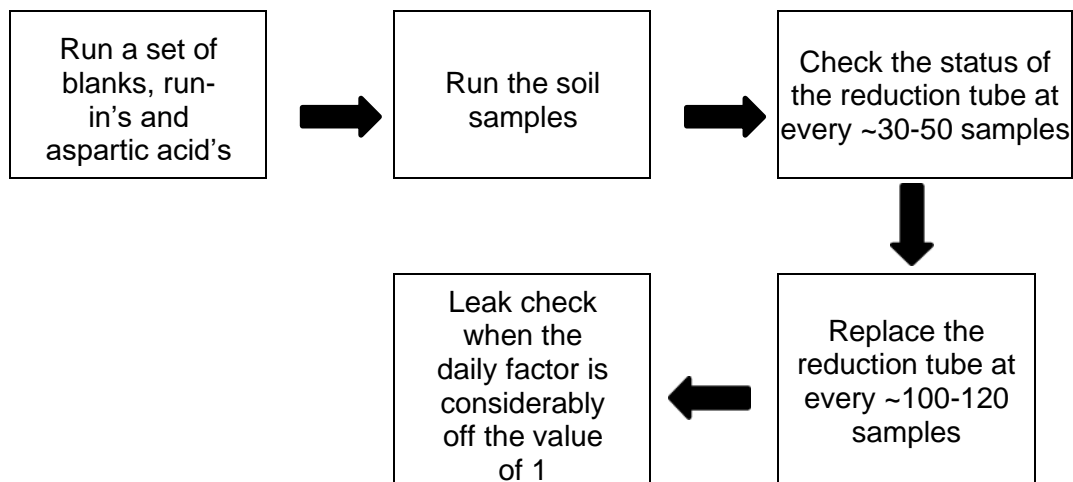


SOP: Elemental Analysis of Soil Carbon and Nitrogen

Overview:

This standard operating procedure (SOP) describes a protocol for high-temperature (900-1200 °C) combustion of soils to determine total carbon (C) or nitrogen (N) content (%) via the Elementar Vario MAX cube ® (Langenselbold, Germany). Key consumables are aspartic acid, crucibles, quartz wool, zinc, copper, copper oxide, tungsten, corundum balls, cotton, Sicapent ®, wire mesh rondes, spacers, spare tubes, oxygen gas, and helium; and key safety considerations are the use of heat protective gloves (capable of holding ≥ 950 °C) when doing maintenance work and manipulating reduction/ combustion/ post-combustion/ drying tubes. Soils that are ground to pass a <2 mm sieve are typically used.



Safety:

All standard safety protocols and online safety training via UIUC [Division of Research Safety \(DRS\)](#) are required.

Personal protection (PPE) for this procedure include:

Body Protection: Laboratory coat

Hand Protection: Nitrile gloves when manipulating samples/ aspartic acid, heat protective gloves (capable of holding ≥ 950 °C) when doing maintenance work and manipulating reduction/ combustion/ post-combustion/ drying tubes

Instrumentation & Consumables:

Sample preparation (blanks, run-ins, aspartic acid and soils)

- Crucibles (steel)
- Aspartic acid ($C_4H_7NO_4$, MW: 133.11)

- Analytical balance (three decimal places sensitivity)
- Spatula

Maintenance work (replacing parts)

I. Reduction tube

- Corundum balls
- Wire mesh ronde
- Wire mesh separators (10)
- Tungsten (MW: 183.84)
- Copper oxide (CuO, MW: 79.54)
- Copper (MW: 63.55)
- Quartz wool
- Zinc (MW: 65.38)

II. Combustion tube

- Spacer
- Wire mesh ronde
- Corundum balls
- Copper oxide (CuO, MW: 79.54)

III. Post-combustion tube

- Spacer
- Wire mesh ronde
- Copper oxide (CuO, MW: 79.54)
- Platinum catalyst
- Quartz wool

