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STANDARIZATION OF THE $NaHCO_3$-EXTRACTABLE P IN SOILS OF THE ICARDA REGION

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Standardization of the $NaHCO_3$ Extractable-P in Soils of the ICARDA Region

1. Introduction

There is an increasing evidence in the ICARDA region that levels of available soil phosphorus, an important element of plant growth, are often low and substantial responses for phosphate fertilizer are frequently reported.

Soil tests have been used extensively to determine plant available nutrients and can be further used to determine the amount of fertilizers needed for optimum crop yields, when soil tests are calibrated with results of fertilizer field experiments and environmental variables.

The $NaHCO_3$-extractable P by soils (Olsen method) has been found by many workers, as a specially good test to determine the available P in calcareous soils which dominate in the region.

The details of the $NaHCO_3$ method had been given by Olsen et al in Circular No.939 of the United States Department of Agriculture in (1954). It is always worth referring to that publication to consider the detailed studies made by the authors on that procedure and the many environmental and physio-chemical factors which affect the results of the analyses and its interpretation. Among those variables one could cite, the effects of time of extraction, pH of the extracting solution and the method of extraction; and finally the calibration of the soil test of available P to expected response to phosphate fertilization with plants grown in pots or in the field.

The present study will concentrate on results of analyses obtained in the (13) soil laboratories of the region which participated in the survey and the analyses of (12) soil samples sent to them. Possible reasons for the variability of results obtained by the laboratories will be discussed, based on the questionnaire concentrated on the details of the procedure used and the minor or major changes made by each laboratory on the original Olsen method.

2. Objectives

The main objectives of the survey study are:

1. To assess the variability among laboratories of the region in results obtained when determining the available-P in soils by the Olsen method, using standard soil samples.
2. To assess the need for a workshop on the methods and procedures of soil analyses used in the various countries of the ICARDA region.

3. Participating Countries and Laboratories

Thirteen soil laboratories in various scientific institutions located in eight different countries participated in the present survey.

A list of these institutions is given below;

Country	Name of Institution and Address
Tunisia	Ministere de l'Agriculture, Direction des Sols, Tunisie.
Tunisia	Institut National de la Recherche Agronomique de Tunisie. Ariana, Tunisie.
Tunisia	Ecole Superieure d'Agriculture du Kef, Le Kef.
Egypt	Desert Research Institute, Mataria - Cairo.
Cyprus	Agricultural Research Institute, Ministry of Agriculture and Natural Resources, Nicosia.
Pakistan	Pakistan Agricultural Research Council, Islamabad.
Pakistan	National Agricultural Research Center, NARC, Islamabad.
Saudi Arabia	The Ministry of Agriculture and Water. Regional Agricultural and Water Resources Center. Al-Ryad.
Jordan	The University of Jordan, Faculty of Agriculture, Amman.
Jordan	The Ministry of Agriculture, Direction of Research and Extension, Amman.
Morocco	Midamerica International Agricultural Consortium, Project Aridiculture, Settat.
Syria	The Arab Center for the studies of Arid Zones and Dry Lands (ACSAD), Damascus.
Syria	The Soil Directorate, Ministry of Agriculture, Damascus.
Syria	The International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo.

4. Major Properties of the Soils Tested

The soils chosen for the present survey were collected from experimental sites in Syria, where fertilizer experiments have been conducted in sites extending between the arid and semi-humid region.

Some of the characteristics of the soil samples sent to various laboratories are given in Table (1).

Table (1) Some characteristics of soil samples sent to various laboratories for P-Olsen analysis.

