlation with growth parameters of the corn plant was investigated. The results showed that increasing the pH of $NaHCO_3$ solutions: soil ratio, shaking period and concentration of $NaHCO_3$ solution significantly increased the extracted P from the soils, but could not significantly increase the correlation between P extracted from the soil and growth parameters of the corn plant. The highest correlation was obtained between modified Olsen method (with 4-hour shaking period and the solution: soil ratio of 6) and shoot dry weight ($r=0.75$, $p<0.01$) which was not significantly different ($\Delta r=0.08$) using Olsen's test (0.5hour shaking time and solution: soil ratio of 20). The results of this study showed that P buffering capacity (PBC) could be the best method for predicting the available P by the corn plant.

INTRODUCTION

Corn is the highest-yielding cereal and regarding the total value of the production, it is considered the third world's cereal product after wheat and rice. Precise extracting and measuring the amount of phosphorus (P) which the plants will uptake during growth period is impossible before its cultivation, though it is possible to extract the P from its different chemical fractions from the soil by different extractants which have statistically good correlation with the uptake P by plants (Kamparth and Watson, 1980). Paauw (1971) observed high correlation coefficient between P extracted by Paauw method (deionized water with H_2O: soil ratio of 60 and 1-hour shaking time) and P content of wheat shoot in several soils of US, Australia and Britain in 4 to 7.7 pH range. Matar and Samman (1975) reported that the correlation coefficient between P extracted from the calcareous soils of Syria by Olsen's test and wheat yield was lower than other methods. They attributed it to quite different properties and constituents of studied soils. (Moody et al., 1990) from south east of Australia, in soils with 5.4 to 7.3 pH range and in a field experiment, reported that the P extracted by $CaCl_2$ 0.01 M had the closest positive correlation with soybean yield ($r=0.96$, $p<0.01$), though correlation coefficient was 0.59 for Olsen's method. They stated this finding could be attributed to soil properties variability and more effect of solution P on the availability of P. Soon (1990), in soils of the north west of Canada in 5.2-7.4 pH range, reported that Kelowna method was better than the Olsen's method for soil available P testing due to extracting several nutrients plus P simultaneously. Humphreys et al. (2001) from Ireland reported that rye dry matter had a significant correlation with P extracted by Morgan and Olsen's tests (r was 0.89, 0.81, $p<0.01$, respectively). Kulhanek et al. (2007), in the soils from Czech Republic with 5.8-7.1 pH range, reported that P extracted by distilled water had the close correlation with the P uptake by the barley plant ($r=0.81, p<0.01$) and Olsen-P had a non-significant linear correlation ($r=0.25^{ns}$). This was due to the inability of $NaHCO_3$ solution in extracting soil organic P (Franzan et al., 1999). Delgado et al. (2010) reported that Olsen-P had significant correlations with plant growth parameters in soils which have almost uniform physical and chemical characteristics. Molinna et al. (2012) compared the Soltanpur's method with Olsen's test in Chili in 4.8-8.3 pH range soils and recommended the Soltanpur's method for all studied soils except Andosols which had very low available P. Moody (2011), in Australian soils in 4.6-8.7 pH range, reported a significant correlation between Olsen - P, Colwell-P and P extracted by filter paper impregnated to iron oxide method and concluded that these three methods could be used for Australian soils. Reviewing the previous studies, Menon et al. (1997) reported that in calcareous soils, available P extracted by filter papers predicted plant response better than Olsen's test.

Since 1954, P extraction by Olsen's test has been one of the most used tests for non-acidic soils (Delgado and Scalenghe, 2008). Olsen's method (1954) is mainly based on phosphate solubility in calcareous soils though this method has been used in acid and neutral soils. In this method, it is believed that phosphate is replaced by bicarbonate, carbonate and hydroxide ions (Barrow and Shaw, 1976). High pH of this method decreases the activity of soil solution Ca^{2+} by precipitating as calcium carbonate, resulting in dissolution of Ca-P and also Fe and Al-P compounds in calcareous and acid soils, respectively (Demetz and Insam, 1999). As an extractant, $NaHCO_3$ acts through pH and ion effect to remove soil solution inorganic P, plus some organic P and some labile solid-phase P compounds such as phosphate adsorbed to free lime, slightly soluble calcium phosphate precipitates, and phosphate loosely adsorbed to Fe and Al oxides and clay minerals.$NaHCO_3$ also removes labile organic P forms that may be readily hydrolyzed to inorganic forms and contribute to plant-available P or be re-assimilated by microorganisms (Demetz and Insam, 1999).

In many countries, a 0.5 M solution of $NaHCO_3$ at pH 8.5 is widely used as a soil test for P. Most of workers have used this pH and concentration but the shaking time has varied from 30 minutes (Olsen et al., 1954) to 2 hours (Piper and DeVries, 1964) up to 16 hours (Colwell, 1963).

Stone (1971) reported that drying soil samples increased the amount of P extracted by Olsen's method in 30-minute shaking time, though such effect was delaminated by increasing shaking period up to 16 hours. (Maclean, 1965) reported that the extractable P by Olsen's method increased by increasing the pH and temperature of $NaHCO_3$ solution, shaking period and soil: solution ratio. (Stone, 1971) reported that increasing the concentration of $NaHCO_3$ solution from 0.1M to 1M decreased the secondary P adsorption by the soil though it did not have a significant effect on initial replacement. Olsen and Sommers (1982) reported that increasing the shaking period usually increases the amount of extractable P by $NaHCO_3$ solution. Increasing temperature of solution will also increase the amount of extracted P. (Olsen et al., 1954) reported that extracted P increased by 0.43 mg P kg^{-1}soil for each 1ºC increase in temperature between 20ºC and 30ºC in soils testing between 50 and 40 mg P kg^{-1} soil.

