International Rice Research Institute
Grain Quality and Nutrition Center
Analytical Service Laboratory

Standard Operating Procedure

PLANT AND SOIL SAMPLE PREPARATION PROCEDURES
QM-AD036-MP24

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PLANT AND SOIL SAMPLE PREPARATION PROCEDURES

1.0 Scope and Application

This laboratory operating procedure covers both plant and soil sample preparation procedures for samples that are to be submitted to ASL for analysis.

2.0 Summary

The procedure includes instructions on how to prepare plant and soil samples free from any kind of contamination that may affect the composition or result of analysis, as well as the proper way of drying, grinding, and storing the samples to preserve their physical and chemical integrity.

3.0 Interferences and Comments

3.1 On decontamination procedure for plant materials

3.1.1 When proper sampling techniques have been utilized, decontamination should be minimized.

3.1.2 Decontamination is generally not necessary where tissue has been exposed to frequent rainfall and/or not exposed to nutrient or fungicidal sprays (Jones et al., 1991). Small plants that have been splattered with soil are the exception to this rule.

3.1.3 Excessive washing is likely worse than no decontamination since soluble elements, including B, K, and N, are likely to leach from the tissue.

3.1.4 Samples should be dipped quickly in the wash and rinse solutions. Sonneveld and van Dijk (1982) recommended a time of 15 seconds.

3.1.5 Relatively high concentrations of Al (>100 mg kg⁻¹), Fe (>100 mg kg⁻¹), and Si (>1.0%) are strong indicators of contamination (Jones et al., 1991). Titanium (Ti) has also been suggested as an indicator of soil or dust contamination (Cherney and Robinson, 1982).
3.2 On drying

3.2.1 Drying times longer than 24 hours may be required depending on the type and number of plant samples in the dryer.

3.2.2 Drying at temperatures under 80°C may not remove all combined water (Jones et al., 1991) and may result in poor homogenization and incorrect analytical results.

3.2.3 Drying temperatures above 80°C may result in thermal decomposition and reduction in dry weight (Jones et al., 1991).

3.2.4 Enzymes present in plant tissue are rendered inactive at temperatures above 60°C (Tauber, 1949). As a result, air drying may not stabilize samples and prevent enzymatic decomposition. Samples should, therefore, be properly dried as soon after taking the sample as possible.

3.2.5 Quick drying of a limited number of samples can be accomplished using a microwave oven provided the samples are turned often and the drying process is closely monitored (Carlier and van Hee, 1971; Shuman and Rauzi, 1981; and Jones et al., 1991).

3.2.6 If samples absorb significant amounts of moisture during grinding, additional drying may be required prior to weighing for analysis. Drying time required will vary. Dry to constant weight by making periodic weighings.

3.3 On size reduction/grinding

3.3.1 Uniform grinding and mixing are critical in obtaining accurate analytical results.

3.3.2 Exercise care when grinding very small samples or plant material that is pubescent, deliquescent, or that has a fibrous texture. These samples are difficult to grind in Wiley mills and the operator should allow sufficient time for the sample to pass through the screen to ensure homogeneity. In these instances, experience has shown that Cyclotec or equivalent high-speed grinders are preferable.

3.3.3 Most mechanical mills contribute some contamination of the sample with one or more elements (Hood et al., 1944). The extent of contamination depends on condition of the mill and exposure time (Jones and Case, 1990). Grier (1966) recommended use of stainless steel for cutting and sieving surfaces to minimize contamination.
3.3.4 Routine maintenance should be performed on mills to ensure optimum operating conditions. Cutting knives or blades should be maintained in sharp condition and in adjustment.

4.0 Safety

Safety precautions are indicated in the use of equipment and facilities that should be properly followed to avoid any undue injury or unwarranted incident.

5.0 Apparatus and Materials

5.1 Medium-stiff nylon bristle brush.

5.2 Plastic containers suitable for washing and rinsing tissue samples.

5.3 Forced-air oven equivalent to Blue M Model POM-166E.

5.4 Standard and intermediate Wiley-type mills equipped with 20-, 40-, and 60-mesh screens and stainless steel contact points.