Sample No. No.	Site	$CaCO_3$ (%)	O.M. (%)	PH (1:1)	E.C. (1:1) mS/cm	Texture
1	Ibtyn	41	0.76	8.4	0.23	clay
2	Himo	27	1.54	8.2	0.30	clay
3	Himo	27	1.54	8.2	0.30	clay
4	Saalo	19	1.04	8.4	0.52	clay loam
5	Chzeilan	24	1.22	8.3	0.26	clay loam
6	Sarakeb	30	1.20	8.2	0.26	clay
7	Suran	36	1.04	8.2	0.22	clay
8	El-Ghab	25	2.30	8.4	0.25	clay
9	Izraa	13	0.64	8.3	0.22	clay
10	Gleen	4	0.78	8.2	0.20	clay
11	Tel Hadya	25	0.85	8.2	0.40	clay
12	Lattamneh	29	0.97	8.2	0.40	clay

5. Results of Analyses

The results of analyses of the Olsen-P by various laboratories are given in Table (2), with each participating laboratory being given a serial number. Results between laboratories have been assessed by conducting a pair-samples T-test (Table 3) and by computing the regression equations among any two participating laboratories. The degree of significance of the slopes and intercepts of the regression between pairs of laboratories have been estimated. You will find in Tables (4), (5) and (6) the values of coefficients of determination, slopes and intercepts matrix among all laboratories results obtained; with the degree of significance.

The results obtained between any two laboratories were compared according to the following principle.

First, the probabilities T-test among any pair of results obtained by participant laboratories, have been used to establish the laboratories which showed any significant differences among their results.

Second, the systematic differences among two sets of results, were judged from the slope of the regression and the degree of its deviation from 1. Therefore if the slope of the regression is significantly different from one, there are systematic differences among these two sets of results.

Third, the consistent differences among any two sets of results, have been judged from the value of the intercept and its degree of significance. So if the value of intercept is significantly different from zero, there are then consistent differences among these two sets of results.

Table (2) Results of $NaHCO_3$ extractable P in soil samples as conducted by various laboratories of the region.

Lab No.	Concentration of $NaHCO_3$-P in Various Soils (ppm)												Overall Mean
	1	2	3	4	5	6	7	8	9	10	11	12	
1	5.9	12.7	11.5	7.5	3.9	6.8	4.1	24.6	1.7	1.6	10.4	16.8	8.97
2	7.6	12.4	11.1	8.0	5.6	7.1	6.2	22.6	3.8	3.5	9.9	14.6	9.37
3	5.7	15.5	10.4	8.6	3.6	7.4	5.3	27.8	2.2	5.6	16.7	16.6	10.45
4	6.0	14.0	13.0	7.0	4.0	6.0	6.0	26.0	2.0	3.5	9.0	15.0	9.29
5	5.4	12.7	10.7	7.1	3.7	5.0	2.7	22.0	1.3	0.5	8.2	13.8	7.76
6	6.0	-	12.7	8.5	4.9	6.8	4.2	26.6	1.3	0.1	10.8	17.5	-
7	5.4	13.0	13.0	7.4	3.7	7.2	4.2	24.0	1.5	1.6	9.3	16.0	-
7	5.9	15.0	13.0	8.6	3.5	6.3	4.0	29.0	1.6	1.3	11.0	17.0	9.27
8	-	17.0	10.0	-	-	-	7.0	-	-	5.0	13.0	15.0	-
9	9.6	17.0	16.2	10.9	5.2	8.7	5.7	31.8	6.1	2.6	12.7	24.9	12.61
10	4.0	7.0	6.0	4.5	2.0	3.0	2.0	19.5	1.0	0.5	9.0	12.0	5.88
11	7.6	12.4	12.1	8.1	20.7	6.5	5.7	15.9	4.8	5.7	8.5	12.1	10.00
12	8.0	14.0	14.0	9.0	3.0	8.0	4.0	29.0	2.0	0.0	11.0	17.0	9.91
13	7.0	15.7	14.8	10.5	7.4	9.2	6.1	27.5	3.9	03.5	13.5	18.3	11.45

Table (3) The pair-samples T-Test matrix among results obtained by participant laboratories in the region, (2-Tail Probabilities).