It has repeatedly been reported that the Olsen's test is less successful in predicting the available P for plants in regions where the soils have quite different constituents and subsequently high variability in their properties (Delgado and Torrent, 1997). Zalba and Galantini (2007) reported that when Olsen's test was modified by Colwell (1963) with increasing shaking period up to 16 h, the effect of P buffering capacity (PBC) on the extractable P by $NaHCO_3$ solution decreased. From Canada, (Soon, 1990), examining soils with 5.2-7.4 pH range, reported that PBC and P-resin had close correlation coefficients with barley plant responses. Investigating several soils of the central regions of Iran in 7.6-8.8 pH range, Shirvani et al. (2005) reported that the obtained PBC from adsorption isotherm equations had a significant relationship with P uptake of wheat in 70-day cultivation period in greenhouse conditions. Van Rotterdam et al. (2012) reported that at least two parameters were required for measuring the P supplying potential: first the quantity of adsorbed P by soil which can be returned to the soil or quantity factor (Q) and the other one is the P concentration in soil solution or intensity factor (I). The best prediction is achieved when Q is obtained by Olsen's method and I by 0.01M $CaCl_2$. Intensity factor (I) also shows the ratio of P which can leached from the soil profile. Q/I show the soil capacity for buffering intensity factor. (Van Rotterdam et al., 2012) used this method in the Netherland soils with 5-7.5pH range and could improve the prediction of P fertilizer requirement in grassland.

Since Olsen's test is the most used method in world's calcareous soils and also in Iran, and there is little published information on the effects of varying the conditions of the soil test, this research aims at studying the effects of varying the extraction conditions such as the period of shaking, the concentration of $NaHCO_3$ solution and the pH on the amount of P extracted and the correlation coefficient of extracted P with corn plant responses.

MATERIALS AND METHODS

Soil samples (0–30 cm) were collected from East Azerbaijan province (Iran). They were air dried, ground in a stainless steel mill to pass a 2 mm sieve, stored in airtight polyethylene containers, and analyzed by the following procedures:

Soil texture by hydrometer (Gee and Bauder, 2002), pH of 1:2 extract (soil: 0.01 M $CaCl_2$) by glass electrode (Richards, 1954), organic carbon (OC) by the wet oxidation (Nelson and Sommers, 1996), CaCO3 equivalent (CCE) by neutralization with hydrochloric acid (HCl) (Allison and Moodie, 1965). The active $CaCO_3$ equivalent (ACCE), which is mostly fine particle size calcite, was determined by the NH_4-oxalate method (Drouineau, 1942). EC of a 1:2 extract (soil: water) by electrical conductivity meter (Rhoades, 1996) and available P by $NaHCO_3$ solution (Olsen et al., 1954). Then, an experiment was done in 25 types of soil with three replications in greenhouse conditions. Five seeds of corn plant (*Zea mays* L.) single cross 704 variety were cultivated in the pots including 3-kg soils and they thinned to three plants after two weeks. The soil's moisture was maintained between 80 to 100% of field capacity during a two-month growth period. Some nutrients except P were used according to soil testing and customary fertilizer recommendations. For this, 120 mg urea/kg, 10mg /kg zinc sulfate ($ZnSO_4.7H_2O$) and 5mg/kg of soil iron as FeEDDHA were applied. Since soils have enough available potassium, potassium fertilizer was not used. After two months, the shoot and roots were harvested, dried and powdered. Digestion of plant samples was done by wet oxidation method (Waling et al., 1989) and the P concentration measuring in them with yellow method (Olsen and Sommers, 1982). The used extractants are presented in Table 1.

Having chosen the best extractant in 25 studied soils, in the rest of this research, the alteration effect of shaking period from 0.25 hours to 16 hours, concentration of $NaHCO_3$ from 0.25 to 1 M, $NaHCO_3$ pH from 7.5 to 9.5, soil: solution ratio from 1:5 to 1:100 on the amount of extracted P and correlation coefficient between extracted P and plant responses were investigated.

The best correlation coefficient was chosen from among these alterations and was implemented as combinational. Finally, optimum conditions for studied soils were concluded. Measuring the concentration of P in extracts was done as molybdo phosphoric acid colorimetric (ascorbic acid) using spectrophotometer which was posed by (Murphy and Riley, 1962) and modified by Watanabe and (Olsen, 1965). The graph was drawn by Excel and statistical analysis of data was carried out by SPSS software.

Table 1. Salient properties of the extractants used in this study.

Method	Concentration and chemical composition	pH	Soil: solution	Shaking period (minute)	References
Distilled water*	H_2O	-	1:10	5	Olsen and Sommers (1982)
$CaCl_2$ 0.01 M*	0.01M $CaCl_2$	-	1:10	5	Olsen and Sommers (1982)
Paauw*	Deionized water	-	1:60	60**	Paauw (1971)
Olsen	0.5M $NaHCO_3$	8.5	1:20	30	Olsen et al. (1954)
Colwell	0.5M $NaHCO_3$	8.5	1:100	960	Colwell (1963)
Soltanpour and Schwab	1M NH_4HCO_3+ 0.005M DTPA	7.6	1:2	15	Soltanpour and Schwab (1977)
Morgan	0.5M CH_3COOH + 0.7M $NaC_2H_3O_2$	4.8	1:5	15	Morgan (1941)
Kelowna*	0.25M CH_3COOH + 0.015M NH_4F + 0.25M $NH_4C_2H_3O_2$	-	1:10	15	Qian et al. (1994)
Paper strips	Types of Whatman filter paper coated with iron oxide	-	1:40:1***	960	Chardon et al. (1996)

* The extractants did not adjust pH; **Before the 60-minute shaking, the soil was soaked in demonized water for 22 hours;

***1 g of soil+40 mL of $CaCl_2$ 0.01 M+paper strip impregnated with iron oxide with sides 2 cm*10 cm.

RESULTS AND DISCUSSION

Properties of the soils are presented in Table 2. In the studied soils except soils No. 8 and 15, which had 35.1 and 5.7 % gypsum respectively, there was not gypsum. Table 3 presented the growth parameters of the corn plant. Among 25 studied soils, the highest linear correlation was observed between P extracted by Paauw method and P content of corn root ($r=0.52$, $p<0.01$). Meanwhile, P extracted by filter paper (Whatman451) impregnated with iron oxide method had a negative and significant relationship with shoot and root P content (r equals 0.44 and 0.48, $p<0.05$, respectively). In this research, the least linear correlation coefficient was observed between P extracted by Paawe method and dry weight of corn shoot ($r=0.01^{ns}$). At first glance, it seems that none of the studied extractants in this research could predict the response of corn plant, but deleting soils number 2 and 5 due to their probable abnormality, this correlation increased significantly.

The results after this removal are presented in Table 4. According to this table, it can be observed that P extracted by Colwell, Kelowna and Olsen's methods had close correlations with shoot dry weight of corn (0.75, 0.67, 0.65, $p<0.01$, respectively).

