

Statistical Evaluation of the Analytical Techniques Employed in the Purdue Soil Analysis Laboratory¹

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Considerable research has been conducted to determine the best quantitative methods for determining the nutrient elements available in soils for plant growth. To the authors' knowledge, no work has been reported, in which the routine analytical techniques were investigated for a possible source of variation.

Even when the most accurate chemical methods are used to determine the nutrient elements, variations between duplicate analyses exist. Therefore, this work was undertaken to determine the source of variation in pH, phosphorus and potassium determinations due to the analytical techniques used by this laboratory in the routine analysis of soil samples.

This work does not attempt to explain the factors underlying the variations.

The results of triplicate analyses of 4 randomly selected soil samples are presented in table 1, to illustrate the variations which have been found to exist in the laboratory routine.

Table 1. Results of Triplicate Analysis of 4 Randomly Selected Soil Samples

Sample No.	pH			lbs. P ₂ O ₅ /acre			lbs. K ₂ O/acre		
33,356	5.9	5.8	5.9	420	400	400	145	125	125
33,361	5.9	6.1	5.8	280	260	240	125	145	125
33,371	7.2	7.0	7.1	850	900	900	90	125	110
33,381	5.6	5.6	5.8	220	200	200	70	70	90

Even though there are considerable variations in certain analyses, these variations are seldom of the magnitude which would influence the type and amount of fertilizer to be applied based on these soil test results. Yet these variations are present and this source should be located so that closer duplication can be achieved.

Experiment 1

Soil samples are received at the laboratory in various physical conditions. They may be received wet or dry, pulverized or hard and cloddy. The physical conditions in which they are received influence some of these variations.

After the soil sample has been received at the laboratory, it is dried, crushed in a double roller type crusher and sieved through an 8 mesh sieve. All soil passing through the sieve is retained and portions are analyzed for phosphorus, potassium and pH determination. All soil

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remaining on the sieve is discarded. The percentage of soil sample passing through the 8 mesh sieve ranged from 35 to 100 percent with the average being near 50% of the original sample.

Therefore, this experiment was conducted to determine if the coarse discard fraction differed from the finer retained fraction.

Twenty-eight consecutive soil samples were crushed, both the fine fraction passing the 8 mesh sieve and the coarse fraction retained on the sieve were retained and further crushed separately to pass a 20 mesh sieve. To determine the variations in pH between the retained and the discarded fraction, a 1:1 soil water suspension and a glass electrode was used. Each analysis was replicated five times.

A significant difference² between the fractions was found on 5 of the 28 soils, and a highly significant difference³ on 4 of the 28 samples. These differences between fractions ranged from 0 to .4 pH units, with the discard fraction always having either an equal pH or a higher pH than the retained fraction.

Phosphorus content was determined colorimetrically (1) on each of the fractions of the 28 soil samples. Highly significant differences between the fractions were found in 14 and significant differences in 2 of the 28 samples. The discarded fraction had a higher phosphorus content in 9 of the 16 samples which showed significant differences between fractions.

Of the 28 soil samples, four had highly significant differences between fractions in the potassium content (1) and 2 differed significantly. In 5 of the 6 soils differing significantly in potassium, the higher potassium content was associated with the discarded fraction.

This experiment indicates rather conclusively that the presently discarded fraction is of significant importance, and the entire soil sample should be crushed and analyzed, especially since the discarded fraction frequently represents 50 percent of the original sample.

Experiment 2

After the soil sample is crushed, the fraction passing through the 8 mesh sieve is analyzed for phosphorus and potassium. To remove these nutrients from the soil, a 5 ml. volume measuring spoon of soil is added to an extraction bottle and the extracting reagent (1) is added simultaneously to a tray containing 12 bottles with their samples. These are then shaken for 2 minutes, filtered and each determination is made on a 2 ml. aliquot of the soil extract.

Some variations in the phosphorus and potassium content are probably due to variations in taking the 5 ml. volume of soil for extraction. The variation between technicians was investigated by sieving a Chalmers silt loam soil through a 60 mesh sieve and having each technician sample the soil 12 times in each technician's normal procedure. Phosphorus and potassium were then determined in the routine procedure to determine the amount of variation. A significant difference was found to exist between technicians.