Lab No.	2-Tail Probabilities												
	1	2	3	4	5	6	7	8	9	10	11	12	13
1													
2	0.386												
3	0.037	0.214											
4	0.004	0.876	0.134										
5	0.001	0.000	0.005	0.002									
6	0.172	0.939	0.249	0.742	0.003								
7	0.178	0.901	0.103	1.000	0.001	0.446							
8	-	-	-	-	-	-	-						
9	0.000	0.009	0.051	0.002	0.000	0.000	0.000	-					
10	0.000	0.000	0.000	0.000	0.003	0.001	0.000	-	0.000				
11	0.563	0.670	0.832	0.690	0.181	0.714	0.708	-	0.268	0.016			
12	0.060	0.497	0.524	0.284	0.004	0.186	0.074	-	0.000	0.001	0.969		
13	0.000	0.002	0.138	0.000	0.000	0.000	0.000	-	0.132	0.000	0.418	0.008	

Table (4) The coefficients of determination (R^2) matrix among results of $NaHCO_3$-extractable P in soils obtained by thirteen soil laboratories of the region.

Lab No.	1	2	3	4	5	6	7	8	9	10	11	12	13
1													
2	.990**												
3	.665*	.596*											
4	.922**	.943**	.379										
5	.981**	.979**	.577*	.969**									
6	.989**	.996**	.618**	.944**	.988**								
7	.981**	.981**	.575**	.969**	.999*	.989**							
8	.862*	.832*	.907**	.622	.774*	.835*	.775*						
9	.977**	.971**	.543*	.936**	.959**	.956**	.959**	.771**					
10	.958**	.964**	.681	.828*	.899**	.951**	.903**	.916**	.925**				
11	.796*	.791*	.291*	.924**	.888**	.812*	.886**	.446	.811*	.615			
12	.963**	.972**	.574	.958**	.991**	.987**	.992*	.775**	.921**	.894**	.867		
13	.978**	.970**	.681	.919**	.986**	.987**	.986**	.845*	.924**	.915**	.830*	.989**	

*, ** Significant at 5 and 1% respectively.

Table (5) The slopes of the regression lines matrix among results of NaHCO$_3$-extractable-P in soils obtained between any combination of the thirteen laboratories of the region.

Lab No.	1	2	3	4	5	6	7	8	9	10	11	12	13
1													
2	1.402**												
3	.937	.643											
4	1.237	.887$_{**}$	.860$_{*}$										
5	1.093	.776$_{**}$	.830*	.853$^{*}_{**}$									
6	.875	.624$_{**}$	.685	.674$_{**}$	.795$^{**}_{**}$								
7	.973$_{*}$	.691	.746	.759	.890	1.111							
8	1.396$_{**}$	.979$_{**}$	1.312$_{**}$	.978$_{**}$	1.221$_{**}$	1.571$_{**}$	1.372$_{**}$						
9	.683$_{**}$	.484	.515	.527	.616	.771$_{**}$	.692$_{*}$	.425*					
10	1.516$_{*}$	1.080$_{*}$	1.198	1.127$_{**}$	1.345$_{*}$	1.721$_{*}$	1.513$_{*}$	1.011$_{*}$	2.173$^{**}_{*}$				
11	1.745	1.238$_{**}$		1.444$_{**}$	1.649$_{**}$	2.000	1.850	.983$_{*}$	2.552$_{**}$	1.039$_{**}$			
12	.854	.609$_{**}$	.659$^{**}_{**}$	.668$_{**}$	.784$_{**}$	.981	.881	.536	1.210	.530$_{**}$	.433$^{**}_{*}$		
13	.972	.689	.688	.744	.885	1.109	.995	1.625	1.382	.616	.483	1.126**	

*, ** Degree of significance at 5 and 1% level successively of the slope obtained which are different from one between any combinationof two laboratories.

Table (6) The intercepts of regression lines matrix among any two sets of results of the $NaHCO_3$-extractable P in soils obtained between any combination of the thirteen laboratories of the region.

