

Protocol – OHHW
CHNS/O Analyser 2400 Series II
Perkin Elmer Company
Prepared by Soung Ryu & Gwen Tenney
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BACKGROUND:

Take soil samples at each of the 4 PVC stakes and the center rebar for each subplot. This makes a total of 5 samples to composite together in a Whirl-pak to minimize high sample variability.

Mix samples thoroughly in the whirl-pak and **freeze soil** samples if they will not be processed immediately.

Grind a portion of the composited soil (~10 grams should be plenty**) until the sample looks homogeneous (~1 minute).

*(NOTE: **Ground litter or roots** can also be used in the CHN analyzer. These samples should also be dried before grinding).*

ENCAPSULATING:

AD-4 Autobalance is normally turned on already. Hit “Range” and see 20mg on the machine’s screen to set the output at 3 decimal places. Leave at 1000 mg when you are finished.

Calibrate with a weight of 10mg** found in the drawer below the balance. Slide open the glass door and put the weight on the right balance and leave nothing on the left balance. Close the door and lower the pedestals supporting the balances. If the machine doesn’t read 10000 then type in the number “10000” and hit “calibrate.” Raise the pedestals and open the glass door. *(NOTE: the pedestals should always be up when the glass door is open because the machine is delicate and the balances need to be supported when exposed to the drafty room.)*

Tare with a squished tin cup on the left balance and another empty but unsquished tin cup on the right balance. Hit “autotare.”

Scoop a small amount of ground soil into the empty tin cup and place the cup back on the right balance to measure it. *(NOTE: The amount of material depends on its nutrient content and if it’s soil or litter. Generally, samples should be about 5mg, but may sometimes range up between 15-50 mg. Using too much material is harder on the CHN machine and requires replacing tubes more often).* Be sure to also encapsulate and standards you are using like **apple leaves** (use ~5 mg especially when processing roots; the certificate of standard content is digitized in another document) and **k-factors** (using ~3 mg).

Squish the filled tin cup without touching the cup or the samples with your fingers (your skin oils would contaminate the sample). Be sure not to puncture the cup, but also be sure the cup is folded over enough to keep the sample inside. This takes practice!

Measure and Record the final weight of the squished cup, the sample number (i.e. location and type), and the slot you are placing the encapsulation into. Later, you will add another number describing the slot in the CHN where you moved the sample to. (*NOTE: If the display says "int" do nothing because the machine is still measuring*).

TURNING ON THE CHN:

Turn on gas by opening the two knobs on the helium tank (*NOT* the one in front). The oxygen tank and nitrogen tanks are normally left on. Helium runs out fairly often, but oxygen and nitrogen last many years before needing to be replaced.

Turn on CHN power by flipping the switch on the side. (*NOTE: some directions say to leave the machine on 3-5 hours before making measurements, but we have found that 1-2 hours is generally enough time****).

Enter parameters as the computer asks. Enter in "200" for reduction to say that 200 samples can be run. Enter "200" for combustion to say that 200 samples can be run. (*NOTE: If the reduction tube is gone, numbers for nitrogen will show up as too high. A new reduction tube should last for ~150-300 samples. When the combustion tube has run out, carbon and helium will be too high. A new combustion tube should last for about 1000 samples. If either of these counts run down to zero while performing analyses the machine will automatically stop, so be sure to set them high at ~200 and reset them if you have run 200 samples without turning off the machine*). **VRCP** is also "200." Hit "YES" for **operator** 12345 (unless you want your own operator ID). The oven temperature aka **combustion tube** (parameter 7) should be 925 degrees C (minimum 500 C) and the **reduction tube** (parameter 8) should be 640 C (minimum 500 C). **Fill time** is "parameter" and "9" and should be set at 29 (or 28). If fill time is too high, increase the helium pressure and if fill time is too low decrease helium pressure. There is no absolute right fill time, but it should stay fairly constant on the data printout.

Purge the gasses and check pressure with helium purging for ~120-300 seconds and oxygen purging ~45-100 seconds. The proper air pressure is listed on the gas dials. Helium has to be higher pressure than Oxygen (*NOTE: If there is not enough oxygen combustion will be incomplete and CHN results will vary widely between samples*).

Turn on furnace by hitting "parameters" and code "12" with option "1" to turn on the furnace.

Check to see if temperatures increasing by hitting "Monitor" and "1" for combustion and "2" for reduction. The numbers should be steadily increasing towards 925 and 640, respectively. It normally takes between 30 minutes to an hour for the CHN to reach proper temperature.

Turn on gas saver if not starting measurements immediately. This will allow the machine to warm up without using up gas unnecessarily. Hit "Parameter" and "22" to turn the gas saver on by hitting "1". Make sure you **turn off the gas saver** by hitting option "2" *before* starting to run samples or performing the leak test!!!

Then, wait... and wait... and...

Check temperatures to see if they are 925, 640 and then do a **leak test** to make sure the combustion tube and reduction tube are fitted securely. (*NOTE: make sure gas saver is off first!*) Hit "Diagnostics," then "2" for gas, then "1" for leak test. Tell it to do "1" test on the reduction tube "1." Then, if it says leak test passed, tell it to do "1" test on the combustion tube "2." If the leak test fails you can't run analyses; turn off the machine by "Parameter" and "12" and turn off helium to let it cool so you can adjust the tubes (see below).

RUNNING SAMPLES

There are many ways to see if the machine is functioning well, here is one way.

Run standards before loading samples in case the machine still isn't working correctly.

