

## PREPARATION OF A SOIL REFERENCE SAMPLE AND INITIAL RESULTS ON LABORATORY QUALITY IMPROVEMENT

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### Abstract

The mineral constituents of soils were analysed for their agricultural potential by the Soils and Fertilizers Research Institute (SFRI). Until recently the laboratories had no reference materials to validate the tests. This deficit was overcome by creating a reference soil sample. The first step was to collect about 100 kg of surface soil from an unfertilized area adjacent to a rice paddy in the Red River Delta. The soil was air dried, and roots and pebbles were removed before grinding, mixed thoroughly and divided into two 50 kg portions. One portion was sent to Australia where it was circulated as a proficiency sample to 39 agricultural laboratories as part of a certification procedure run by the Australasian Soil and Plant Analysis Council (ASPAC). The inter-laboratory means and coefficients of variation for a wide range of tests were returned to SFRI where they are being used in method validation and quality control. The retained portion was divided into 100 jars each of ~500 g and the nitrogen content (semi-micro Kjeldahl) was used to assess the homogeneity of the material within jars (4 replicates) and between jars using the within-jar means. The mean N content for all jars was 0.067% and the CV was  $\pm 6.22\%$ , and the corresponding ASPAC statistics were 0.066% and  $\pm 12.29\%$ . Other parameters were analyzed and compared with the ASPAC inter-laboratory proficiency results as a part of method validation. For example, there respectively means for SFRI and for ASPAC (in parentheses) were: for the pH of 1 g/5 mL suspension in water 7.96 (8.04); for organic carbon, 0.46% (0.44%); for total-P as  $P_2O_5$ , 0.09% (0.08%); and for available K, 4.24 mg/100 g as  $K_2O$  (4.7 mg/100 g). However, the agreement for other parameters, such as cation exchange capacity, was poor: 13.2 cmol/kg (10 cmol/kg) and the causes are being investigated. Finally, it is planned to develop additional reference materials to complement the Hanoi reference soil. These materials will strengthen the quality of testing at SFRI and provide objective measures of that quality into the future.

**Keywords:** ASPAC, homogeneity, reference sample, SFRI

### INTRODUCTION

The Soils and Fertilizers Research Institute (SFRI) is the peak body for soil classification and agronomic testing in Vietnam. The SFRI consequently operates laboratories in a number of locations, with the central facility being located in Hanoi. The organization has developed a comprehensive suite of test methods that are officially recognized (SFRI, 1998). Precision and accuracy are important requirements of chemical tests and can be achieved by skilled analysts using validated test methods and replication to reduce random errors. The validation of test methods includes measures of precision and accuracy, and these measures rely on reference materials, i.e. on homogeneous materials of known composition.

The International Organization of Legal Metrology defines reference samples as materials or substances for which one or more characteristic(s) is homogenous and the material is made to standardize equipment, evaluate a method or to define the composition of materials. The standard values of reference samples are ideally created following a technical protocol with

perfect accuracy and reproducibility. Under some conditions the composition is certified and approved by official agencies.

Agronomic properties of soils are operationally defined and as such are unsuitable for certification. Nonetheless, reference materials of a suitable standard can be prepared and used to validate operationally defined test methods. This is the process we describe here for a reference soil sample. To our knowledge this is the first example of a reference soil sample being developed and used in Vietnam.

### MATERIALS AND METHODS

#### Materials

For effective long-term use, the key requirements for a soil reference sample are homogeneity and a large quantity (about 200 kg). We selected an alluvial soil from the Red River delta that had been fallowed for a long time. This soil was taken from Tho An commune, Dan Phuong district, Hanoi capital.

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## Methods

### *The soil*

*Sample collection:* Followed by TCVN 7538-1-2006 (Vietnam Standard No 7538-1-2006). The soil surface was cleared of contaminants (gravels, roots, fallen tree leaves etc.), and 200 kg of surface soil was removed. The soil was put into the clean boxes and transported to the laboratory.

### *Sample treating methods:*

Step 1 - Air dry, pass through a 10 mm sieve. Sort out all extraneous matter (rock fragments, gravels and plant debris, because the latter contain much more organic carbon and nitrogen than the soil matrix).

Step 2 - Evenly mix the sample by making a cone shape and dividing into 4 parts. This was repeated many times until the sample appeared homogenous. Then it was once again shaped as a cone and divided into two parts. One part (50 kg) was sent to Australia and the rest was kept in SFRI to be treated in the 3<sup>rd</sup> step.

Step 3 - Divided into jars: grind the soil to pass through a 0.5 mm sieve, spread it in a thin layer and dry at 60 °C. Continue mixing by coning and quartering several times, then divide the sample, weigh and put into 100 jars, each jar contain 500 g of soil. Label the jars by SFRI - 2014, Soil, No 01 to No 100.

*Storing method:* A chest freezer was purchased to store reference samples at -20°C to maintain their integrity. Dry at 40°C and mix before using. Sample uses a long, narrow tool to avoid over sampling the surface layer.