Lab No.	1	2	3	4	5	6	7	8	9	10	11	12	13
1													
2	-3.82**												
3	-1.36	+2.038											
4	-2.630	+0.809	+2.919										
5	+1.035	+3.486**	+4.892	+3.178*									
6	+0.952	+3.410	+4.715	+3.191*	-0.019								
7	+0.068	+2.796*	+4.158	+2.422**	-0.884**	-1.002							
8	-5.076	-0.734	-2.198	-0.479	-5.026	-6.646	-4.661						
9	+0.402	+3.047*	+4.523	+2.759*	-0.467	-0.512	+0.468	+4.721*					
10	+0.241	+2.903	+3.854	+2.877	-0.489	-0.741	+0.433	+4.236*	+ 0.334				
11	-6.514	-1.859	+0.357	-3.434	-7.364	-8.582	-7.259	+1.328	-10.087	+3.642			
12	+1.007	+3.444**	+4.842	+3.139*	-0.040	+0.014	0.935*	+5.062*	+ 1.172	+0.725	+4.824**		
13	-2.042*	+1.320	+2.058	+0.934	-2.772*	-3.411*	-2.121	+2.976	- 3.113	-1.218	+3.396	-3.439*	

*, ** Degree of significance at 5 and 1% level successively which are different from 0, between any combination of two laboratories.

The results obtained in laboratories (1, 2, 3, 4, 6, 7 and 12) are highly correlated with each others with r = +0.98 to 0.99, and no significance differences among their results were observed based on the T-probability tests (see Table 3). In Figure (1) which relate results obtained in lab (12) and (2) would show very clearly that, although the set of results are highly correlated with r = +0.972, it is obvious that the slope is different from one, leading to a systematic decrease in the available P estimated for soils with high P values; and to the contrary higher values were obtained by lab (2) for soils with low P test values. That would illustrate the weakness in considering the coefficients coefficients alone as the only criteria used to compare two sets of laboratory results.

In addition, the laboratories (9) and (13) had a very high correlation coefficients between results with that obtained by the seven above mentioned laboratories with (r = 0.90 to r = 0.98). But their overall means have been significantly higher by more than 20 to 25%; and the T-probability tests among their results with others, were proved to be highly significantly different (at 1% leve).

In addition, the slopes for regression equations obtained between Lab (9) and any other lab, have been very different from (1). Figure (2) illustrates the relationships between results obtained in lab (9) and (2). There is a significant trend to obtain higher results for available P in Lab (9) as compared to Lab (2); however there are highly correlated (r = +0.971).

Similarly the comparison between two sets of results obtained by any two laboratories could be done accordingly.

Finally, the overall results obtained in laboratory (11) are less correlated with results obtained by other laboratories, and largely due to the analyses of samples 5 and 8 which resulted in very different values from that obtained in other laboratories of the region.

6. Discussions

Although the $NaHCO_3$ method for extracting the available Phosphorus in soils has been described fully by the authors (4), some variations have been always introduced by individual laboratories, to suit their own working conditions or which have given better correlations with phosphorus responses on local soils.

Although there is no need to emphasize that all laboratories should follow closely the procedure described by the authors, it is however important to consider the effect of various factors which might have some significant effect on the performance and results of their analyses.

