World Agroforestry Centre TRANSFORMING LIVES AND LANDSCAPES	STANDARD OPERATING PROCEDURE		No of pages 5
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	Title: Soil Sample Processing at Regional Laboratories		

I. SCOPE AND APPLICATION

This standard operating procedure for AfSIS soil processing is designed for use by AfSIS regional laboratories. The procedure covers sample reception and logging, soil sieving and weighing, soil sub-sampling, soil sample storage, and shipping of subsamples to the AfSIS reference soil laboratory "Soil-Plant Spectral Diagnostic Lab" at the World Agroforestry Centre (ICRAF) in Nairobi.

All samples from the field (sentinel sites, agronomic trials, legacy samples) are transported to regional laboratories for processing and processed using this standard operating procedure. In some cases soil drying may be conducted in a country prior to transportation to the regional laboratories to avoid changes in soil properties from storage of wet samples. In this case only the soil drying step of this protocol is implemented prior to transportation of samples to the regional laboratories.

II. RELATED SOPS

i. A separate guide is available for data management and submission

ii.

III. PRINCIPLE

Sampling and sample pretreatment forms an important stage of the analytical process of soils because conclusions about a site are often based on the analysis of representative soil samples collected during this stage. Due to high spatial variation in the soil, it is important that the process followed should lead to collection of a sample that is true representative of the site. In addition, errors in sampling and sample pretreatment can exceed analytical error and can influence the final results of a soil analysis.

Soil collected from field is a mix of gravel (fraction of the soil with a diameter of > 2 mm), sand (fraction between 0.05 mm and 2.0 mm), silt (fraction between 0.002 mm and 0.05 mm) and clay (fraction smaller than 0.002 mm or 2µm). Since the contribution of gravel fraction to the dynamics of soil nutrient cycles and supply to plants is minimal, they are excluded from the analysis and reporting of plant available nutrients. During the preparation of soil samples, this fraction should not be crushed and pulverized, because then this fraction is considered as part of the fine soil and result in low values of the analyses due to dilution effect. However, aggregates of >2 mm size, formed by fine particles have to be crushed in order to pass through a 2 mm sieve and included in the analysis. Normally, soil sampling procedures lead to collection of more than required amount of soil and to get a representative sample of necessary volume, there

is a need to follow standardized and consistent procedures. For this aim it is necessary to use the method of coning and quartering.

IV. EQUIPMENT

a. Drying trays
b. Oven
c. Metal boxes
d. Balance
e. Sieve 2mm
f. Wooden rolling pin

V. MATERIALS

a. Plastic sheet
b. Markers
c. Brown paper bags size 5
d. Plastic zip-lock bags
e. Particulate respirator (eg., N95 of 3M make)
f. Nitrile gloves

VI. PROCEDURES

a. Soil sample reception and logging

- Lay out the samples received in order of labeling and check against field sheets. Samples collected within the AfSIS project have field descriptors in electronic format already entered in the AfSIS database.
- Make detailed notes in a laboratory record book of any labeling discrepancies or problems due to damaged sample bags or lost samples. In case of AfSIS samples enter them in the comments field of the logging database.
- To the extent possible, resolve the problems noticed at this stage. This is particularly important for samples from the auger holes used for soil mass determinations, where complete recovery of the sample is required.
- Assign the Sample Serial Number (SSN) starting from 000,001 up to 999,999. Write the assigned SSN number on the sample bags using a permanent marker. Including the thousand separator (comma) helps in avoiding recording errors. The number should be prefixed with the laboratory location code assigned by AfSIS (sel for Selien; sot for Sotuba; chi for Chitedze; iia for IIAM Maputo).
- Next copy the field descriptors on the sample bag plus the assigned serial number to a sample logging sheet (Appendix 1). For AfSIS sentinel site samples, the field descriptors should already be in the database. For other samples, ancillary data on the recording sheet will need to be entered into the database.

b. Drying

- Spread the soil out as a thin layer into shallow trays or plastic or paper sheets. It is important to ensure that no material from a sample is lost or discarded, as weights of soil fractions are to be recorded on processing.