2. Significant difference signifies significance at the 5% level.

3. Highly significant difference signifies significance at the 1% level.

Experiment 3

In an attempt to learn why the difference between technicians found in experiment 2 might exist, this experiment was initiated to investigate sampling techniques of the 5 ml. soil portion. This experiment was carried out by only one technician to remove variation between technicians as a variable. The following sampling techniques were used:

1. Technique normally employed by the technician.
2. Dip the sampling spoon into the soil, and level off the excess soil with a spatula.
3. Dip the sampling spoon into the soil, tap the handle of the spoon once on the handle with the edge of the spatula to compact the soil and then level-off the excess soil with the spatula.
4. Same technique as 3, except handle was tapped three times instead of once.

Each sampling technique was replicated 12 times on the soil used in experiment 2. Phosphorus was determined by the routine procedure to determine the variation. A highly significant difference due to sampling methods was found. The average phosphorus content, ranged between the highest and lowest values and the least significant differences at 5 percent, are shown in Table 2 for each technique.

Table 2. Average Phosphorus Content, Range and LSD at 5% for Sampling Techniques

	1	Sampling Technique		4
		2	3	
Ave. P_2O_5 /acre	274.2	281.0	286.0	297.6
Range-lbs./acre	40	48	36	32
LSD at 5% = 8.92 lbs. P_2O_5 /acre				

Since the range for technique 4 was the least, it was used in the remainder of the study.

Experiment 4

The particle size of the soil sample might influence the amount of soil which a sampling spoon might hold, which in turn might influence the phosphorus or potassium content.

To study this problem, a Chalmers silt loam soil was dry fractionated into 7 size classes: 8-20 mesh, 20-40, 40-60, 60-80, 80-100, 100-200, and finer than 200 mesh. The Chalmers soil was selected because it is a prairie soil, high in organic matter and therefore has very stable dry aggregates, which do not crush easily. A 5 ml. volume sample was taken from each fraction, weighed and analyzed for phosphorus and potassium. These were replicated 5 times.

The average sample weight, average phosphorus and potassium content are presented in Table 3. Differences in weight, phosphorus, and potassium content were all highly significant.

Table 3. Average Weight, Phosphorus and Potassium Content for Each Size Fraction

Weight of Sample	Fraction Size Classes (sieve mesh sizes)						Less than 200	5% LSD
	8-20	20-40	40-60	60-80	80-100	100 to 200		
grams/5 ml.	4.1758	4.2972	4.4614	4.5049	4.4301	4.4199	4.7835	.0717
#P ₂ O ₅ /acre	212.8	237.4	240.4	259.8	264.6	289.8	295.6	18.00
#K ₂ O/acre	119.4	132.2	136.6	132.4	140.6	146.2	145.0	5.57

Table 3 shows that the average weight, phosphorus and potassium content were dependent on particle size.

To determine if the phosphorus and potassium content were associated with the change in sample weight, or if a real difference due to fraction existed, all phosphorus and potassium contents were adjusted to a 5 gram sample weight.

The phosphorus and potassium contents when adjusted to 5 gram sample weights are shown in table 4.

Table 4. Average Phosphorus and Potassium Content When Values Were Adjusted to 5 Gram Sample for Each Fraction

	Fraction Size Classes (sieve mesh sizes)					100 to 200	Less than 200	5% LSD
	8-20	20-40	40-60	60-80	80-100			
#P ₂ O ₅ /acre	254.4	276.4	269.2	288.4	298.6	327.8	309.8	21.7
#K ₂ O/acre	143.0	154.6	153.2	146.8	158.8	165.4	145.6	5.3

The phosphorus and potassium contents were highly significant when they were adjusted to a 5 gram sample.

Since both phosphorus and potassium content appear to be dependent on the fraction size, even though the sample weight is equal, regressions and correlations were calculated for both phosphorus and potassium on fraction size.

The phosphorus content followed a linear regression on fraction size, with $b = .327$ and had a correlation coefficient, $r = .873$. The regression and correlation were both significant. Potassium content and fraction size had a correlation coefficient, $r = .217$, which is not significant.

The data in table 4 shows that the fraction size which is sampled was very important in both phosphorus and potassium analysis. In coarse crushed samples, many fine particles could settle to the bottom of the sample box. This would leave the coarse fraction near the surface. These would have a greater chance of being included in the volume of soil being analyzed, and might result in large variations in the test results, especially phosphorus.

