DIBANET workshop for Biomass Sampling and Analysis
University of Limerick, Ireland
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From Wet Unground Samples to Dry Sieved WU→DS
Rev: 1.0 (1-2-10)

This procedure is for samples that will be prepared in a dry sieved form for analysis later. It is for biomass that is in a wet form.

Equipment Needed:

- Analytical balance.
- Drying oven, with temperature control of 105 ± 3$_o$C
- Cutting device (e.g. chipper or mill).
- Sieve Shaker that provides motion in both horizontal and vertical axes- for optional sieving step, alternatively the sieves can be shaken by hand.

Chemicals and Materials Needed:

- Sample trays for drying (e.g. party trays)
- Sieve Set, No. 20 (850 μm), No. 80 (180 μm) stackable sieves with lid and bottom pan. Sieves and bottom pan should be 8.9 cm (31/2 in.) in height.
- Porcelain crucibles (50ml)
- Plastic sample bottles for storing the DS and DF fractions.

THINGS TO ASK BEFORE STARTING:

- Do we already have NIR-NUMBERS for these samples?
- Is the total weight of the sample important?
- Is it important that some of the original sample is retained and not dried?
- How many samples will we be preparing this day? Referred to as "X"

DAY BEFORE:

1. 2X large crucibles should be placed in the oven at 105C.
2. If the samples are in the freezer they should be taken out of the freezer the night before in order to defrost and reach room temperature. The bags **should not** be opened.
3. Have numerous (2X or more) clean biomass air-drying trays ready for use the next day – make sure these trays have letters labeled on them

DAY 1 (Wet Analysis):

1. Take a "WU-DS - From Wet Unground Samples to Dry Sieved Samples" Data Recording Sheet. **Make sure you know whether the total sample weight for this sample is important**. This will influence which WU-DS data recording sheet is used.
2. Take all of the bags for one sample (usually only 1 bag but could be more) and record its Universal Code, the date it was sampled, and any other codes (e.g. AM1X1) in the appropriate boxes in the first available column.
3. If it does not already exist, you will need to get a **unique NIR number** for each sample and record this on the Data Recording Sheet.
    a. Go to the EXCEL file called "***ISI-SCAN CODES 3***" (on the desktop) and pick the next number after the last sample according to the species being sampled, e.g. if the last one was 22 take 23.
    b. Put the Universal Code (e.g. PTRB1M) that this ISI SCAN number represents in the EXCEL file. Remember that all new samples will have new ISI SCAN codes but that the same ISI SCAN code will be used for a sample in various states (e.g. wet unground, wet ground, dry unground, dry ground).
4. Take out 2 large crucibles (for each sample analysed) from the oven and place in the dessicator. Record the number of these crucibles. These should not be weighed (and samples not added) until about 20 minutes after removal from the oven.
5. **If the sample is in a state that can be put directly in the NIR cell** with no air gaps or spaces (e.g. mushroom compost, peat) proceed to step 6. If not you will need to reduce the particle size so that it can pack efficiently in the cell. This can be done in the following ways:
    a. **FOR STEMS/WOODY MATERIAL - CHIPPING:**
        i. Open the chipper – Push in the release valve and twist to open the door. The press the red button and then the blue button.
        ii. Make sure that the chipper is clean and dry. Use the air-pressure/hair drier and tools (brush, hand, ruler, wet cloths) accordingly.
        iii. Close the chipper, making sure that the appropriately sized mesh (20mm for most stems) is in place, and secure the valve.
        iv. Make sure the collection tray is clean and below the chipper and that the plastic funnel is connected properly to the chipper.
        v. Start the blades on the chipper (green button).
        vi. Put an appropriate amount of biomass standing up over one side of the chipper chute.
        vii. Push the sample down with the wooden tool and make sure that the tool stays down while the samples are being chipped.
        viii. Take the prodding tool with the plastic side upwards and use to poke at the mesh of the chipper to avoid blockages. Make sure that the prodding takes place at different locations and angles to ensure there are no blocked areas.