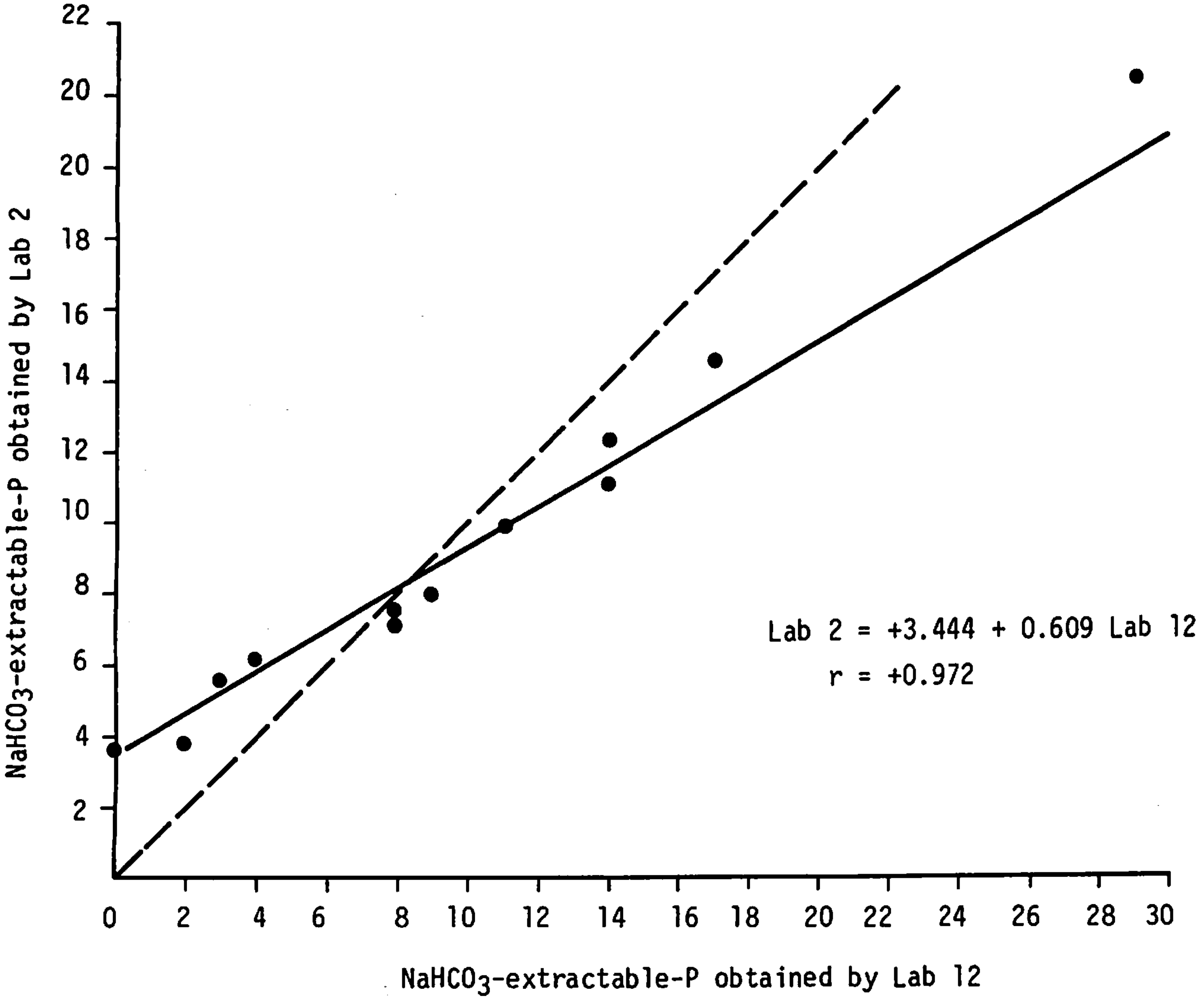

Fig. (1) The relationship between the available-P for 12 soil samples obtained by two laboratories of the region.