Thirty nine ASPAC laboratories participated in analyzing total N on the reference soil. During ASPAC inter-laboratory proficiency testing this material was identified as ASS1506-2 Hanoi Soil.

### *Homogeneity testing*

Nitrogen (N) is poorly distributed in soil, being present in inorganic forms and biotic detritus. In addition, the subsample size for N analysis is relatively small. Consequently, N-homogeneity is a rigorous test of homogeneity for soil reference materials. However, before the SFRI semi-micro Kjeldahl test could be used to estimate homogeneity, the test had to be validated.

To acquire precise analysis, the instruments and methods should be tested and calibrated before use. The balances were tested using standard masses, and volumetric glassware was tested by weighing the water contained or dispensed (1 mL of water considered as exactly 1 g). At SFRI a semi-micro Kjeldahl digestion followed by a semi-automated ammonia distillation (model UDK 132 - Italia) are used to

determine N. The method was tested by measuring N recovery from a highly pure organic amine, TRIS (hydroxymethylaminomethane), with the formula  $(\text{HOCH}_2)_3\text{CNH}_2$  (Gold Biotechnology, USA). We refer to this compound as TRIS and its theoretical N content is 11.57%.

*Homogeneity assessment method:* Test the homogeneity between the jars by analyzing four replicates/jar for Kjeldahl N contents, following TCVN 6498:1999 (for soil sample). Total 100 jars, i.e. 400 analyses were conducted.

### *Data analysis*

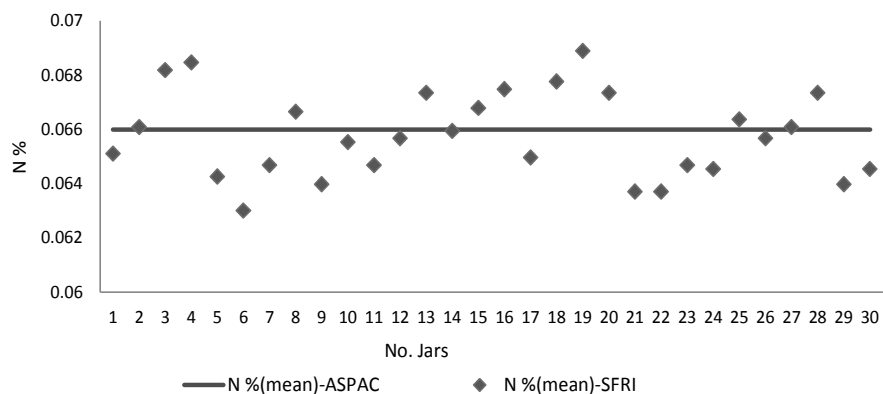
The mean and coefficient of variation (CV) of 4 replicates/jar was calculated for each of the 100 jars, i.e. the within jar statistics. The within jar means were then used to calculate a between jar mean and CV. For comparison with the ASPAC results, we analyzed 20 replicates from one jar for several parameters.

## RESULTS AND DISCUSSION

### *Homogeneity*

The instrument calibrations were all accurate; however, the recovery of TRIS for the method of sample digestion used at SFRI (solutes 2 g of mixture Se: K<sub>2</sub>SO<sub>4</sub> (with the ratio of 1:100) as catalyst in 5 ml concentrated H<sub>2</sub>SO<sub>4</sub>; digest for 3 hours at 300 °C), was only 91.66%. Increase the used amount of H<sub>2</sub>SO<sub>4</sub> to 10 ml and of the catalyst mixture to 3 g, also change the ration of the mixture Se: K<sub>2</sub>SO<sub>4</sub> to 1:300; and increasing the digestion temperature to 400 °C gave a recovery of 99.31% in 1 hour. This result validated the semi-micro Kjeldahl method and showed that it was suitable for use in homogeneity testing.

The within jar coefficient of variation (CV) varied widely: 30 jars had a CV less than 1%, 51 jars had a CV of 1–3% and 19 jars had a CV over 3%. These CVs did not meet the target of less than 1%. The mean of N content for the 100 jars was 0.067%, with a CV of 6.22%. However, with better mixing and sampling techniques, both the within and between jar CVs decreased, as shown for the between jar data for jars 1–30 (Figure 1), and for the replicated results for seven other tests (Table 2). Consequently it was inferred that the reference material was acceptably homogenous for use. Nonetheless, for some jars the means fell into the tails of the distribution. We propose to mix the contents of one low and one high jar, replace the mixed material into the jars and retest for N to determine whether the contents should be retained or disposed of.



**Figure 1.** Within jar mean N content for 30 jars of SFRI – 2014, Soil, No 01. Each data point is the mean of 4 replicates/jar for the 30 first jars measured at SRFI. The line represents the ASPAC proficiency testing mean for 15 laboratories that used a Kjeldahl N method for the material identified as ASS1506-2 Hanoi Soil.

The results in Figure 1 also show that the SFRI and ASPAC N means do not differ within experimental uncertainty. This confirms the usefulness of TRIS to validate the Kjeldahl method for soil analysis.