Although P extracted by other methods had a significant relationship with plant parameters, such relation could not be a proper prediction for growth indices of corn plant due to its low accuracy. According to Table 4, in this experiment, P extracted by distilled water, 0.01M $CaCl_2$ and Morgan's method did not have significant correlations with none of the indices of corn plant. P extracted by Paawu and filter paper (Whatman451) methods had negative and significant correlations with P concentration of shoot and root as well as P content of root while other extractants did not have a correlation with these parameters. In Pi method, the best correlation was in a method in which Schilcher and school filter paper No.589 were used, though due to the expensiveness of this paper, it is better to use Whatman filter papers 40 and 42 in Iran and similar countries since the P extracted by them had good correlations with shoot dry weight of corn plant ($r=0.59$, $p<0.01$, in both of them).

Table 2. Characteristics of calcareous soils used in this study.

Property	minimum	maximum	mean	std. deviation	Coefficient of variation (%)
CCE (%)	007.00	35.00	21.5	9.15	43
pH^{*}	007.08	08.05	8.1	0.19	2
pH^{**}	007.06	08.04	8.1	0.23	3
pH^{***}	007.03	08.02	7.7	0.23	3
O.C (%)	000.16	01.93	1.04	0.55	53
Sand (%)	015.57	67.78	38.94	18.48	47
Silt (%)	015.59	43.24	29.93	8.04	27
Clay (%)	012.39	57.27	31.11	12.55	40
ACCE (%)	001.47	10.78	5.1	2.79	55
EC (dS/m)	000.13	04.16	0.69	1.01	146
Total P(mg kg^{-1})	409.2	1070.07	720.4	180.6	25

*Distilled water to soil in a1:1 ratio;** Distilled water to soil **in a2:1** ratio;***$CaCl_2$ to soil in a 2:1 ratio

Table 3. Corn plant parameters

Growth parameter	minimum	maximum	mean	Std. deviation	C.V (%)
Root Dry weight (g/pot)	0.32	1.85	1.03	0.4	39
Shoot Dry weight (g/pot)	2.15	10.1	5.71	1.93	34
Root P concentration (mg/g)	0.19	0.66	0.46	0.12	26
Shoot P concentration (mg/g)	1.98	5.15	4.06	0.78	19
Root P content of (mg/g)	0.13	0.81	0.47	0.2	43
Shoot P content of(mg/pot)	9.36	33.13	22.44	6.59	29
Relative growth (%)	21.29	100	56.58	19.15	34
Stem diameter(mm)	4.4	10.39	6.94	1.38	20
Plant height(cm)	63.2	114.1	97.34	14.29	15

Table 4. Correlation coefficients (r) for linear relationships between extracted P and the corn growth parameters at 23 examined soils (after removal of soils number 2 and 5).

Extraction methods	Dry weight of Shoot	Shoot P concentration	P content of Shoot	Root P concentration	P content of root
Distilled water	0.37^{ns}	-0.28^{ns}	0.23^{ns}	-0.21^{ns}	-0.05^{ns}
$CaCl_2$, 0.01 M	0.37^{ns}	-0.31^{ns}	0.14^{ns}	-0.22^{ns}	-0.06^{ns}
Paauw	0.19^{ns}	-0.62^{**}	-0.21^{ns}	-0.57^{**}	-0.56^{**}
Olsen	0.67^{**}	-0.14^{ns}	0.56^{**}	-0.05^{ns}	0.14^{ns}
Modified Olsen 1	0.73^{**}	-0.21^{ns}	0.58^{**}	-0.13^{ns}	0.08^{ns}
Modified Olsen 2	0.75^{**}	-0.20^{ns}	0.59^{**}	-0.15^{ns}	0.03^{ns}
Modified Olsen 3	0.75^{**}	-0.19^{ns}	0.60^{**}	-0.15^{ns}	0.05^{ns}
Colwell	0.70^{**}	-0.26^{ns}	0.48^{*}	-0.23^{ns}	0.03^{ns}
Soltanpour and Schwab	0.47^{*}	-0.29^{ns}	0.26^{ns}	-0.16^{ns}	-0.01^{ns}
Kelowna	0.65^{**}	-0.25^{ns}	0.42^{*}	-0.23^{ns}	-0.02^{ns}
Morgan	0.38^{ns}	-0.25^{ns}	0.18^{ns}	-0.12^{ns}	-0.10^{ns}
Pi (Whatman 40)	0.59^{**}	-0.25^{ns}	0.41^{ns}	-0.17^{ns}	0.01^{ns}
Pi (Whatman 41)	0.55^{**}	-0.25^{ns}	0.38^{ns}	-0.16^{ns}	0.01^{ns}
Pi (Whatman 42)	0.59^{**}	-0.24^{ns}	0.43^{*}	-0.11^{ns}	0.10^{ns}
Pi (Whatman 541)	0.08^{ns}	-0.60^{**}	-0.34^{ns}	-0.57^{**}	-0.56^{**}
Pi (Schleicher and Schuell 589^3)	0.60^{**}	-0.33^{ns}	0.37^{ns}	-0.15^{ns}	0.04^{ns}

ns * and ** are non-significant and significant at 0.05 and 0.01 levels of probability respectively Pi: Iron oxide impregnated filter paper method. Modified Olsen 1: The Olsen method just with the bicarbonate concentration was 1M. Modified Olsen 2: The Olsen method just the shaker period was 16 hours. Modified Olsen3: The Olsen method just the shaker period was 4 hours and soil: solution ratio was 1:60

Under this research conditions, the extracting methods based on $NaHCO_3$ solution had better relationships with growth parameters of the corn plant. Although Colwell's method had a higher correlation coefficient with shoot dry weight compared to the Olsen's method, this difference was not significant ($\Delta r=0.08$, $p>0.05$). The pH, concentration of $NaHCO_3$ (C), solution: soil ratio, and shaking period were altered according to Table 5 in this study and then, the correlation coefficient of obtained data from these alterations was evaluated with plant growth parameters including shoot and root dry weight, P concentration of shoot and root and also content of shoot P. These alterations were done in 12 soils from among 25 soils. According to Table 6, it can be observed that alteration of the extraction conditions of Olsen's method affected correlation coefficients of extractable P and growth indices of corn.

Table 5. Statistical characterization of extracted P (mg kg^{-1}) with the Olsen and modified Olsen's methods.