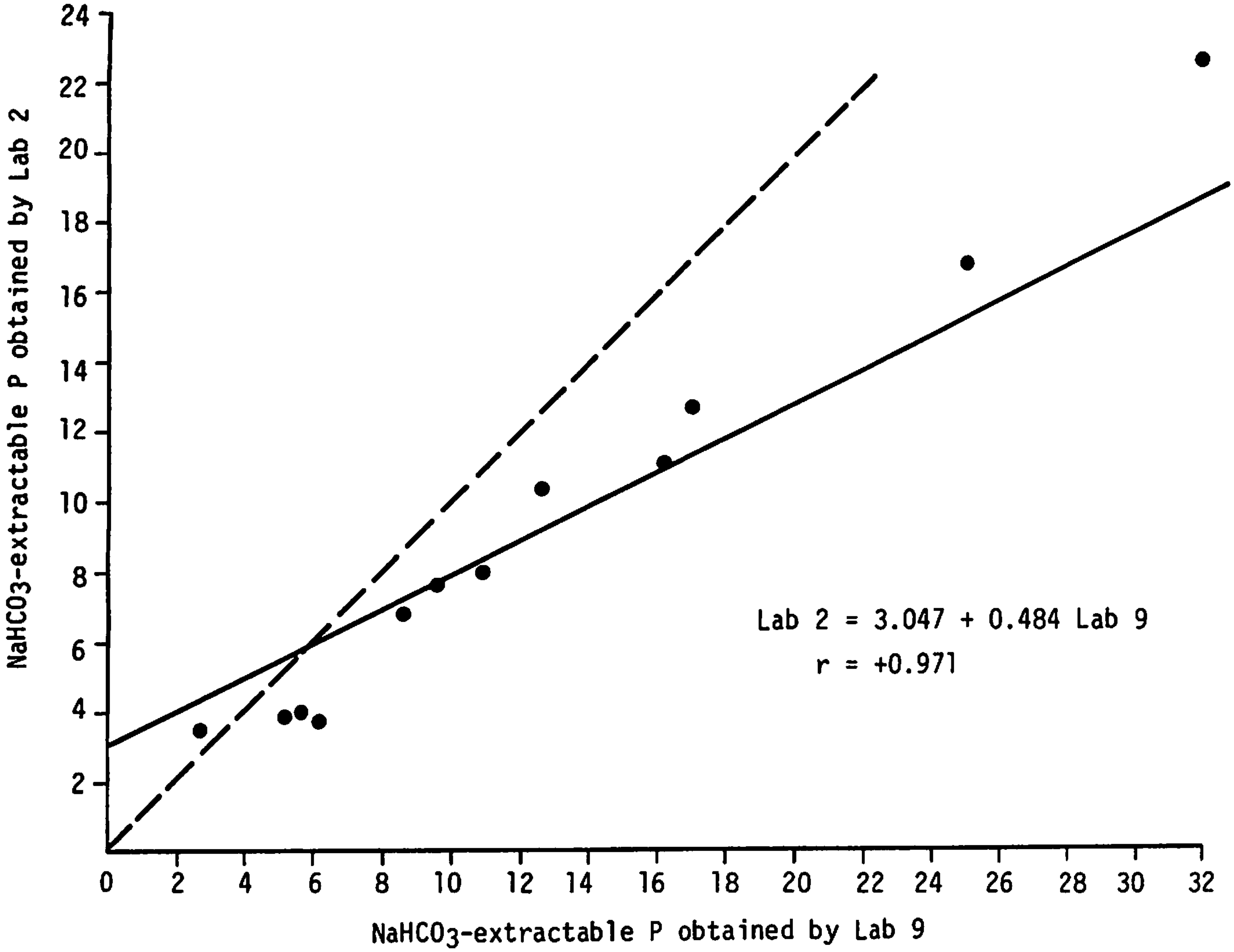

Fig. (2) The relationship between the available-P for 12 soil samples obtained by two laboratories of the region.

6.1. Soil Preparation

Most laboratories in the region, used 2 mm air-dried soils for P analysis; except one laboratory which dried its samples at 100 °C and for 18 hours. No laboratory in the region used field-moist soils for P determinations. Field-moist and air-dried samples gave quite similar results as tested in the ICARDA Soil Lab.

The time elapsed between the soil drying and preparation and the chemical analysis of available P ranged between 2 and more than 30 days for various laboratories in the region. Ageing of soil samples could have some significant effect.

6.2. The $NaHCO_3$-Extraction Solution

Although 0.5 Molar solution adjusted to pH = 8.5 is required, there are several laboratories in the region which still do adjust the pH of the extraction solution; it is obvious that when the solution is exposed to the air or has been prepared for some time, the pH will change and consequently its extraction power of the soil phosphate will be altered, usually leading to higher values of extractable-P.

Olsen et al assumed that the level of extractable-P in relation to crop response would be more uniform at pH = 8.5 for the group of soils tested.

6.3. The Soil to Extracting Solution Ratio

Most laboratories in the region used a soil/solution ratio of 1/20 as suggested by the original procedure except one laboratory which used 1/10 ratio.