ix. Repeat for all the sample. If necessary occasionally spread out the sample on the collection tray so that it is even. If any excessively large pieces come through these can be put through the chipper again. If you find that the tray is becoming full it may be necessary to transfer the material to the bench to allow further chipping.
x. Once chipping is complete stop the rotor and open the chipper.
xi. Remove any sample that remains in the chamber, attempting to transfer to the collection tray if possible. Remove the mesh and clean with hands.
xii. Transfer all of the chipped sample to the bench.

b. FOR LEAVES/SHEATHS etc – SHREDDING:

i. Take a box or container and place below the shredder, ensuring that there is no gap through which sample could be lost.
ii. Turn on the shredder and put the sample through the shredder.
iii. Empty the contents of the box onto the bench for further analysis.
iv. If the material is still not in a suitable state for NIR scanning then it can be put through the chipper with the 6mm mesh (see (a) above).

6. If the total weight of the sample is important:

a. If the sample is still in the sample bag and the sample bag (with sample) weighs less than 600g:
   i. Weigh the bag on the scales and record the scales used.
   ii. Empty the contents of the bag onto the wooden platform.
   iii. Weigh the bag.
b. If the sample is already on the bench (i.e. if it has been chipped) or the sample bag (with sample) weighs more than 600g:
   i. Empty the contents of the bag onto one side of the wooden platform.
   ii. Take the weighing boat (making sure it is clean) and then put on the scales (replacing the lid for the cardboard box). Press TARE
   iii. Take the weighing boat and fill with the sample from the collection tray. Put on the scales and put the lid on. Record the weight on the sheet.
   iv. Transfer the sample from the weighing boat to the other side of the weighing platform (i.e. it should be separate from the unweighed material).
   v. Repeat steps ii-iv for the material that has not yet been weighed.

7. **If the total weight of the sample is NOT important (e.g. PEAT) –** Making sure that the wooden platform/bench in the centre of the lab is clean, empty the contents of the sample bag(s) on to this platform.
8. Mix the sample on the wooden platform **thoroughly** (1 minute). You will then need to decide if this sample is to be subdivided. How the sample is then subdivided will depend on how much sample is available and if you want to keep some WU for future analysis:
   a. **First Priority** - You will need to take enough material to spread evenly (and thinly) across at least one (and preferably about 3) “Party” trays (for drying), for the **wet unground (WU)** NIR analysis and subsequent drying

and wet chemical analysis. i.e. we would like to have at least 200g for WU analysis and drying.

b. **Second Priority** – The rest (if any) can be stored, if desired, by putting in sealable bags which then go in the freezer.

9. **NIR ANALYSIS OF WET-UNGROUND MATERIAL** - Take the part that has been reserved for the wet unground (WU) analysis.
    a. Mix this thoroughly on the bench and spread thinly across the bench.
    b. Put a representative sample of this into the large NIR sample cell (make sure the cell is clean and dry and that the cell for "wet" biomass is used) and fill the cell and put the lid on.
    c. Put the cell in the XDS (without touching the transport), making sure it is in correctly. Press "Scan". Save this scan in the appropriate product group. For the Sample Number box put the NIRNUMBER-WU-1 (or 2 or 3 depending on the number of the scan) e.g 22-WU-1. In the comments box put the UNIVERSALCODE. Also put any other things that were on the card in the sample bag (e.g. date and original code) or any other comments that are in the ISI SCAN CODES 3 file. Also make a note of the number/letter of the NIR cell that is used. Then take the sample out of the cell and put back with the rest of the sample.
    d. Steps a-c should be repeated for scan WU-2 and WU-3. **Each time take the sample out of the cell and mix with the rest of the sample reserved for Wet Unground analysis and then the cell should be filled again.**
    e. Clean the NIR cell afterwards with a tissue if it is wet and make sure it is dry.
10. Moisture Content Determination – MUST BE DONE IMMEDIATELY AFTER NIR ANALYSIS OF WU:
    a. Take one of the large numbered crucibles out of the dessicator.
    b. Weigh it to the nearest 0.1mg, record the scales that were used.
    c. Put in a subsample of the WU-NIR-analysis subset (that which was just used for NIR scanning) and fill the crucible (but do not have any material over the edges) and weigh to the nearest 0.1mg in the same scales.
    d. Place crucible in the oven and dry at 105C overnight.
    e. Repeat a-d for a second large crucible.
11. Air-drying of remainder of sample:
    a. Take a numbered tray, record the number of the tray according to the sample. Weigh the tray.
    b. Add the remaining material from the WU-NIR subset (unground biomass) to it, make sure that the biomass is not deeper than 1 cm. Weigh the tray.
    c. If there is too much material then repeat steps (a)-(b) for more trays.
    d. Put the trays on a bench and allow to dry.
    e. Record the time and date it was put in.
    f. Each day record the weight of the trays and move the sample around so that it will dry properly (i.e. turn it over).
    g. The drying is complete when the weight does not change significantly between days.