Component changed	minimum	maximum	mean	std. deviation	Coefficient of Variation (%)
Olsen*	3.14	17.84	8.53	5.06	57
0.25M	2.18	11.87	5.89	2.95	50
0.75M	3.34	21.3	9.55	5.68	59
1M	4.3	24.38	11.05	6.36	58
pH 7.5	3.27	16.56	8.05	4.18	52
pH 8	3.03	16.71	8.36	4.45	53
pH 9	3.81	19.68	9.66	5.17	54
pH 9.5	3.85	22.14	9.85	5.6	57
Solution: soil= 5	1.81	12.74	5.73	3.35	59
Solution: soil= 10	2.1	14.24	6.7	3.82	57
Solution: soil= 40	3.31	21.17	9.25	5.24	57
Solution: soil= 60	4.37	21.23	10.65	5.44	51
Solution: soil= 80	4.97	25.09	13.23	6.93	52
Solution: soil= 100	5.4	25.15	14.07	6.61	47
0.25 h	2.93	12.82	6.31	3.13	50
1 h	3.27	19.78	9.08	5.2	57
2 h	4.32	19.83	9.85	5.32	55
4 h	4.65	26.05	12.45	6.69	54
8 h	5.13	31.71	13.75	7.84	57
16 h	5.54	38.15	15.68	8.93	57
1M and solution : soil= 60	5.66	30.64	13.38	7.81	58
Solution : soil= 60 and4 h	6.05	36.69	16.96	9.05	53
1M and 4 h	7.94	42.6	18.16	10.88	6
4 h and 1M and solution: soil= 60	11.51	56.39	25.46	12.61	50

* Concentration was 0.5 M, pH=8.5, shaker period 0.5 hours, soil: solution was 1:20

Changing the extraction conditions of Olsen's method did not have any significant effect on extractable P correlation with P concentration of root and shoot and also the content of P. The highest linear correlations were used in any alteration method for combinational selection of changing conditions which included the 1M concentration, solution: soil ratio of 60 and shaking period of 4 h. Although the shaking period of 16 h had the highest correlation coefficient, but due to decreasing the extraction time, and the quickness of the extraction procedure and insignificance of the difference of linear correlation coefficient with 4h, the shaking time of 4 h was preferred. Due to non-effectiveness of $NaHCO_3$ solution pH on correlation coefficient value of extracted P with plant shoot dry matter (Table 6); it was removed from the combination with the selected condition. As it was considered, when at Olsen's method, the shaking period increased up to 16 h; the linear correlation coefficient of extracted P with shoot dry weight of the corn increased. Similar results were observed for shaking time of 4 h and solution: soil ratio of 60.

Generally, it cannot be claimed that altering specific conditions led to a significant increase or decrease of the correlation coefficient between extractable P by $NaHCO_3$ solution and growth parameters of the corn plant. Finally, Olsen's method with 1M concentration of $NaHCO_3$ solution and 16h shaking period and solution: soil ratio of 60 and shaking for 4 h was chosen for continuing the experiments. Colwell believed that at different regions of Australia, due to different land suitability, climates and parent materials nature, there were different conditions; therefore, each region can have its own suitable extracting method. Colwell (1963), in Australia, reported a higher correlation compared to the Olsen's test by alternating the shaking period to 16 h and ratio of solution: soil of 100:1. Meanwhile, Colwell (1963) reported that correlation coefficient between plant responses and extractable P was more than the customary Olsen's method. In the majority of the studies, the effect of extraction conditions alteration on extractable P amount was studied and there has been limited reports related to the manner of changing the statistical correlations with plant parameters. According to tables 5 and 6, it can be concluded that increasing the extractable P by $NaHCO_3$ solution increased its correlation with plant parameters (though such increase is not high) and this result conforms to Colwell's (1963) report. Meanwhile, according to Table 6, it can be concluded that alteration of specifications of $NaHCO_3$ extractant had an independent effect on the correlation of extracted P and growth parameters of corn plant since no increase was seen in these correlation coefficients by combining the components which led to increasing the correlations.

Table 6. Correlation coefficients (r) for linear relationships between extracted P by modified Olsen's methods and corn growth parameters.

Component changed	Shoot dry weight	Shoot P concentration
Olsen	0.72^{**}	0.61^{*}
0.25M	0.62^{*}	0.64^{*}
0.75M	0.69^{*}	0.52^{ns}
1M	0.74^{**}	0.58^{*}
pH7.5	0.7^{*}	0.59^{*}
pH8	0.66^{*}	0.56^{ns}
pH9	0.7^{*}	0.62^{*}
pH9.5	0.67^{*}	0.56^{ns}
Solution: soil= 5	0.67^{*}	0.5^{ns}
Solution: soil= 10	0.69^{*}	0.56^{ns}
Solution: soil= 40	0.76^{**}	0.58^{*}
Solution: soil= 60	0.76^{**}	0.63^{*}
Solution: soil= 80	0.65^{*}	0.62^{*}
Solution: soil= 100	0.66^{*}	0.63^{*}
0.25 h	0.65^{*}	0.61^{*}
1 h	0.71^{**}	0.59^{*}
2 h	0.73^{**}	0.60^{*}
4 h	0.77^{**}	0.64^{*}
8 h	0.75^{**}	0.59^{*}
16 h	0.77^{**}	0.59^{*}
1M and solution :soil= 60	0.73^{**}	0.53^{ns}
Solution :soil= 60 and4 h	0.77^{**}	0.61^{*}
1M and 4 h	0.77^{**}	0.57^{ns}
4 h and 1M and solution :soil= 60	0.75^{**}	0.54^{ns}

ns, * and ** are non-significant and significant at 0.05 and 0.01 levels of probability respectively.

Effects of extraction conditions alterations on the extractable P by $NaHCO_3$ solution