6.4. Method of Extraction

Different types of linear or orbital shakers with different rates of shaking are used in the laboratories of the region. For linear shakers the rate of shaking ranged between 90 and 233 cycles per minute and for orbital shaker, 30 to 100 revolutions per minute. Olsen et al (4) has advised, in their original procedure, a constant rate of shaking during P extraction to eliminate the differential effects of shaking frequencies and time.

Throughout the laboratories of the region, it has been adopted a shaking time of 30 minutes.

6.5. Use of Activated Charcoal

It is suggested by the original procedure to add activated charcoal to the soil-extracting solution mixture to adsorb any organic coloured material which could interfere with the determination of available P. Most soil laboratories in the region do not use activated charcoal except for very coloured solution extracts. However, some laboratories in the region still use activated charcoal either from well-known companies, or brought from local markets and washed with water and $NaHCO_3$.

It has been found by ICARDA Soil Lab, that washing local activated charcoal to remove traces of soluble P is a time consuming process and need a large volume of $NaHCO_3$ solution and distilled water. In addition, the activated carbon (Darco-G-60) suggested by Olsen et al, releases phosphorus when treated with $NaHCO_3$, and requires a pretreatment with the $NaHCO_3$ solution by leaching with distilled water and drying prior to its use.

Consequently, it was found that $NaHCO_3$-extractable P for arid or semi-arid soils, as is the case in most rainfed areas of the ICARDA region, could be conducted without the addition of carbon black; and differences in results obtained with or without the addition of carbon black had been little affected. However it migh be safer and worthwhile to add carbon black for soils rich in organic matter, where soil extracts with $NaHCO_3$ are dark coloured.

6.6. Temperature of Extracting Solution

The data obtained by Olsen et al (4), were obtained at 25 + 1 °C. It was found that extractable-P increased approximately 0.43 parts per million, for each degree rise between 20 and 30 °C for soils between 5 and 40 ppm of P.

Many of the soil laboratories in the region are not temperature-controlled, and temperatures in the lab could vary between 10 °C (winter) and more than 35 °C (summer). Consequently, the extraction capacity of the $NaHCO_3$ solution will change with time of the year; and results of available P in soils would depend on the season and time of the year. In addition, it was found that the molybdenum blue colour develops at a rate which depends on the degree of temperature and the $SnCl_2$ concentration. Thus the analysis of the extract solution should be carried out under constant temperature conditions whenever possible.

Along this line, it is suggested to run the extraction of available P under a more or less uniform use laboratory temperature of 25 + 2 °C. But if this is not possible, at least adjust the temperature and the pH of the $NaHCO_3$ to the desired temperature prior to its immediate addition to the soil samples. Others (6) have suggested the need to study the effect of change of temperature on extraction and apply a correlation factor or factors.

6.7. Type of Filtration

Most soil laboratories in the region extracted the soil extracts by gravity, using very many types of filter papers ranging between qualitative to very fine pore filter papers. The type of filtration could have a double effect. On one hand, the degree of clarity of solution would depend on the type of filters, but on the other hand, the contact time between soil and extracting solution would depend on the type of filter. It takes a longer time to obtain a clear soil extracts when a very fine pore filter is used as compared to qualitative filters. Consequently, the time of contact between soil and extracting solution is longer with fine pore filter and possible higher P values could be obtained. It would be advisable to use large filters to hold an ample amount of the soil extract needed for analysis and transfer to the filter the supernatant liquid only.

The Soil Lab at ICARDA obtained more reproductive results by using the fine pore filters and during filtrating using vacuum pump, to control the time of contact between soil and extracting solution.

In addition, one laboratory in the region found that filter papers could be a second source of P contamination; and all filter papers used are usually double washed with acid prior to filtration of the $NaHCO_3$ extract.

6.8.1. Automation and simultaneous determination of N, p and K in $NaHCO_3$-extracts of soils

In recent years, many soil laboratories in the region have automated with success the determination of P in the $NaHCO_3$-extract using an auto-analyzer. Sometimes NO_2/NO_3 as well K are analyzed simultaneously.