- Break up clods as far as possible to aid drying. Take care to avoid crushing gravel sized particles.
- Great care should be taken at all stages to ensure sample labels remain with the samples.
- Exercise care to avoid contamination from dust, plaster or other potential contaminants during drying, as soils are subjected to trace element analysis.
- Air dry the samples in shade. Drying can also be done in a large room, custom-made solar dryer, or a forced-air oven at 40° C.
- Drying time will depend on the condition of the samples and ambient conditions, but the samples should be thoroughly dry (i.e. constant weight).

c. Weighing and sieving (regular samples)

- Sample processing procedures are different for regular samples and samples taken for cumulative mass. For instructions on cumulative mass samples see the section titled **cumulative mass samples** in this SOP and follow the steps below for regular and all other samples.
- Weigh the whole dried soil sample to 0.1 g using a calibrated top-pan balance and record the weight. Exercise care to avoid loss of sample.
- Mix the dry sample thoroughly while still on the drying tray.
- Spread the sample onto a plastic sheet on a solid table.
- Using a wooden rolling pin, crush the sample to pass through a 2 mm (US sieve size 10) certified sieve.
- While crushing, remove any plant materials (e.g. roots) and any possible pieces of gravel (making sure they are gravel and not soil aggregates) and place in a separate pile (the coarse fraction).
- Pass the crushed sample through the 2 mm sieve. DO NOT use the sieve as a grinder; i.e. do not rub or mash the soil on the sieve, but shake the sieve gently to allow the soil to pass through. If a large amount of soil needs to be sieved, it is easier to do it in small batches rather than all at one time.
- Place whatever remains on the sieve back onto the plastic sheet and crush again gently. Then pass again through the 2 mm sieve. Make sure that all soil materials are crushed, but do not attempt to crush gravel and rocks.
- Transfer anything that now remains on the sieve into the coarse fraction pile. Retain the coarse fraction for subsampling.
- The whole sample should be processed and no material should be discarded. You will remain with two fractions:
  - The coarse fraction (>2 mm), which cannot pass through the sieve.
  - The soil fines (<2 mm), which have passed through the sieve.
- Weigh the coarse fraction and record the weight to 0.1 g and record the data in “Sample Logging Sheet”. The logging sheet is given as Appendix 1 to this SOP.
- Enter the weight of the coarse fractions into the AfSIS database. The weight of the fine fraction is calculated in the database.
- Clean off the bench with a damp cloth to remove soil dust, so as to prevent contamination from one sample to another.

d. Sub-sampling of fine fractions

- If the weight of the soil fines is much greater than 350 g, subsample the soil fines using coning and quartering (Appendix 2) or a sample divider (riffle box) to give about (not less than) 350 g of soil.

- Continue the coning and quartering technique on all samples to obtain a representative 20 g subsample of soil fines for shipping to ICRAF Soil-Plant Spectral Diagnostic Lab in Nairobi for specialized spectral analysis.
- Place the subsample in a zip-lock polythene bag labeled with the SSN and "Fines". See Appendix 3 for sample shipping procedures.
- Place the remaining 350 g sample of soil fines into a strong size 5 brown paper bag, labeled with the SSN, site, cluster, plot, depth_std code, and std_fines. Example: sel000,001 Kiso1.1.TOP.std_fines.
- Store the excess soil fines in a labeled bag for possible use in future.
- Selected samples of the 350 g soil fine samples are shipped to ICRAF Soil-Plant Spectral Diagnostic Lab in Nairobi (Appendix 3) for reference analyses. Soil fines samples not shipped should be stored at the regional laboratory in case they are needed for future analysis.

e. Sub-sampling of coarse fractions

- The coarse fraction of the samples selected above for reference analyses (i.e. the coarse fraction of the 350 g samples) is to be sub-sampled before shipping to the ICRAF Soil-Plant Spectral Diagnostic Lab
- Crush the coarse fraction as much as possible to homogenize the sample before sub-sampling
- Use coning and quartering (Appendix 2) or a riffle box to obtain a 20 g subsample of the coarse fraction
- Place the subsample in a zip-lock polythene bag labeled with the SSN, site, cluster, plot, depth_std code, and "coarse". Example: sel000,002. Kiso1.1.TOP.Coarse".
- Ship the sample to ICRAF Soil-Plant Spectral Diagnostic Lab in Nairobi following the procedure outlined in Appendix 3
- Place the remainder of the coarse fraction into a labeled paper bag and store at regional laboratory for possible future analysis.

f. Selection of 32 samples of 350-g soil fines to be shipped to ICRAF Soil-Plant Spectral Diagnostic Lab -Nairobi