5.5 Cyclotec or equivalent high-speed grinder.

5.6 Medium bristle brush.

5.7 Vacuum system.

5.8 Airtight plastic storage containers.

5.9 Storage cabinet located in cool, dark, moisture-free environment.

5.10 If samples are placed in a cool (4°C), dark, dry environment, storage life is indefinite (Jones et al., 1991).

5.11 Coin envelopes can also be used for sample storage, however, somewhat greater care must be exercised in handling to prevent absorption of moisture. Collecting the ground sample in the envelope and immediately placing into a dessicator cabinet or dessicator will minimize moisture absorption.

6.0 Reagents and Standards

6.1 Deionized water.
6.2 0.1 to 0.3% detergent solution (non-phosphate)

7.0 General Procedure for Sample Preparation, Handling and Storage

7.1 Decontamination

7.1.1 Plant materials must be clean and free of extraneous substances including soil and dust particles, and foliar spray residues that may influence analytical results. Generally, the elements most affected by soil and dust particles are Fe, Al, Si, and Mn. Foliar nutrient spray and fungicide residues can affect several elements and should be taken into account in the decontamination process and when evaluating the analytical results. The decontamination process must be thorough while still preserving sample integrity. Therefore, decontamination procedures involving washing and rinsing should only be used for fresh, fully-turgid plant samples.

7.1.2 Examine fresh plant tissue samples to determine physical condition and extent of contamination. Unless leaf tissue is visibly coated with foreign substances, decontamination is usually not required except when Fe (Wallace et al., 1982) Al, Si, or Mn are to be determined (Jones and Case, 1990).

7.1.3 When Al, Si, Mn, and Fe are not of primary interest, plant leaves should be brushed briskly to remove visible soil and dust particles.

7.1.4 When plant samples show visible residues from spray applications and when Al, Si, Fe (Wallace et al., 1982), and Mn are elements of interest, leaves should be washed in a 0.1 to 0.3% detergent solution (Ashby, 1969 and Wallace et al., 1980) followed by rinsing in deionized water. The wash and rinse periods should be as short as possible (Sonneveld and Van Dijk, 1982) to avoid danger of N, B, K, and Cl leaching from the tissue (Bhan et al., 1959).

7.1.5 After decontamination, samples should be dried immediately to stabilize the tissue and stop enzymatic reactions.

7.2 Drying

7.2.1 Water is removed from plant tissue to stop enzymatic reactions and to stabilize the sample. Removal of combined water also facilitates particle size reduction, homogenization, and weighing.

7.2.2 Fresh sample is pre-washed at the greenhouse with tap water, then rinse with RO water 4x.

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7.2.3 Excess water is blotted off using a paper towel or clean cheesecloth.

7.2.4 Separate or loosen tissue samples and place in cloth bags or perforated paper bags. Do not tie samples together.

7.2.5 Place bags in forced-air oven and allow to dry at 80°C for 12 to 72 hours. Drying time depends on the type of samples and the amount of samples in the oven.

7.3 Size-reduction/Grinding

7.3.1 Plant tissue samples are reduced to 0.5- to 1.0-mm particle size to ensure homogeneity and to facilitate organic matter destruction.

7.3.2 After drying, samples should be ground to pass a 1.0-mm screen (20 mesh) using the appropriate Wiley Mill. A 20-mesh sieve is adequate if the sample aliquot to be assayed is >0.5 g. However, if the sample aliquot to be assayed is <0.5 g, a 40-mesh screen should be utilized (Jones and Case, 1990).

7.3.3 To obtain homogeneous powders, samples are finely ground, using a Cyclone Udy Mill with stainless steel screen to pass through a 20-mesh sieve. Large samples are first ground through a standard Beater Cross grinder and are then reduced by quartering to a manageable size. These are then ground by the Cyclone Udy Mill or Intermediate Wiley Mill. These samples are used for the determination of N, P, K, Ca, Mg, Na, and other elements.