DAY 2 – MOISTURE CONTENT OF SAMPLES

1. Take the large crucibles (for the moisture content of the wet unground material) out of the oven and put in a dessicator. About 20 minutes later weigh these crucibles to the nearest 0.1mg.

DAY "Y" (when the weight of the trays stops falling) - NIR ANALYSIS AND SIEVING OF DRIED SAMPLES:

1. These steps will occur when the weight of the trays with the samples on does not change significantly between 2 days. From now on the procedure is identical to a DU-DS procedure.
2. Take all of the trays for one sample and empty the contents onto a bench. Mix the sample on the bench thoroughly.
3. NIR Scanning:
    a. Take a representative sample from the bench and put in the NIR cell. Make sure that the cell is clean and (very important) dry and that the NIR cell that is for "dry" biomass is used.
    b. Scan the sample
    c. **Consult the Data Recording Sheet and ISI-CODES 3 document for the appropriate NIR-NUMBER for the sample.** For the Sample Number box put the NIRNUMBER-DU-1 (or 2 or 3 depending on the number of the scan) e.g 22-DU-1. In the comments box put the SAMPLENUMBER-DU-1(or 2 or 3 depending on the number of the scan). Also put any other things that were on the card in the sample bag (e.g. date, original code), and things that may be in the comments column for the sample on the ISI SCAN CODES 3 file, also note the latter/number of the NIR cell that is used. Then take the sample out of the cell.
    d. **Steps a-c should be done three times (3 scans) with the sample being taken out of the cell each time and mixed with the rest of the dry unground material.** Clean the NIR cell afterwards with the brush and air gun.

4. **PARTICLE SIZE REDUCTION:**
    a. The next steps are necessary to reduce the particle size of the sample down from the DU state to the DG state (less than 850 υm). The DG state comprises the DS fraction (the fraction used for wet chemical analysis and has a particle size 180υm < x < 850υm) and the DF fraction (which has a particle size < 180υm). **IT IS VERY IMPORTANT THAT THE PROPORTION OF THE DS FRACTION IS MAXIMISED. THEREFORE THE EXACT METHODS OF PARTICLE SIZE REDUCTION WILL VARY ACCORDING TO THE SAMPLE OF INTEREST.**
    b. There are several options for reducing particle size:
        - Using the chipper (with different mesh sizes)
        - Using the mill.

- Using a mortar and pestle.
- Other options…..

c. This is a general summary of the steps involved for particle size reduction:
   i. Sieve the sample (see below for sieving instructions).
   ii. Remove the DG fractions (DS and DF).
   iii. Take the DB fraction (x > 850υm) and cut it somehow (e.g. chipper, mill, etc.)
   iv. Repeat steps (i) to (iii) until there is no DB left. It may be useful to record the weight of DB, DS and DF material for each iteration of these steps.