#### Comparing some SFRI and ASPAC results

The reliability of some methods has been tested at SFRI by analyzing 20 replicates side-by-side. The parameters tested include:  $\text{pH}_{\text{H}_2\text{O}}$ , organic carbon (OC), total N, total and available phosphorus (P), total

and available potassium (K), and cation exchange capacity (CEC). The results generally compare well with ASPAC values (Table 3). However, with total K content, the results are very different between SFRI and ASPAC. Since the ASPAC results were from only 2 labs, the value here is not strong; nonetheless the data deserve further investigation. The relatively small CVs (Table 2) support the earlier statement that N is a robust test of homogeneity.

**Table 2.** Comparing analysis results between SFRI and ASPAC

Parameters	Indexes	SFRI	ASPAC Median (n labs)	Comparison with ASPAC
$\text{pH}_{\text{H}_2\text{O}}$	No. Reps	10	8.04 (37)	Acceptable
	Mean	7.962		
	SD	0.017		
	CV%	0.212		
OC (%)	No. Reps	20	0.44% (33)	Acceptable
	Mean	0.459		
	SD	0.010		
	CV%	2.183		
Total N (%)	No. Reps	20	0.064% (39)	Acceptable
	Mean	0.064		
	SD	0.003		
	CV%	4.312		
Total $\text{P}_2\text{O}_5$ (%)	No. Reps	20	P 0.036% (22) $\text{P}_2\text{O}_5$ 0.08%	Acceptable
	Mean	0.090		
	SD	0.002		
	CV%	2.073		
Bray-2 P (mg/kg)	No. Reps	20	No corresponding data but Bray-1 is 1.4 mg P/kg (8) or 3.2 mg $\text{P}_2\text{O}_5$ /kg	Acceptable
	Mean	33.93		
	SD	0.017		
	CV%	0.494		

**Table 2.** Comparing analysis results between SFRI and ASPAC

Parameters	Indexes	SFRI	ASPAC Median (n labs)	Comparison with ASPAC
Total K - K <sub>2</sub> O %	No. Repts	20	K 0.14% (2) K <sub>2</sub> O 0.17%	Neither acceptable Note that for ASPAC n = 2
	Mean	0.638		
	SD	0.006		
	CV%	0.895		
Available K as K <sub>2</sub> O (mg/100 g)	No. Repts	18	Exch. K (cmol/kg) 0.10 (33) or 4.7 mg K <sub>2</sub> O/100 g	Acceptable
	Mean	4.243		
	SD	0.040		
	CV%	0.949		
CEC (meq/100 g or cmol/kg)	No. Repts	20	No corresponding data but Σ exacts ~10 cmol/kg (25)	No direct comparison The values are similar
	Mean	13.160		
	Standard deviation	0.186		
	CV%	1.415		

### Using the reference samples

From October 2015 to June 2016, the analysis group continued using the reference samples together with other samples for quality control in daily works. Some of the results are shown in Table 3.

Generally, the difference in results of one sample for one parameter at difference time of analyzing may reveal some problems that need to be fixed in analysis techniques of technicians.

**Table 3.** Results of reference sample using

Indexes	pH <sub>KCl</sub>	Total (%)				Avail. P (mg/100g)	Avail. P (mg/100g)	CEC in soil (meq/100g)
		OC	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	
n	20	22	31	19	15	14	15	31
min	6.90	0.360	0.050	0.088	0.770	7.760	2.590	5.900
max	7.21	0.520	0.076	0.122	1.090	9.759	2.820	11.740
Mean	6.85	0.439	0.064	0.098	1.024	8.279	2.723	9.306
median	7.09	0.438	0.064	0.096	1.040	8.073	2.710	9.700
SD	0.102	0.033	0.005	0.009	0.073	0.593	0.064	1.416
CV%	1.49	7.49	7.72	9.29	7.14	7.16	2.35	15.2

## CONCLUSION AND RECOMMENDATION

### Conclusion

We created, homogenized and characterized a large quantity of a soil reference sample. Nitrogen appears to be a robust index of homogeneity and TRIS a suitable primary standard for Kjeldahl digestion. ASPAC reference values can be used as targets to validate test methods. Lastly, storage of the material at -20 °C should maintain its integrity indefinitely, and allow it to be used for a considerable period for quality control and further test validation.

### Recommendation

Test whether the utility of the material can be extended by blending the contents of pairs of jars that fall in the opposite tails of the between container distribution. The idea is to mix the contents of one low and one high container, replace the mixed material into the jars. This will be done until both tails have been blended. The blended material will be retested for N. If the composition falls within the main body of the distribution the jars will be retained, if not they will be disposed of.

Use the reference material to validate other soil tests, such as heavy metals, and for quality control. For this purpose, an acceptable range will be set as control limits, i.e. to trigger investigation and correction of the cause(s) of unacceptable bias and variability.

Lastly, prepare and characterize a more acidic soil with a higher concentration of available P, and a plant reference material, to extend the scope of test validation and quality control.

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