7.3.4 For the determination of Fe, Mn, Cu, and Zn, the samples are ground in the Stainless Steel Beater Cross grinder or an agate or porcelain mortar to avoid metallic contamination.

7.3.5 After grinding, the sample should be thoroughly mixed and a 5- to 8-g aliquot withdrawn for analyses and storage.

7.3.6 Using a brush or vacuum system, clean the grinding apparatus after grinding each sample.

7.4 Storage

7.4.1 After particle size reduction and homogenization, samples should be stored in conditions that will minimize deterioration and maintain sample integrity for weighing and follow-up analytical work.
7.4.2 Ground samples are transferred to tightly capped glass jars or sealed polyethylene bags, labeled clearly, and stored for further analysis.

7.4.3 Containers should then be placed in a cool, dry place for storage.

7.4.4 For long term storage, ground samples should be thoroughly dried, sealed, and placed under refrigerated conditions (4°C) until the required analysis can be completed.

7.4.5 Before being weighed for analysis, samples should be oven-dried overnight at 60°C for B and 80°C for other determinations. If a sample is dried at 60°C for B, it can be used for other determinations after drying at 70°C. For analysis, the material is sub-sampled by quartering.

8.0 Plant sample preparation for Kjeldahl N analysis, macro- and micronutrient analysis by acid digestion and ICP-OES

8.1 Oven-dry samples at 80°C. Large dried samples are first ground using stainless steel beater cross grinder and subsampled by quartering to a manageable size. To obtain homogeneous powder, samples are finely ground using stainless steel cyclone Udy Mill or Wiley Mill and allowed to pass through a 20-mesh or 0.841 mm stainless steel sieve to avoid metallic contamination. After grinding, the whole sample must be mixed thoroughly.

8.2 In between samples, the mill is thoroughly cleansed with a stiff-bristled brush or compressed air. Additional step of sample pre-flushing is recommended before collecting the ground sample for analysis in order to avoid cross-contamination.

8.3 Submit at least 5g of ground sample placed in a coin envelope (available at ASL), label envelope with the following information:

| Sample ID: ________________ |  
| Sender: _________________ |  
| Grinder used: ____________ |  
| Who did the grinding: _________________ |  
| When grinding was done: _________________ |  

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9.0  **Plant sample preparation for total C, C-13, total N and/or N-15 analysis by Dumas method**

9.1  Grind oven-dried plant samples using stainless steel ball mill (SSBM) to talcum powder fineness (0.177 mm or #80 mesh). Ask ASL staff for assistance on how to use the SSBM.

9.2  Submit *at least 2 g* of ground sample placed in a coin envelope (available at ASL), label coin envelop as in 8.3.

10.0 **Plant sample preparation for boron analysis by dry ashing and ICP-OES**

10.1 Samples must be oven-dried overnight at 60°C to prevent B volatilization. If a sample is dried at 60°C for B, it can be used for other determinations after drying at 80°C. Follow the same grinding procedure as 9.1 and sample labeling as 8.3.

10.2 Once samples are ready for submission, customer may now avail of online Request for Analysis (RFA) using ASL website at [http://asl.irri.org/lims/](http://asl.irri.org/lims/) where forms for RFA, Sample IDs and Charge Slips are available. Fill out the required forms and submit to ASL using your ASL account. New customers should create an account with us first before availing of the online request system.

