***SMAPVEX12 Laboratory Protocol***

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2. ***Hydra Probe Lab Calibration***

**INTRODUCTION**

A broad cross-calibration of probes is essential to check the inter-variability of the instruments and ensure consistency under controlled environment. A two-step assessment will be carried out.

***STEP A:***

I Fill a 400ml beaker with distilled water.

II Submerge the probes in water, ensure that all the tines are completely submerged, and allow stabilising for one minute.

III Take the dielectric permittivity reading.

Typically, distilled water has a dielectric permittivity of about 80 and a range of 80 + 5 is considered acceptable.

***STEP B:***

I Prepare air-dry soil of different textures (preferably heavy and light texture).

II For heavy texture soil, weigh about 1kg of soil to a tray and add 200g of water. Mix thoroughly.

III Weigh a 926cm3 plastic container (MC) and add 840g of the moist soil. Pack the moist soil to 700cm3 volume.

IV Insert the probes, ensure that all the tines are buried in the soil. Record the dielectric permittivity reading and the volumetric water content.

V Take a small sample of the soil (about 100g) for gravimetric water content (GWC) analysis. Weigh the container before putting the sample in it and re-weighed.

VI Place in an oven set at 105°C for 24 hours.

VII Weigh the oven dry soil.

VIII Calculate the bulk density of the soil, multiply this with the GWC

IX Repeat the analysis, but this time, with more water (350g) to the 1kg soil.

For light texture soils, 100g of water should be added to 1kg soil in the initial analysis and can be increased to 200g when the analysis is repeated.

Calculation

Compare the volumetric water content from the analysis to the output from the instrument using root mean square error. A linear regression line can be used to generate a line of best fit using θ = a (ε)0.5 + b, where θ is volumetric soil moisture content and ε is the real dielectric value.

1. ***Calculation of biomass and canopy water content***

Wet weights of the plant samples are to be taken as soon as possible after collection, as plant matter can degrade quickly. To slow this process, keep samples in a cool shaded place or a cooler if possible until samples can be weighed. A scale will be located at a central location with a maximum driving time of 25mins from any field such that teams can bring biomass samples for weighing as soon as convenient to do so, ideally after each field is sampled. One individual will be tasked with weighing and drying all the samples. This person will also identify and record the phenological stage of the plant.

One plant sample from each field will be separated into component parts (leaves, stem, fruit) and these individual parts will be weighed separately. The separation of these plant components will be completed in the field, by the field crews.

Wet weight in the field laboratory

* Ensure that the balance is level
* Tare (zero) the balance
* Record a standard weight measure first ( see protocols for handling a standard weight)
* Leave plant sample in paper and plastic bag
* Place sample on the balance and record the weight on data sheet
* If plant sample is too large to sit on the balance, a larger flat surface (pan, cardboard) can be placed on the balance, then the balance should be tared (zeroed) prior to measuring the plant sample
* For samples that are partitioned, weigh each component as above and record on data sheet.
* For all samples, remove plant from paper bag and record number of leaves, phenological stage (BBCH scale)
* Store weighed samples for transportation to ROC for drying.
* Record average weight of ten paper bags for each size of bag used for the samples and record on data sheet
* Record average weight of ten plastic bags for each size of bag used for the samples and record on data sheet

Vegetation Drying

After the wet weight has been determined, remove the plastic bag and place the plant sample and paper bag into the drying trailer. Dry plant samples for 72 hours at 30°C. Ensure that the plant samples are completely dry before determining the dry weight. If unsure, place the sample back in the drying room.

Dry weight in the ROC

* Ensure that the balance is level
* Tare (zero) the balance
* Record a standard weight measure first (see protocols for handling a standard weight)
* Leave plant sample in paper bag. Place sample on scale and record weight in grams.
* Determine the size of paper bag used then weigh 10 dried paper bags. Record the weight of these 10 bags and find the average weight. This average weight is the bag weight to be used in calculation.