Experiment 5

After the 5 ml. volume of soil has been sampled, extracting reagent is added, shaken and filtered. A 2 ml. aliquot of the resulting soil extract

is pipetted into test tubes for phosphorus and potassium determination. Since an automatic pipette is used to remove the aliquot, it is not rinsed between samples. Therefore, this experiment was conducted to determine the amount of phosphorus carried over from one sample to the next. The phosphorus carry-over was determined by rinsing the pipette with 2 ml. of distilled water and determining the phosphorus in the distilled water. All phosphorus found in the distilled water was being carried over in the pipette to the next sample.

The amount of phosphorus carried-over in the pipette when pipetting a sample with a known phosphorus concentration is shown in figure 1. This figure shows the amount of carry-over to be a significant source of variation in phosphorus analyses.

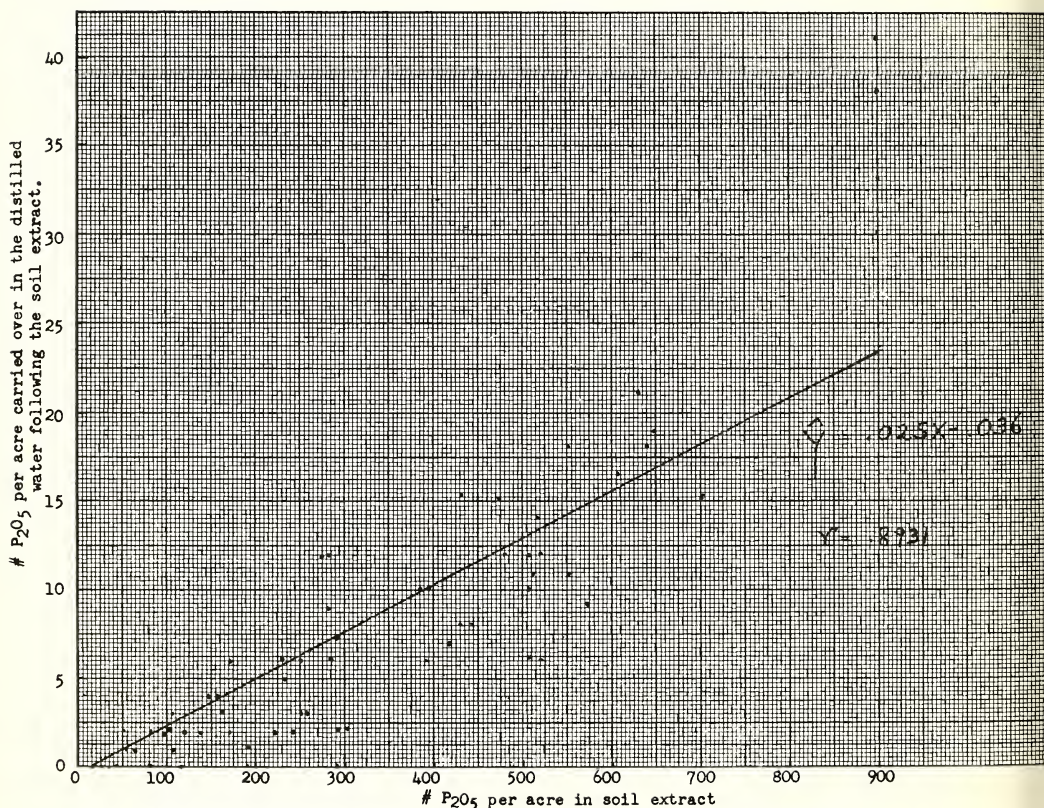


Fig. 1. Pounds per acre of P₂O₅ carried over in the transfer pipette when pipetting a soil sample extract of known P₂O₅ content.

Experiment 6

As table 1 shows, the pH values of the soils varied only slightly between duplications. It was thought that since the water is added to the soil and is allowed to stand for a period of time before making the determinations, this might be a source of variation.

The pH was determined with a glass electrode on several soils at various intervals between 5 minutes to 8 hours after adding the water to the soil. No difference in pH was found due to allowing the water to be in contact with the soil for as long as 8 hours.

Summary

This study has indicated that numerous factors present in the routine now used are sources of variation between duplicates of the same soil sample. Numerous additional factors remain to be investigated. When all the factors causing variation have been studied and corrected, closer duplication should result.

Literature Cited

1. SPAIN, J. M. and WHITE, J. L. Procedure for the Determination of Phosphorus and Potassium Soil Testing. Unpublished date, Purdue University. March 1960.