Fig. 1 shows the mean of extracted P by $NaHCO_3$ solution with different ratios of solution to soil. According to Figure 1, it can be observed that in this experiment, increasing the ratio of $NaHCO_3$ solution to soil from 5 to 100 increased the amount of extracted P as exponential significantly ($p<0.05$). Barrow and Shaw, (1976a) reported that the main reason for decreasing the extracted P by increasing the ratio of soil to the $NaHCO_3$ solution was due to decreasing the concentration of bicarbonate ion. Increasing the ratio of solution to soil increased the amount of extractable P and such increase was as exponential which was also reported by other researchers (Barrow and Shaw, 1976a; Westerman and Ehlers, 1986). Barrow and Shaw (1976a) reported the increasing process of extractable P as exponential after incubation of soils in 4.2-6 pH range with 300mg Pkg^{-1}soil for 380 days. In their research, the ratio of solution to soil has been varied from 2 to 100. Westerman and Ehlers (1986) reported $P=4.19\ (solution:\ soil)^{0.19}$ and $P=28.4\ (solution:\ soil)^{0.15}$ equations with r^2 equal to 0.97 and 0.98 respectively for two soils by stabilizing other conditions. In their research, the ratio of solution: soil was increased from 10 to 100 and soil samples were prepared from Idaho State of US, which were non-saline, non-sodic with 3 to 15% calcium carbonate equivalent. According to Fig.2, it can be observed that increasing the extractable P by 0.5M $NaHCO_3$ solution was a natural logarithm by increasing the shaking period from 15 min to 16 h. In this figure, the points which do not have common letters had a significant difference with probability level of 5% by Duncan test. Barrow and Shaw (1976b) explained the effect of changing the characteristics of $NaHCO_3$ solution on the P extraction with two terms of initial replacement and partly re-absorbed. They reported that these two values increased by increasing the shaking time which finally led to increasing the extracted P by $NaHCO_3$ solution. (Barrow and Shaw, 1976b) investigated the effect of shaking period (up to 150 h) in different ratios of $NaHCO_3$ solution: soil in soils with 4.2-6pH range for 756 days of incubation with 400 mg

Pkg^{-1}soil, and as a result, the equation of $P=363 (1+35.1/t)^{-0.276}$ was presented. In this equation, P represents the amount of extractable P by $NaHCO_3$ solution and t represents the shaking time. In another research, Westerman and Ehlers (1986) reported that increasing the shaking time to 120 minutes led to linear increasing of extracted P- which did not conform to the results of the current research. Therefore, they concluded that the effect of shaking time depended on the concentration of soil P. According to Fig. 3, it can be observed that increasing the concentration of $NaHCO_3$ from 0.25M to 1M increased the extracted P from studied soils as exponential.

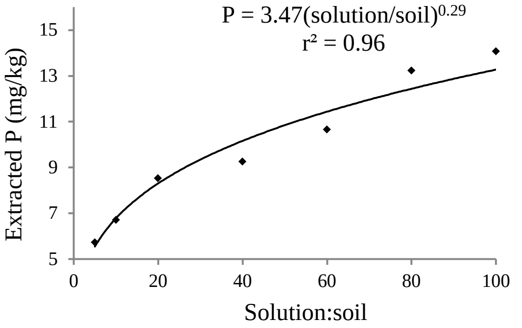


Fig. 1. Effect of $NaHCO_3$ solution: soil ratios on P extracted for 12 calcareous soils.

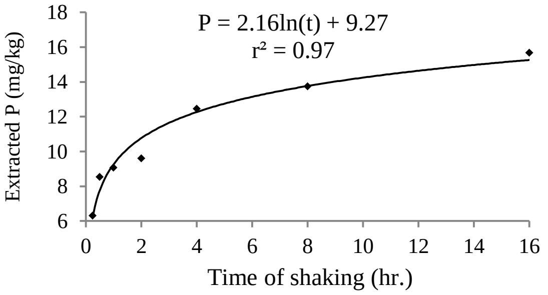


Fig. 2. Effect of shaking period on the P extracted by $NaHCO_3$ solution for 12 calcareous soils.

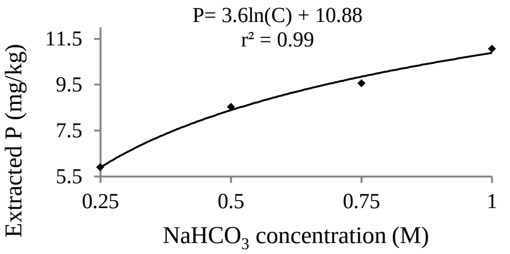


Fig. 3. Effect of $NaHCO_3$ solution concentrations on P extracted for 12 calcareous soils.

Barrow and Shaw (1976b) reported that the amount of extracted P by $NaHCO_3$ increased by increasing its concentration in pH=8.5 due to decreasing the amount of re-adsorbed of phosphate. This result conforms to the report of (Westerman and Ehlers, 1986). Also, Barrow and Shaw (1976b) reported that increasing the concentration of $NaHCO_3$ led to increasing the extracted P and this increase was obvious in high ratios of solution to soil. Fig. 4 shows that extracted P by $NaHCO_3$ solution increased by increasing the pH of solution as linear though such increase was not significant. Barrow and Shaw (1976b) reported that in presence of bicarbonate, the most effect of increasing pH was on initial replacement and had little effect on the phosphate re-adsorbed. This effect may largely be due to more increasing of the concentration of hydroxide (OH^-) ion to increasing the concentration of carbonate ions. The importance of hydroxide in replacing phosphate may be due to increasing the negative charge of soil particle surface (Hingston et al., 1968). Similar results were also reported by (Cowling et al., 1987; Barrow and Shaw, 1976b). By increasing pH of $NaHCO_3$ solution, Ca^{2+} is precipitated as $CaCO_3$ and led to dissolution of calcium phosphates (Lindsay, 1979). Westerman and Ehlers (1986) reported that extractable P by $NaHCO_3$ by changing the pH from 8 to 8.5 did not change although when it reached 9, considerable increase was observed since when pH was more than 8.3, the activity of Ca^{2+} decreased and led to dissolution of calcium phosphate and therefore, P concentration increased in the solution (Lindsay, 1979). Cowling et al. (1987) studied the effect of increasing pH of$NaHCO_3$ solution and reported that increasing pH from 8.5 to 9.5 increased the amount of extracted P as linear.

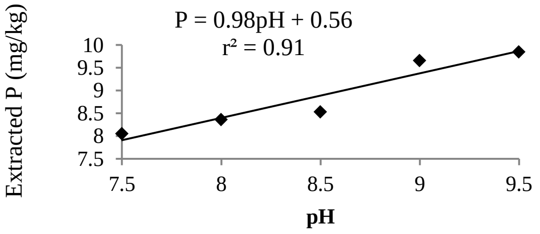


Fig. 4. Effect of $NaHCO_3$ solution pH on the extracted P for 12 calcareous soils.