- Select the topsoil (0-20 cm) and subsoil (20-50 cm) sample of PLOT 1 from each of the 16 clusters of a sentinel site (32 samples in total).
- If PLOT 1 has the subsoil sample missing due to a depth constraint, then select PLOT 2 for both topsoil and subsoil for that cluster instead.
- If PLOT 2 has the subsoil sample missing, then go to PLOT3, etc.
- If a subsoil sample from entire Cluster is missing, then select and an additional Plot from an adjacent cluster to compensate. For example if all plots in CLUSTER 2 are missing then select PLOT 2 and PLOT 3 from CLUSTER 3.
- Ship the samples to ICRAF Soil-Plant Spectral Diagnostic Lab following the procedure in Appendix 3

g. Cumulative mass samples

- Processing of cumulative soil mass samples is shown in the flow diagram in Appendix 4 and involves following steps
- Place the entire soil in a bowl on receipt of the sample at the laboratory
- Air dry the sample in shade. Drying can also be done in a large room, custom-made solar dryer, or a forced-air oven at 40° C.

- Weigh the entire sample
- Sub sample about 50 g soil into a metal container and record the weight
- Dry the sub-sample in an oven at 105^0C for 48 hours
- Weigh the dried sub-sample and record the weight in the sample data sheet given as Appendix 5.
- Load the data into the database

VII. CALCULATIONS

All the calculations are automated in the database management system

VIII. QUALITY ASSURANCE/QUALITYCONTROL

Not applicable

IX. DATA VALIDATION

Not applicable

X. HEALTH AND SAFETY

- Wear nitrile gloves to reduce the incidence of skin contact with potentially contaminated soil and to reduce the risk of cross-contamination
- Wear respirator that covers the mouth and nose to filter out harmful dust particles. Inhaling such particles irritates the nostrils and sinuses and can lead to lung diseases.
- Refer to the site-specific Health and Safety Plan for other safety concerns and applicable personal protective equipment

XI. REFERENCES

XII. APPENDIX

Appendix 1: Sample Logging Sheet

SSN	Sampling date	Cluster	Plot	Depth_std code	Depth_ top	Depth_ bottom	Total air dried soil weight	Coarse fragments

Appendix 2: Coning and quartering procedure

Coning and quartering procedure
1. Place the sample on a large cleaned surface or heavy-duty plastic sheeting.

2. Thoroughly mix the soil sample and spread the sample into a conical pile.

3. Further mix the soil by circumventing the cone symmetrically, repeatedly taking a spatula-full of soil from the base and transferring the soil to the apex of the cone.

4. Ensure the spatula is large enough to reach to centre of the cone. Circumvent the cone twice.

5. Flatten the cone to a height of about 1 cm.

6. Use a flat spatula or ruler, divide the pile into quarters along two lines intersecting 90° to each other.

7. Select one pair of opposite quarters as the sample to be retained.

8. If the sample is still too large then repeat the procedure from the beginning.

Appendix 3: Sample shipping procedures

In case AfSIS and those projects following AfSIS protocols, the following sub-samples should be shipped to the ICRAF Soil-Plant Spectral Diagnostic Lab, Nairobi from each site:

- A 20 g sub-sample of fine fractions for all soil samples.
- 32 samples of 350g soil fines for 0-20 and 20-50 depths from Plot One (or whichever plot has both top and subsoil samples)
- The corresponding 20 g sub-sample of coarse fraction of the 32 samples of soil fines. If there are no coarse fragments, that is fine.

1. Communicate and inform ICRAF Soil-Plant Spectral Diagnostic Lab prior to shipping to ensure all legal requirements are met and to ensure the safety of the samples.
2. In advance of shipment, send the details of your samples to the ICRAF Soil-Plant Spectral Diagnostic Lab at ICRAF Headquarters, Nairobi to: Keith Shepherd (k.shepherd@cgiar.org) copied to Elvis Weullow (e.weullow@cgiar.org). The information required is
    a. A description of the material (e.g. air-dried 2 mm-sieved soil samples)
    b. Number of soil samples for shipping
    c. Total weight of the soil in the batch
    d. Name, institutional address and fax number of the scientist shipping the samples.
3. Obtain a phytosanitary certificate from your country's plant inspectorate authorities or, if this is not possible, a letter from the relevant government authority indicating that the soils are specifically meant for research purposes only and have no commercial value.
4. Send the phytosanitary certificate or letter to the ICRAF Soil-Plant Spectral Diagnostic Laboratory.
5. Based on the above documentation, the ICRAF Soil-Plant Spectral Diagnostic Laboratory will obtain the import permit from the Kenya Plant Health Inspectorate Service (KEPHIS) and a scanned copy of the permit[1] will be mailed to you.
6. The samples should be shipped together with a copy of the KEPHIS permit and a copy of the phytosanitary certificate or government letter from the source country. Failure to do so may result in the samples being destroyed by KEPHIS!
7. The soil samples to be shipped should be carefully double-packed into strong polythene bags that cannot be easily ripped or damaged in transit, and packed into strong shipping cartons.