Plant water content (PWC) will be calculated as:



For wider spaced row crops (corn, soybeans, sunflower, canola etc.) plant water content will be scaled to an area basis (grams of water per m2) according to:



Narrow spaced low biomass crops are already collected on an area basis (0.25 m2). Ensure that the sampling area is identified prior to calculations. Thus the total plant water content is easily scaled to g/m-2 by applying a factor of 4

1. ***Lab procedures and calculation for volumetric water content***

Calibration of field instruments is vital to the success of any research. Validating the output of these soil moisture instruments increases the level of confidence in the result obtained. A bulk density core sampler will be used to collect soil samples and three soil moisture measurements will be taken about 15 cm from the sampling point. The sample must be bagged immediately to minimize moisture loss and taken to the lab for analysis. Proper labeling of the zip-lock bags as well as soil containers used in the lab is very important.

***Step 1:*** Calculate the total volume of the bulk density core (all cores are expected to be uniform): *VT = π r² h*

***Step 2:*** Weigh empty container and record as *MC*. Note the container ID.

***Step 3:*** Empty the soil sample from zip-lock bags into the container and weigh. Ensure that all soil particles are emptied from the bag and record this as *WSMC*. Find the actual weight of wet soil by subtracting *MC* (mass of the container) from *WSMC* and record this as *WS*.

***Step 4:*** Place the container with wet soil in an oven set at 105°C for 24 hours.

***Step 5:*** After oven-drying for 24 hours, reweigh the container with the dry soil and record as *DSMC*. The mass of dry soil (without the container) is simply the difference between *DSMC* and *MC*. Record this as *DS*.

Calculations:

1. Find the mass of soil moisture content by subtracting *DS* from *WS*. Record this difference as *SM*.
2. Calculate the volumetric water content (*VWC, m3 m-3*) by dividing *SM* by *WS*.
3. Determine the bulk density (ℓb, g cm-3) by dividing *DS* by the *VT*.

Definition of Terms:

*VT* = Total soil volume (volume of the core) *r* = Internal radius of core sampler

*h* = Height of core sampler *MC* = Mass of container

*WSMC* = Wet soil + container *WS* = Wet soil

*DSMC* = Dry soil + container *DS* = Dry soil

*SM* = Mass of water in soil sample *VWC* = Volumetric Water Content

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Field ID** | **Container ID** | **VT** | **MC** | **WSMC** | **WS** | **DSMC** | **DS** | **SM** | **VWC** | **ℓb** |
| F\_1\_12 | 1 | 98.2 | 28.5 | 188.5 | 160.0 | 138.5 | 110.0 | 50.0 | 0.313 | 1.11 |
| F\_1\_13 | 2 | 98.2 | 28.3 | 179.3 | 151.0 | 125.8 | 97.5 | 53.5 | 0.354 | 0.99 |

Table 1: Preview of volumetric water content and bulk density calculation

1. ***Handling Standard Weight and Scale Calibration***

The use of known standard weights is very important before determining the unknown weight of a sample. It is used to check the accuracy and precision of the weighing balance. Standard weights should be placed in a dustproof container (e.g. weight case with internal lining or a clear plastic container) with lid. Depending on the type of balance, precision should range from 0.01g to 0.1g. Weighing should be done in an environment with relatively still air.

* Ensure that the balance is level
* Place the lid on the balance and tare (zero) the balance
* Without touching the standard weight (use lifters or forceps), place it on the lid
* Compare this to the known weight of the standard.

If the difference is greater than + 0.1g, check the claim of the standard weight by using another weighing balance. Establish the standard weight by finding the average of 10 replications.

***5. Downloading Logged Data from Hydra Probes to Computer***

Must use MicroSoft (MS) Active Sync:

1. Open Microsoft Active Sync under the “Start” menu of your PC (this can be done with or without you Pocket PC in synchronized to the PC.
2. Click on the “Explore” button. There you will see the file names that you

saved the logged data to when using the HydraMon. It is likely in a .csv file. Click on this file and it should open in MS Excel which presents your logged data in a tabular format for review and analysis.

***6. Particle Size Distribution – Pipet Method***

INTRODUCTION

There are many different types of pre-treatment procedures for soil samples that are being prepared for particle size analysis. This procedure includes a standard Hydrogen peroxide digestion for the removal of organic matter and an overnight holding period to assess any potential salinity problems. If a sample is highly saline, the sample will floculate and need to be repeated.

The initial target weight for samples is 10 g. This provides a standard sample size for determining the amount of hydrogen peroxide required for organic matter removal.