5. Scanning the dry ground (DG) material:

a. At this stage all of the original DU should be in a DG state. Empty all of the DG sample onto the bench and mix thoroughly. If it is separate as DS and DF fractions (after sieving) these need to be mixed together to give the DG fraction.
b. Transfer the dry ground material to the sample cell (you should be wearing plastic gloves for this so not to give moisture to the sample). The cell should be dry and clean and the cell that is designated for "dry" samples should be used.
c. Scan the sample.
d. **Consult the Data Recording Sheet and ISI-CODES document for the appropriate NIRNUMBER for the sample.** For the Sample Number box put the NIRNUMBER-DG-1 (or 2 depending on the number of the scan) e.g 22-DG-1. In the comments box put the UNIVERSALCODE. Also put any other things that were on the card in the sample bag (e.g. date, original code) and anything that is in the comments section for the sample on ISI SCAN CODES 3 file, also make a comment of the number/letter of the NIR cell that is used. Then take the sample out of the cell.
e. **Steps b-d should be done two times (2 scans) with the sample being taken out of the cell each time and mixed with the rest of the dry ground material before transferring sample to the cell again.**
f. Once finished transfer the material from the cell back to the tray. Clean the NIR cell afterwards with the brush and air gun.

6. Sieving:

a. Find a "sieve-set" (top (850υm), middle (180υm) and bottom (bottom pan) that is dry, clean and where the mesh is not blocked with particles.
b. Put some of the DG on the "top" sieve but do not put too deep a layer of DG here. If there is too much material then more than one sieve set can be used or it will be necessary to sieve parts of the sample in sequence until all the sample is sieved.
c. Put the sieve set(s) on the AS 200 digit and start the shaker on a 20minute run.
d. Once the run has completed. Take a tray, weigh it and make a note of the weight and empty all of the DS fraction from the middle sieve(s) onto this. Do the same for the DF fraction (in the bottom pans). Then weigh the DS and DF trays so that we will know the proportion of DS and DF fractions.

e. Then transfer the DS fraction to a container. Label this container UNIVERSALCODE-DS and NIRNUMBER-DS.
f. Then transfer the DF fraction to a container. Label this container UNIVERSALCODE-DF and NIRNUMBER-DF.

g. These fractions can then be stored for future wet chemical analysis.

7. **Scanning the dry sieved (DS) material:**
    a. Empty the DS sample onto the bench and mix thoroughly.
    b. Transfer the material to the sample cell (you should be wearing plastic gloves for this so not to give moisture to the sample). The cell should be dry and clean and the cell that is designated for "dry" samples should be used.
    c. Scan the sample.
    d. **Consult the Data Recording Sheet and ISI-CODES document for the appropriate NIRNUMBER for the sample.** For the Sample Number box put the NIRNUMBER-DS-1 (or 2 depending on the number of the scan) e.g 22-DS-1. In the comments box put the UNIVERSALCODE. Also put any other things that were on the card in the sample bag (e.g. date, original code) and anything that is in the comments section for the sample on ISI SCAN CODES 3 file, also make a comment of the number/letter of the NIR cell that is used. Then take the sample out of the cell.
    e. **Steps b-d should be done two times (2 scans) with the sample being taken out of the cell each time and mixed with the rest of the dry ground material before transferring sample to the cell again.**
    f. Once finished transfer the material from the cell back to the storage container.

<u>Instructions for using the mill:</u>

1. Make sure that the mill is clean, use the air gun and/or wet tissues for this. If tissues are used make sure that the mill is dry before it is used to cut the sample.
2. Take a brown collection bottle and make sure it is clean. Put this in place below the mill
3. Put the dry unground sample through the mill. Be careful not to put too much in at any time or the mill will overload
4. If there is too much dry unground material to fit in one collection bottle then use another (clean) one or empty the contents of the bottle onto a tray and then put the bottle back under the mill.