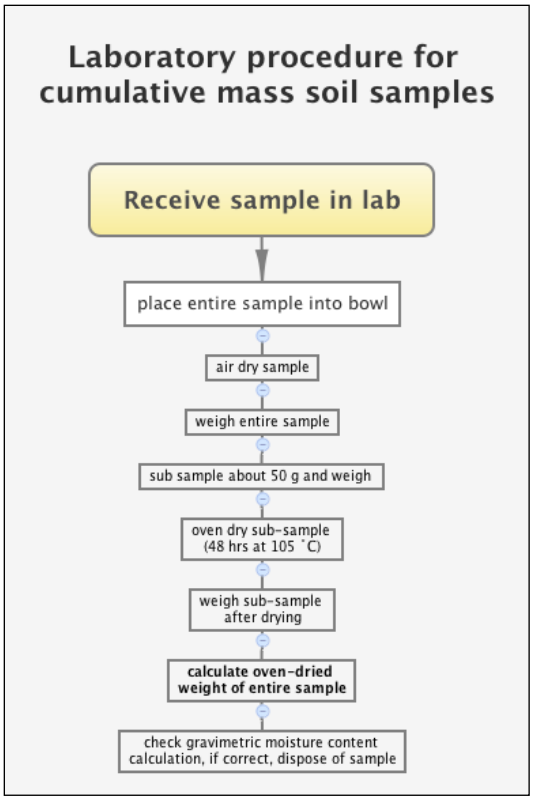
[1] KEPHIS also issue a quarantine (Q) label that the ICRAF Soil Lab will retain for clearance purposes.

8. Also have the shipping agent repack the consignment. Secure packing is critical because damaged packets will be destroyed by KEPHIS and our agreement with KEPHIS may be revoked.
9. Make sure that the final packing will stand the rough handling at airports.
10. The shipping address is:

    Dr. Keith Shepherd
    Att: Elvis Weullow
    World Agroforestry Centre (ICRAF).
    P. O. Box 30677-00100
    Nairobi, KENYA
    Tel: +254 20 7224000
    Fax: +254 20 7224001

11. Immediately after shipping, fax or email the shipping details (e.g. airway bill number) to Samuel Gaturu (s.gaturu@cgiar.org), Elvis Weullow (e.weullow@cgiar.org), and Mercy Nyambura (m.nyambura@cgiar.org), copied to Dr Keith Shepherd (k.shepherd@cgiar.org). This will allow us to alert the shipping agent's Nairobi office about the arrival of shipment and prepare for their clearance on arrival.
12. ICRAF Soil-Plant Spectral Diagnostic Laboratory will arrange for clearance of the shipment and inspection of the soils by KEPHIS.
13. Upon clearance by KEPHIS, ICRAF will arrange for collection and transport of samples to ICRAF Soil-Plant Spectral Diagnostic Laboratory.
14. At the laboratory, samples will be logged as foreign soils, stored in dedicated foreign soil store and handle them as per the specified procedures for handling foreign soils laid down in their agreement with KEPHIS.
15. Note that the ICRAF Soil-Plant Spectral Diagnostic Laboratory charges US$100 to cover all the expenses involved in sample clearance protocols, including KEPHIS fee, visits to the KEPHIS office, and clearance when the samples arrive. The shipping and permit costs are the responsibility of the sender.

Appendix 4: Flow chart for processing mass samples



Appendix 5: Sample data sheet for mass samples

SSN	Sampling Date	Cluster	Plot	Depth_ top	Depth_ bottom	Total air dried soil weight	Tin ID	Tin weight	Tin_air dried soil weight	Tin_oven dried soil weight