**Table 1. Weight requirement for Plant Analysis.**

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Weight of sample/analysis ( g plant, 0.841 mm, No. 20 mesh)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kjeldahl N</td>
<td>0.2</td>
</tr>
<tr>
<td>Wet ashing – ICP Macroelements</td>
<td>4.0</td>
</tr>
<tr>
<td>Wet ashing – ICP Microelements</td>
<td>4.0</td>
</tr>
<tr>
<td>Dry ashing -  ICP Macroelements</td>
<td>4.0</td>
</tr>
<tr>
<td>Dry ashing -  ICP Microelements</td>
<td>4.0</td>
</tr>
<tr>
<td>HCl extraction –ICP Macroelements</td>
<td>4.0</td>
</tr>
<tr>
<td>Boron</td>
<td>1.0</td>
</tr>
<tr>
<td>Total C &amp; N, 13C &amp; 15N</td>
<td>2.0 (0.177mm, No. 80 mesh)</td>
</tr>
</tbody>
</table>
11.0 Soil sample preparation for pH, EC, exchangeable bases, CEC, particle size analysis, available P, K and B

11.1 The soil required for normal analytical work is fine earth or soil that has been air-dried, crushed and passed through a 2-mm sieve to remove stones, twigs, leaves, stems and roots.

11.2 Dried soil should be ground to 2 mm size using the Rukuhia grinder (RG) located in Soil Grinding Room (Rm.148) (available at ASL).

11.3 Submit at least 500g of ground sample placed in an 8.5-size coin envelop (available at ASL), label envelop with the following information:

<table>
<thead>
<tr>
<th>Sample ID:</th>
<th>_____________</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sender:</td>
<td>_____________</td>
</tr>
<tr>
<td>Grinder used:</td>
<td>_____________</td>
</tr>
<tr>
<td>Who did the grinding:</td>
<td>_____________</td>
</tr>
<tr>
<td>When grinding was done:</td>
<td>_____________</td>
</tr>
</tbody>
</table>

12.0 Soil sample preparation for available Zn & Cu, active Fe & Mn, organic C, Kjeldahl N, total C, C-13, total N and/or N-15 analysis requiring fine samples

12.1 Using Pascall grinder, take an aliquot of the bulk sample for fine soil grinding. Once the soil has been ground to fine powder, it cannot be used for analyses listed in Sec. 11 above.

12.2 Submit at least 50g of fine sample placed in an 8.5-size coin envelop (available at ASL), label envelope following Sec.11.3 procedure.

12.3 Once samples are ready for submission, customer may now avail of online Request for Analysis (RFA) using ASL website at [http://asl.irri.org/lims/](http://asl.irri.org/lims/) where forms for RFA, Sample IDs and Charge Slips are available. Fill out the required forms and submit to ASL using your ASL account. New customers should create an account with us first before availing of the online request system.
Table 2. Weight requirement for Soil Analysis

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Weight of sample/analysis (g soil, 2 mm, # 10 mesh)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (by H$_2$O, KCl, CaCl$_2$)</td>
<td>40</td>
</tr>
<tr>
<td>EC</td>
<td>80</td>
</tr>
<tr>
<td>Olsen P</td>
<td>10</td>
</tr>
<tr>
<td>Bray P</td>
<td>20</td>
</tr>
<tr>
<td>CEC &amp; Exchangeable Bases (Na,K,Ca,Mg)</td>
<td>20</td>
</tr>
<tr>
<td>Exchangeable Al &amp; H$^+$</td>
<td>40</td>
</tr>
<tr>
<td>Available K</td>
<td>20</td>
</tr>
<tr>
<td>PSA (Hydrometer)</td>
<td>240</td>
</tr>
<tr>
<td>PSA (Pipet)</td>
<td>50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Weight of sample/analysis (g soil, 0.177 mm, # 80 mesh)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Available Cu &amp; Zn</td>
<td>40</td>
</tr>
<tr>
<td>Active Fe &amp; Mn</td>
<td>2</td>
</tr>
<tr>
<td>Kjeldahl N</td>
<td>1</td>
</tr>
<tr>
<td>Organic C</td>
<td>5</td>
</tr>
<tr>
<td>Available B</td>
<td>40</td>
</tr>
<tr>
<td>Total C &amp; N, C-13 &amp; N-15</td>
<td>1</td>
</tr>
</tbody>
</table>

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