The total sample size for calculating the mineral fraction percents should be either the dried and weighed sample after pretreatments have been completed or the recovered weight based on the measured sand fraction and calculated combined silt /clay fraction.

**Soil Sampling and Methods of Analysis, Carter 1993**

**47 Particle Size Distribution, Sheldrick and Wang**

**REAGENTS**

Dispersing Agent (2L)

1. -add 15.88 g sodium carbonate and 71.4 g sodium meta-phosphate to 1500 mL of RO water in the 2.5L dispensing bottle, while swirling. Use a mechanical stirrer and a stirring bar to mix the solution.
2. -when dissolved, bring the final volume to the 2 L.

SAMPLE PREPARATION

1. Thoroughly air dry the soil sample and crush all lumps, being careful not to crush any gravel or stones. Sieve off the gravel using a 2 mm sieve. If desired, this gravel weight can be recorded and reported as a percentage of the total soil sample.

**DAY 1 PROCEDURES**

1. Mix the soil thoroughly and weigh out a 10.00 g sample into a 400 mL beaker.
2. Use a water bottle to add approx. 20 mL RO water. Use the spray to break up the sample, with additional swirling of the beaker, if necessary.
3. Place the beakers in the fumehood.
4. Put on gloves / safety glasses, add 10 mL of 30% H2O2 and swirl the beaker to mix.
5. Place a watch glass on top of the beaker.
6. If the sample foams up, you can use a fine spray of water or a couple of drops of 2-octanol to knock back the foam.
7. When the reaction calms down, add an additional 10 mL of 30% H2O2.
8. Swirl and wash down the beaker as necessary.
9. Let samples digest overnight.

**DAY 2 PROCEDURES**

1. Assess the colour of the samples. If it looks like organic matter is still present add an additional 10 mL of 30% H2O2.
2. Set the hotplate dial setting to 85 (this should produce a solution temperature of about 45 C) Place beakers on the hot plate, five 400 mL beakers per hotplate.
3. If a strong reaction occurs, remove the beaker and swirl rapidly.
4. When the reaction subsides return the beakers to the hotplate and continue the digestion.
5. Monitor the reaction and be prepared to knock down any foaming.
6. When reaction is less violent, raise the hotplate dial setting to 115 ( solution temperature about 52 C). Then add an additional 10 mL 30% H2O2.
7. All samples are unique in composition and a precise determination of when complete organic matter removal has been achieved is not clearly defined.
8. Assess sample progress by the decrease in solution colour and the nature of the reaction when additional 30% H2O2 is added. (Note: higher temperatures will increase the thermal breakdown of the peroxide directly to O2 and H2O.)
9. When you have decided the reaction has been completed raised the solution temperature between 90 – 100 C by increasing the dial setting to 230. This will breakdown any remaining peroxide.
10. Don’t allow the beaker to go dry.
11. Remove from the hotplate and allow to cool down.
12. Add RO water to the 300 mL mark and use a rubber policeman to stir the sample. Rinse the soil from the rubber tip back into the beaker.
13. Allow the beakers to sit overnight.
14. Place 2 sets of 10 ea, 50 mL beakers and 1 set of 100 mL beakers in the 105 C oven and dry over night.