Effects of Changes in extraction conditions on the correlation between extracted P by $NaHCO_3$ solution and shoot dry weight of corn plant

According to Fig. 5a, it can be observed that increasing the ratio of solution to soil by 60 led to increasing the correlation coefficient between extracted P and corn shoot dry weight, though in more than that, it led to decreasing such correlation. The reason for such decrease can be explained such that ratios of solution: soil for more than 60 may lead to the release of the non-labile soil P which is not available for the plant. As it can be observed in Fig.5b, increasing the P extracted by $NaHCO_3$ can lead to increasing the linear correlation(r) between extracted P and corn shoot dry weight when the ratio of solution to soil increases from 5 to 60. Fig. 6 shows that increasing the linear correlation coefficient of extracted P by $NaHCO_3$ solution and shoot dry weight of corn due to increasing the shaking time is similar to the available trend in Fig. 2. That means extracted P by 16 h shaking time includes plant's available P which is not extracted in durations less than that. Meanwhile, Fig. 6 shows that this increasing the correlation by 4 h has an increasing trend. But after that, it remains constant. Due to the lack of similar published results in Iranian soils, it is not possible to do the comparison. According to Fig.7, it is observed that the increasing procedure of linear correlation coefficient with increasing the concentration of $NaHCO_3$ is similar to the available procedure in Fig. 3. As it can be seen in this figure, 1M concentration could probably extract more parts of the plant's available P. In this concentration, increasing bicarbonate ions leads to increasing their competitiveness in replacing with phosphate ions in exchangeable places and more decrease of the calcium activity resulted from their precipitation as calcium carbonate. Although the correlation coefficient increased from 0.25 M to 1 M, (from 0.62 to 0.74), firstly, such increase was not very high ($\Delta r=0.12$); secondly, increasing the concentration of $NaHCO_3$ solution is expensive economically. As it can be seen in Fig.8a, changing the linear correlation coefficient with shoot dry weight resulted from the $NaHCO_3$ solution pH alteration does not follow a specific procedure. The correlation of extracted P and shoot dry weight with changing pH of the $NaHCO_3$ solution in 8.5-9.5 range decreased as linear, though such correlation decrease was not significant (Fig. 8b). Meanwhile, in 7.5-8.5 pH range, the correlation was very weak (Fig. 8c).

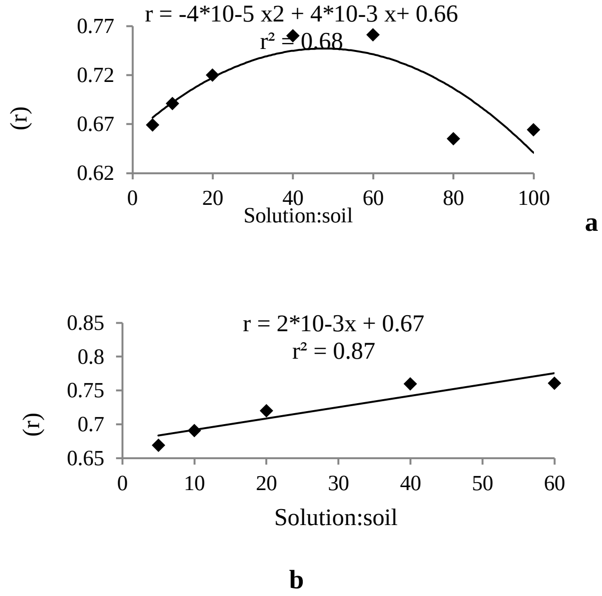


Fig. 5. Effect of solution: soil ratio on the correlation coefficients (r) between extracted P and shoot dry weight of corn (n=7 in"a" and n=5 in "b").

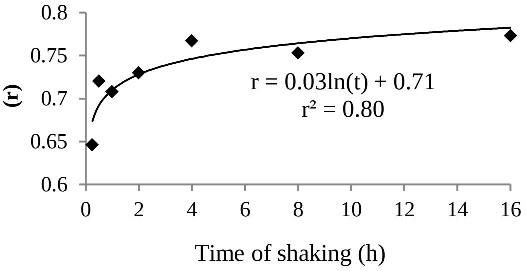


Fig. 6. Effect of shaking period on the correlation coefficients (r) between extracted P and shoot dry weight of corn.

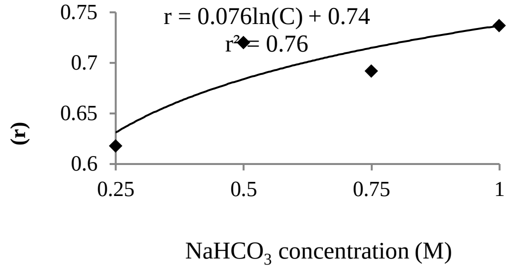


Fig. 7. Effect of $NaHCO_3$ solution concentration on the correlation coefficients (r) between extracted P and shoot dry weight of corn.

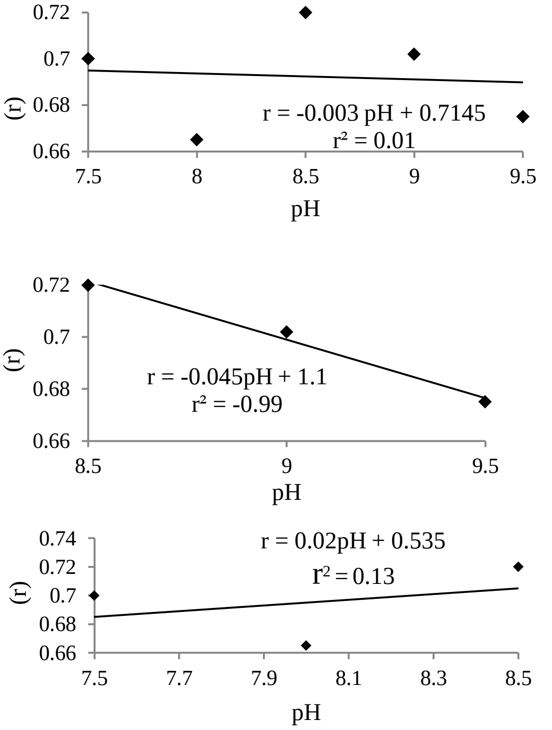


Fig. 8. Correlation coefficients (r) for linear relationships between P extracted by $NaHCO_3$solution with varying pH and shoot dry weight of corn (pH=7.5-9.5 in "a", pH=8.5-9.5 in "b" and pH=7.5-8.5 in "c").

Van Rotterdam et al. (2012) defined the PBC as the best state when it is measured by dividing the Olsen-P on $CaCl_2$-P. In this research, three indices of soil solution P extractants (distilled water, 1M $CaCl_2$and Paawu methods) were used for measuring the PBC in different extractants which can be P quantitative index. Having studied the statistical relations of obtained PBCs with parameters of corn plant, it was observed that none of these linear correlations were close. Meanwhile, the correlation coefficient of second order equation was not significant. For example, fig. 9 and 10 can be referred to.