6.8.2. Modification of the Olsen Method

In one laboratory of the region, the Olsen-Dabin procedure is used where the extracting solution of P is a mixture of 0.5 M-$NaHCO_3$ and 0.5 M-NH_4F. The P determinations have been determined by both the original Olsen method and that of modified-Dabin. A linear regression was found between both results and the regression equations was of the kind:

$$P_{Olsen} = 0.367 + 0.2083\ P_{Dabin}$$

with R = +0.77.

The Olsen modified-Dabin procedure could be used when values of available P as determined by this method are calibrated by field experiments to study response to fertilizer application.

6.9. Type of Colorimetric Methods for Determination of P

Several procedures have been used to determine the soluble P in the $NaHCO_3$-extract. The molybdenum-blue method is still the most widespread either using $SnCl_2$ as reducing agent; or an acidic molybdate solution containing Ascorbic Acid. No apparent differences in results obtained of P determination in the laboratories of the region occurred due to differences in their colorimetric determination of P. A more detailed study for the effect of the reducing agent on final P results are underway at the ICARDA Soil Lab.

6.10. Miscellaneous Factors

- Spectrophotometer glass tubes used:

The optical density for the same blue-coloured solutions, as measured on the spectrophotometer was found quite different when the glass tube is changed. Apparently glass tubes used for spectrophotometeric measurements, although they look alike, have different rate of light transmittancy. Consequently, it was found advisable to use the same glass tube for measurements of the optical density of various coloured solutions including the P standard solutions.

Table (7) Some details of the NaHCO$_3$-P procedure as used by various laboratories in the region.

Lab No.	Soil Drying	Lag Period	NaHCO$_3$ purity	Molarity of NaHCO$_3$	Adjust + of pH of ext.soil	Ratio soil/ solution	Shaker type- -speed	Type Filter	Activated Charcoal	Test of P in Charcoal	Colorimetric Method
1	Air-dry	15 days									
2	Air-dry	10 days	Merck 99.5	0.5	8.5	5/100	Linear 233/CPM	S S/SA710	No	-	Molydb. blue
3	Air-dry	5 days	M&B 99.5%	0.5	8.5	5/100	Linear 145/CPM	No. 40	Yes	Yes	Ascorbic Acid
4	Air-dry	-	M&B limt. 99%	0.5	8.5	5/100	Linear about 90 CPM	Edrol No. 42 slow	No	-	Molybd. Blue
5	Air-dry	7 days	BDH (Analar) >99.8%	0.5	8.5	10/100	Orbital 100 rpm	Whatman No. 42	No	-	Ascorbic Acid
6	Air-dry	-	Chemapol purity?!	0.5	8.5	5/100	Linear 140 CPM	Whatman 40 or 42	Yes	Yes	Heteromolybd. Blue to 880
7	Air-dry	-	-	0.5	8.5	5/100	Linear 140 CPM	2 filters Whatman 40	No	—	Molybd. Blue
8	Air-dry	3-10	Backer 99.7%	0.5	8.5	5/100	Linear 200 CPM	Whatman No. 5	Yes	No	Molydb. Blue
9	Air-dry	3-10	Merck Row Analyzer	0.5	8.5	5/100	Orbital 35 rpm	SvS 3001/	Yes	Yes	Molybd. Blue
10	Air-dry	> 15	Merck 99.5%	0.5	8.5	5/100	Orbital 60 rpm	Macherey Nagel MN713qual.	Yes for dark samples		
11	Air-dry	1	Merck Analar	0.5M	8.2-8.5	5/100	Linear 200 CPM	Whatman No. 42	Yes	Yes	Molybd. Blue
12	Air-dry	15-30	Fluka	0.5	8.5	5/100	Orbital 30 rpm	Normal filter	Yes	Yes	Ascorbic Acid
13	Oven 100°C for 18 hours	2	Labosi	0.5	8.5	5/100	Linear 110 CPM	Whatman No. 40		-	Molybd. Blue

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