**DAY 3 PROCEDURES**

1. Take the 10, 100 mL sand beakers from the oven and place in the dessicator to cool for 30 minutes. Record the empty weight of the beakers.
2. Assess the condition of the solution in the sample beakers. If there is clear layer of solution on the top, decant it to waste. This may indicate there is a salinity problem with that sample.
3. If the solutions are cloudy, skip the decanting step and continue to step 7. If the sample has completely dropped to the bottom of the beaker, with a very clear solution on top, this may indicate a very high salinity level.
4. Decant away the clear solution.
5. Add additional RO water back to the 300mL mark and re-mix the sample with the rubber policeman.
6. Let sit overnight. Repeat until excess salinity has been washed away.
7. Use a pipet to add 10 mL of Dispersing agent to each 400 mL beaker of sample solution. Do all beakers at the same time, to avoid missing one.
8. Use the rubber policeman to wipe down the watch glass and the sides of the beaker.
9. Use the water bottle to aid in transferring all material from the beaker into one of the stainless milkshake cups.
10. Bring the volume of the solution in the cup up to the top of the ridges , about 600 mL.
11. Put on the mixer for 5 minutes. The mixer speed switch should be set to #2 and the rheostat set to about 40%.
12. After 5 minutes, shut off the mixer, remove the cup and let the solution sit for a few minutes.
13. Decant the solution through the #270 mesh sieve and into the 1L plastic cylinder. Silt and clay particles will pass through the sieve, while sand particles and any plant matter will collect on the screen.
14. Continue to add small volumes of RO water to the cup, swirl the solution and pour it through the sieve. This is to wash the silt and clay from the sand particles.
15. Repeat until the sand is clean. Keep observing the collecting cylinder. The volume can not go above 1L.
16. Transfer the sand to a pre-weighed 100 mL beaker. Tip the sieve upside down in the funnel and use at stream of water to wash the sand into the beaker.
17. Beakers containing the sand fraction are dried over night in the 105 C oven.
18. Bring all the cylinders to the 1L mark with RO water.
19. Take the 2 sets of 10 ea, 50 mL beakers from the oven and place in the dessicator to cool for 30 minutes. Record the empty weight of the beakers.

**DAY 4 PROCEDURES**

1. Ensure the solution level is still at the 1 L mark.
2. Check the temperature of the solution.
3. Use the paint stirrer to uniformly mix the sample solution.
4. Aggressively mix the bottom 5 cm with a rapid up and down motion. Continue mixing until you can see that all settle material is back in suspension (note if the samples have sat over a weekend then this will require extra time).
5. Now reduce the mixing speed to a steady smooth up and down motion covering the full depth of the solution. Continue for approximately 20 seconds.
6. Remove the paint mixer and take a 25 mL aliquot, using the glass pipet. This aliquot will contain the silt and clay fraction.
7. Dispense the aliquot into the pre-weighed 50 mL beaker. When the solution has drained, spray water into the pipet and collect it in the beaker. This will dislodge any particles that have settle on the inside of the pipet.
8. Repeat for the remaining samples.
9. Based on the solution temperature, use the chart to determine when to take the second aliquot. This aliquot will contain the clay fraction <0.002 mm. Note that the cylinders can be placed in a water bath to maintain constant temperature.
10. For this second fraction do not re-mix the sample.
11. Lower the pipet tip to the 10 cm depth and collect an additional 25 mL aliquot.
12. Transfer to a pre-weighed 50 mL beaker, as described above.

**Settling Time for 2 µm Clay at various Temperatures (for 10 cm)**

|  |  |
| --- | --- |
| **Temperature °C** | **Settling Time** |
| 20 | 7 hr 40 min |
| 21 | 7 hr 33 min |
| 22 | 7 hr 25 min |
| 23 | 7 hr 17 min |
| 24 | 7 hr 9 min |
| 25 | 7 hr |
| 26 | 6 hr 51 min |

Place beakers in the 105 C oven overnight.

Remove sand beakers from the oven, allow to cool for 30 minutes in the desiccator and then get a final weight of the beaker and sand.

After weighing, use the back of the balance brush to remove the sand from the beaker. Wash the beaker and return it to the tray.

**DAY 5 PROCEDURES**

Remove the 2 sets of 10ea, 50 mL beakers from the oven, allow to cool for 30 minutes in the desiccator and then get a final weight of the beaker and contents.

It is not necessary to wash out the 50 mL beakers. Just tap out any loose material and return the beakers to the tray.

Trays are stored in numerical order in the above counter cupboards.

Calculations:

**Based on recovered weight after pretreatments**

Recovered Wgt =

40 x (silt,clay+beaker wgt(g) – beaker wgt (g) – 0.0109(g)) – (sand+beaker wgt(g) – sand wgt(g)

Note: the mass of dispersing agent in the 25 mL sample aliquot is 0.0109 g

% sand = ( sand + beaker wgt (g) – beaker wgt (g) ) x 100

Recovered sample wgt (g)

% clay = 40 x (clay + beaker wgt (g) – beaker wgt (g) – dispersing agent wgt (g)) x 100

Recovered sample wgt (g)

% silt = 100 - % sand - % clay