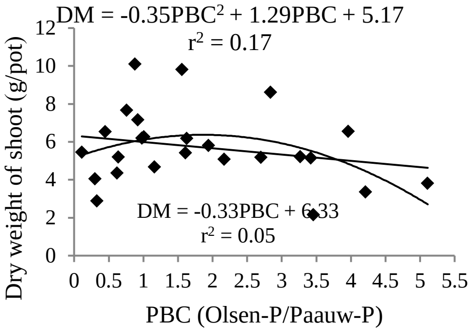


Fig. 9. Correlation between phosphorus buffering capacity (PBC) and dry weight of corn shoot.

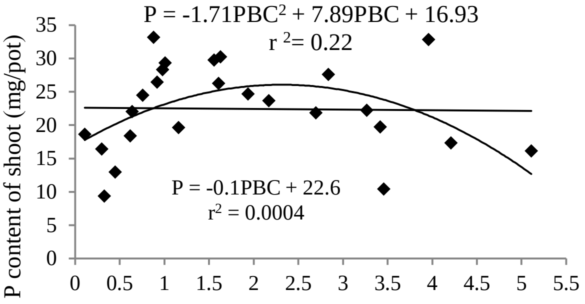


Fig. 10. Correlation between phosphorus buffering capacity (PBC) and corn shoot P content.

The highest correlation was achieved when PBC was calculated by dividing the amount of extracted P with paper strips (Whatman42 and 40) on P dissolved in 0.01M calcium chloride and the shoot P content was drawn (r was 0.56 and 0.54, respectively and $p<0.01$). (Soon, 1990) reported a close and positive correlation between PBC (extractable P with $0.01MCaCl_2$/P-resin) and plant's responses. (Shirvani et al., 2005) reported that the obtained buffering indices from P adsorption equations have significant and inverse relationships with P uptake by wheat in a 70-day cultivation ($p<0.05$, $|r|=0.69-0.74$). Having studied the relationship between PBC and plant indices more, it was observed that increasing PBC (P-Olsen/P-Paawu) by 1.2 lead to a significant increase of the correlation. Such procedure was available in shoot dry weight too but after that, this will be reverse. That means increasing PBC (P-Olsen-P-Paawu) for more than 1.2 decreased the absorbed Pin shoot. Therefore, the relationship between dry weight of shoot and PBC in two parts (PBC less and more than 1.2) was studied and it was observed that in PBC less than 1.2, this relationship follows a cubic equation (Fig.11a) which became a linear relationship by removing two first and last points (Fig.11b).Shoot dry weight and PBC more than 1.2 had a weak and negative linear correlation with each other (Fig.12). The relationship between shoot P content and PBC in two parts (PBC less and more than 1.2) was studied and it was observed that in PBC less than 1.2, this relationship follows a cubic model (Fig.13a) and a very strong linear relationship was obtained by deleting two first and last points (soils with very low and very high Olsen-P) ($r^2=0.83$) (Fig.13b). The shoot P content and PBC more than 1.2 had a weak and negative linear correlation with each other (Fig.14a). Having studied the data due to deviation by SPSS, it was observed that, in PBCs of 3.4 and 3.9 (soils No. 5 and 9), Cook's value is more than 1.

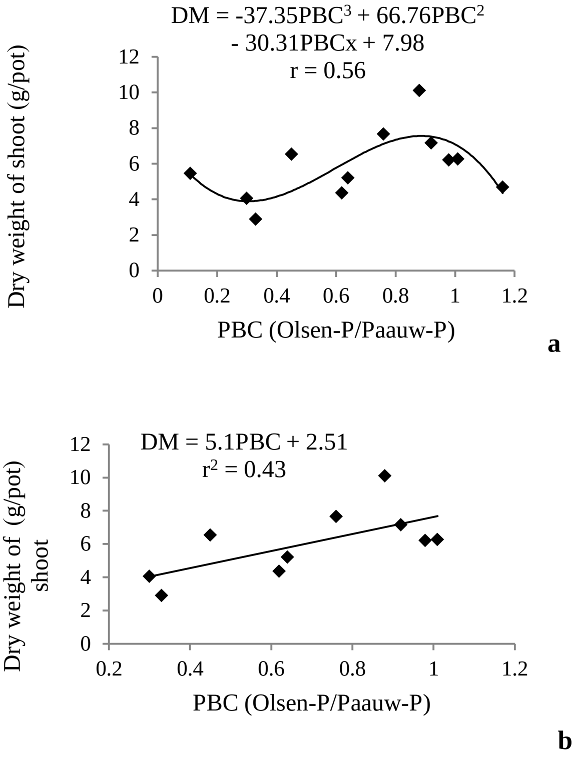


Fig. 11. Correlation between PBC of less than 1.2 and dry weight of shoot (n=12 in "a" and n=10 in "b").

Therefore, these two data were considered as statistically deviated and can be removed. Having deleted these two data, a strong relationship ($r^2=0.89$) was observed between shoot P content and PBC more than 1.2 (Fig. 14b). Bell et al. (2005) reported that the saturation of P and the capacity of soil to P adsorption, which are considered as two quantitative factors of P and P buffering capacity in predicting the plant's available P, better predict the available P of grasses in basalt soils compared to Olsen or other recognized methods. In this research, PBC is defined as the ratio of quantitative ratio to intensity. Therefore, PBC method (P-Olsen/P-Paawu) can be the best method for predicting the growth indices of corn plant. The high correlation of this method with the content of shoot P indicated that PBC linear correlation with shoot P content is 0.91 in a positive relationship and is -0.89 in a negative relationship ($p<0.01$).