Blanks are the first thing to try running. Do a "single run" of 3 blanks to flush out the machine. Drop a squished tin cup (or nothing) into the sample rack beginning with the sample cup that is an open hole, and proceeding counterclockwise. (The turntable moves *AFTER* finishing a sample). Run as a "1" sample because you are running blanks. The printout should show all positive numbers:

C ~ <100	with a difference of <30 between blank samples
H ~ 200-300	with a difference of 100 between blanks
N ~ <50	with a difference of 16 between blanks
Signals ~ 9000	should be fairly consistent between blanks

(*NOTE: If the numbers aren't good, try running more blanks or try a k-factor*).

K-factors should then be run if the blanks are successful and the baseline numbers have stabilized. Start with ~2-3 to be sure the machine is working and then insert a k-factor every ~10-15 samples thereafter so you can be certain your analysis remains accurate throughout. (*NOTE: It is better to make sure k-factors are good before loading the sample rack*). Run k-factors as a "3" sample. Enter in the ID numbers you're using. Letters can be typed by using numerical code (i.e. A is .01 and B is .02 and so on). For our k-factors, the printout should show:

C ~ 71.09
H ~ 6.71
N ~ 10.36
Signals should be fairly constant

LOADING THE RACK

Load samples with about 12 samples if k-factors came out nicely. Insert another k-factor after every 10-15 samples. Run as "autorun" and hit "4" to reset values. The

machine counts the number of samples run, but it will stop running at #120 (two full turntables worth of samples) so be careful! Hit “3” for sample (*NOTE: if you accidentally hit “1” I don’t know how to undo this so just leave a space in the turntable for the blank you just told the machine to run.*) You can add the next set of samples into the rack and enter data while the machine is running. **Check previous entries** by hitting the arrow key to scroll to the sample, then enter in the ru# and look at what you wanted, then go back to “autorun” to resume data entry.

TURNING THE MACHINE OFF

Parameter “12,” and turn the furnace “off,” then flip the off switch and turn off the Helium by closing the two knobs on the helium tank (*NOT* the one in front).

TROUBLESHOOTING and MAINTANENCE

Adjusting the tubes from a failed leak test is potentially difficult and it may need to happen multiple times before the leak test is passed. The combustion tube is on the left and reduction is on the right. **Take out the tubes** by unscrewing the gold bolts on top and bottom (4 total) and then unscrew the wheel on the bottom to lean out the full box. Try simply reinserting the tubes, always being careful to not break them; you may need to change the tubes if the leak test keeps failing.

Check for blockage if leak test fails through three ways. **First, check valves** by taking off the right side panel, which is where all the valves are located. Put your finger in front of the hose coming off valve E; you should feel pressure coming out. If you don’t there is a blockage. **Second, purge** by pressing “purge” and select helium and purge for 300 seconds. Press “Monitor” and code “4.” If you do not see the pressure appear, there is a blockage. **Thirdly, check the 3 filters** because it is possible the filters are blocking the flow of gas, which causes the pressure to go down and the leak test to fail. One is in the bottom of the combustion tube where it goes into the block/crossover. The second is in the bottom of the crossover at the reduction tube. The third is at the top of the reduction tube where it goes into the block.

Change tubes by removing the old tubes (see above). You change the reduction tube more often, but still clean out the top of the combustion tube when changing the reduction tube. Before filling a tube, check to make sure it fits squarely in the machine- some tubes are a bit crooked and *SHOULD NOT* be used. You have to heat the silver gauze to red hot when changing and packing tubes. Use a bunsen burner (available in the lab across from BO 3013). Be sure to change the filter in the reduction tube (it gets black and disgusting). Precut filters are expensive so we cut out a filter to size from paper. Use enough quartz wool because not using enough can cause machine malfunctions. *Be cautious when handling quartz wool by using it in only in the hood because it can lead to lung related diseases.* Extensive instructions are in the chapter 4 of the manual.***

Printing a master list summarizing ID#, weight, and sample# can be done by going to “autotun” and “4” and “option 2” for printing. Thus must be done while the machine is

stopped. Printing this list can be helpful if something is not working right and you want to see exactly where the data went bad. Printing a complete list including CHN values can be done through “print results.”

Message 35 sometimes comes up as an error. We don't know what this message means and the company itself has been unable to provide answers! When the error comes up, just turn the machine off and back on again.

COMPANY CONTACT INFORMATION

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SUPPLIES

Here's the supplies needed to process 1000 samples, as assembled by Soung Ryu.

PerkinElmer 1-800-762-4000		Quantity	Unit Price	
Combustion tube	N241-1264E	1	\$ 145.00	\$ 145.00
EA-1000	N241-0092	1	\$ 170.00	\$ 170.00
Silver Tungstate on Magnesium Oxide	0240-1344	0.05	\$ 420.00	\$ 21.00
Silver Vanadate	0240-1117	0.7	\$ 91.00	\$ 63.70
Silver Gauze		0.5		\$ -
Reduction Tube	N241-1291E	5	\$ 125.00	\$ 625.00
Copper oxide	0240-1092	0.15	\$ 105.00	\$ 15.75
Coper Plus	0240-0117	3	\$ 170.00	\$ 510.00
Silver Gauze		0.5		\$ -
He gas		2	\$ 90.00	\$ 180.00
Filter	0240-0137	0.5	\$ 38.00	\$ 19.00
Tin cups (Standard size 5X8mm)	N241-0155	1	\$ 675.00	\$ 675.00
				\$2,424.45

Additionally, check Costech instead of PerkinElmer for cheaper prices.

Costech 1-800-524-7219		Quantity	Unit Price	
Tin cups (standard size 5X9mm)	41077	4	\$ 26.00	\$ 104.00
				\$ 104.00

IMPORTANT PARAMATERS LIST

Parameter "7"	Combustion Tube
Parameter "8"	Reduction Tube
Parameter "9"	Fill Time
Parameter "12"	Furnace
Parameter "22"	Gas Saver