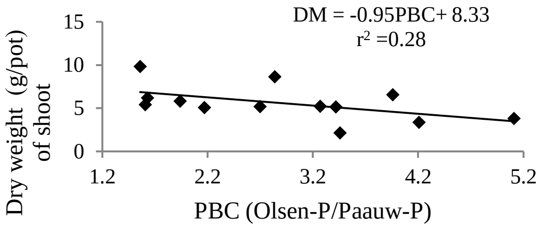
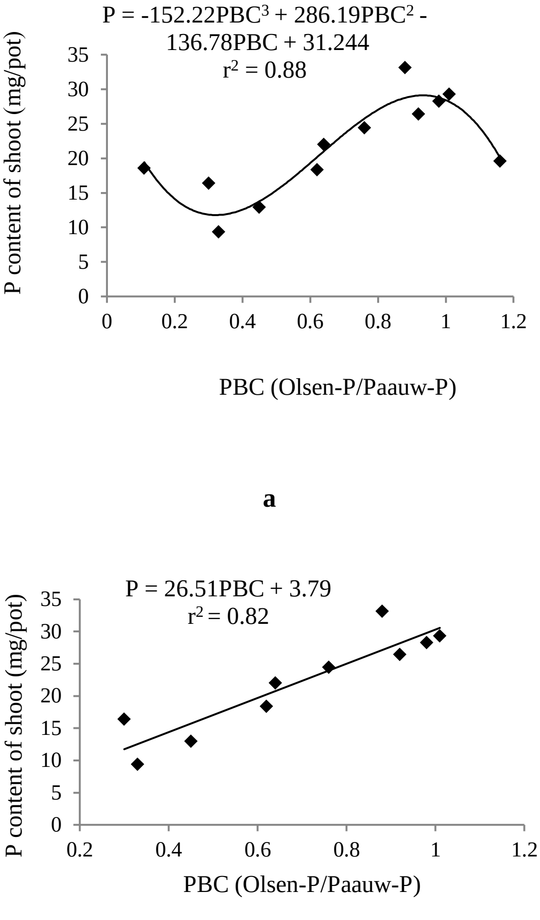


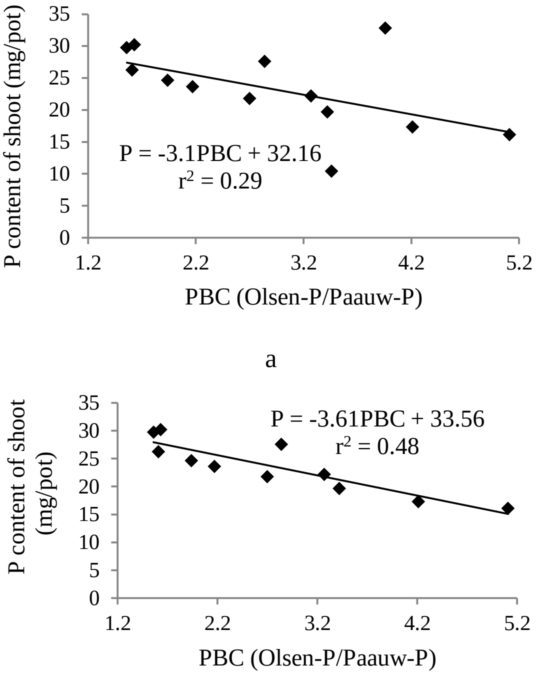
Fig. 12. Correlation between PBC of more than 1.2 and dry weight of corn shoot.



a

b

Fig. 13. Correlation between PBC of less than 1.2 and corn shoot P content (n=12 in "a" and n=10 in "b").



a

b

Fig. 14. Correlation between PBC of more than 1.2 and corn shoot P content (n=13 in "a" and n=11 in "b").

CONCLUSIONS

Under greenhouse conditions of this piece of research and in the studied soils, the extracting methods based on $NaHCO_3$ solution had better relationships with growth parameters of corn plant compared to other methods. According to the results, increasing the concentration of $NaHCO_3$ solution, soil ratio and shaking period led to a significant increase in extracted P, but regarding the increase of pH, the results were not significant. Alteration in P extraction conditions of Olsen's method could not significantly increase the correlation coefficient between extracted P and growth indices of corn plant. Meanwhile, changes of different characteristics of $NaHCO_3$ solution had an independent effect on correlation of P extracted from the soils and growth parameters of corn plant. The results of this experiment showed that PBC method (Olsen-P/Paawu-P) can be the best method for predicting the absorbed P by corn plant. The high correlation of this method with shoot P content can be an indication for it.

ACKNOWLEDGEMENT

We hereby appreciate the Research vice Chancellorship at the University of Tabriz for providing the necessary expenses.

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تحقیقات کشاورزی ایران (۱۳۹۴) ۳۴(۱) ۹۲-۱۰۴

توسعه روش جایگزین آزمون اولسن جهت تعیین فسفر قابل جذب ذرت در خاک‌های آهکی

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اطلاعات مقاله

تاریخچه مقاله:
تاریخ دریافت: ۱۳۹۲/۱۰/۱۰
تاریخ پذیرش: ۱۳۹۳/۱۱/۵
تاریخ دسترسی: ۱۳۹۴/۴/۲۱

واژه های کلیدی:
خاک آهکی
ذرت
آزمون اولسن
فسفر

چکیده- در این تحقیق، برخی عصاره‌گیرهای شیمیایی و روش‌های تغییر یافته اولسن در ۲۵ خاک آهکی سطحی (۰-۳۰ سانتی متر) برای تعیین فسفر قابل جذب گیاه ذرت مورد مطالعه قرار گرفت. در این آزمایش، بیشترین همبستگی بین فسفر استخراج شده توسط روش‌های اولسن و کالول (فسفر قابل عصاره‌گیری با محلول بی کربنات سدیم) و وزن خشک بخش هوایی ذرت مشاهده شد($r=0.67,0.75$ و $p<0.01$). بنابراین اثرات شرایط موثر و متغیر عصاره‌گیری همانند زمان تکان دادن، غلظت و pH محلول بی‌کربنات سدیم بر مقدار فسفر عصاره‌گیری شده از خاک و همبستگی آن با پارامترهای رشد ذرت مورد تحقیق قرار گرفت. نتایج نشان داد که افزایش pH محلول بی کربنات سدیم، نسبت محلول به خاک ، زمان تکان دادن و غلظت محلول بی کربنات سدیم بطور معنی‌داری میزان فسفر عصاره‌گیری شده از خاک را افزایش داد اما نتوانست بطور معنی‌داری همبستگی بین فسفر عصاره‌گیری شده و پارامترهای رشد گیاه را بهبود ببخشد. بیشترین همبستگی بین روش تغییر یافته اولسن (با ۴ ساعت دوره تکان دادن و نسبت محلول به خاک برابر ۶)و وزن خشک بخش هوایی بدست آمد(($r=0.75, p<0.01$)) که البته تفاوت معنی داری با روش اولسن(نیم ساعت تکان دادن و نسبت محلول به خاک ۲۰) نداشت. نتایج این مطالعه نشان داد که ظرفیت بافری فسفر می‌تواند بهترین روش در پیش بینی فسفر قابل جذب ذرت در خاک‌های آهکی باشد.