IV. Drying tubes

- Cotton
- Sicapent ®

Detailed Procedure:

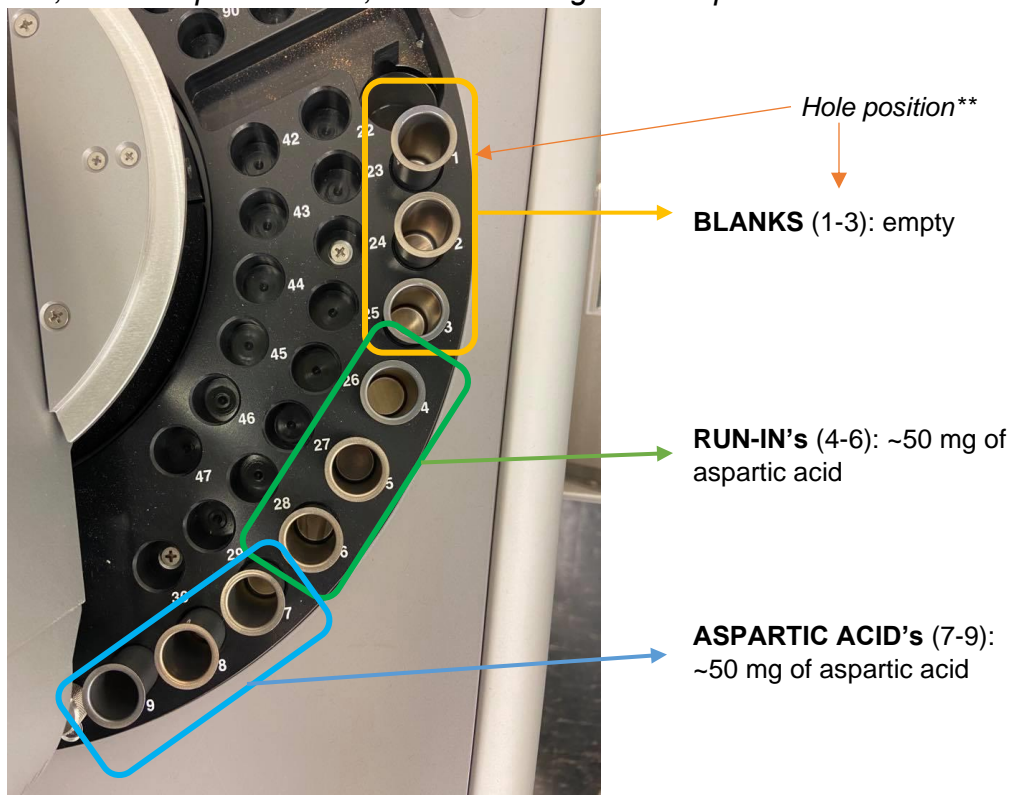
I. C/N determination

1. Sample preparation

**Note that liquid samples could be run, but this protocol focuses on C and N measurement in (solid) soil samples*

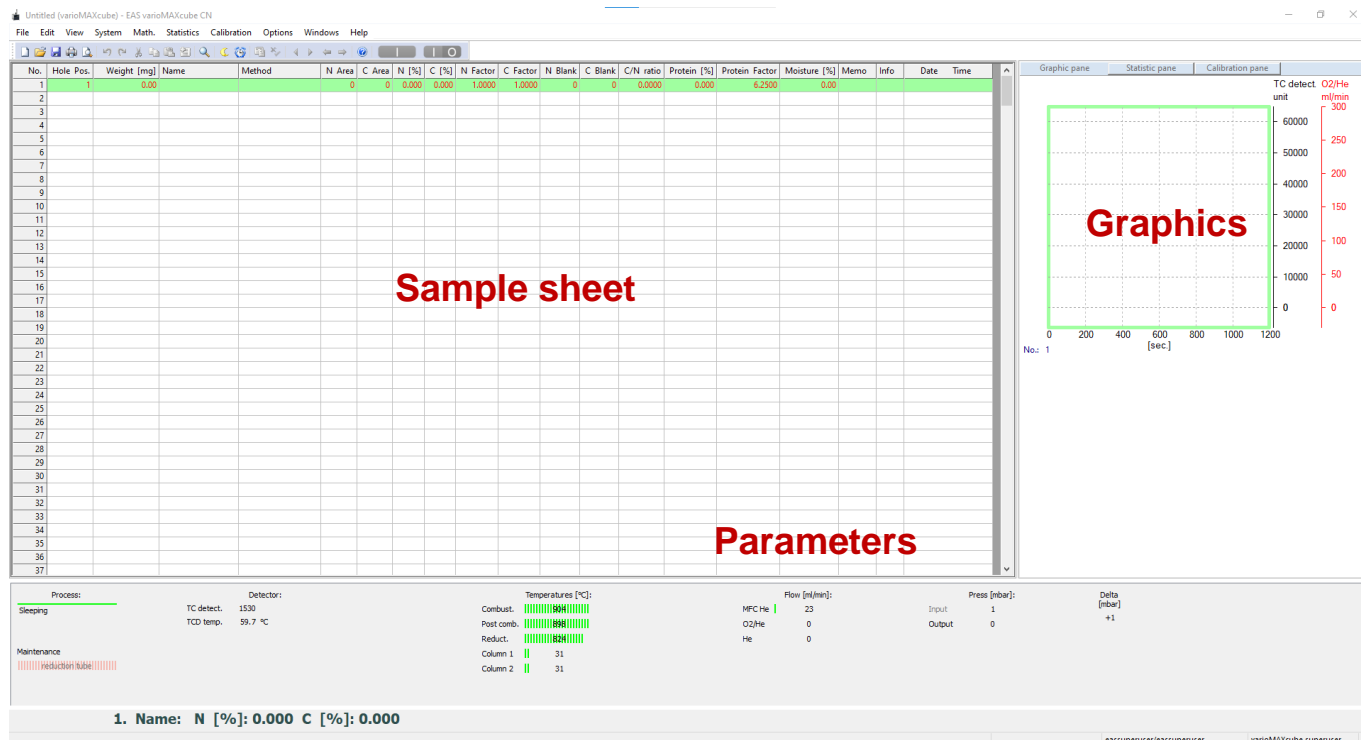
- 1.1. Place three empty crucibles on the position of 1-3 of the sample plate. They will be used as **blank** samples.
- 1.2. Measure ~ 50 mg of aspartic acid into three crucible and place them in sequence after the blanks. They will be used as **run-in** samples. Record the soil mass (mg) of each sample as it will need to be entered into the sample sheet.
- 1.3. Measure ~ 50 mg of aspartic acid into three crucibles and place them in sequence after the run-ins. They will be used as **aspartic acid** samples. Record the soil mass (mg) of each sample as it will need to be entered into the sample sheet.
- 1.4. Measure ~ 250-500 mg of dried soils into each crucible and place them in sequence after the aspartic acid samples (higher end ~350-500 mg recommended for samples low on N). Record the soil mass (mg) of each sample as it will need to be entered into the sample sheet.

Note: The photo below shows the blank, run-in, and aspartic acid samples on the sample plate. At the beginning of the experiment, and every 24 hours, one must run 3 blanks, 3 run-in's, and 3 aspartic acid's, before running soil samples.



2. Software settings

- 2.1. Open the EAS Vario Max Cube program in the desktop.
- 2.2. File > New (a new – empty – sample sheet* will appear) to create a new project:



*Where Hole Pos. corresponds to the position in the sample plate (marked by numbers, can be modified manually), Weight is the mass in mg of the corresponding sample, Name is the arbitrary label of the corresponding sample, and Method is the corresponding measuring technique for each kind of sample (e.g., **blank [O2]** for blanks, **aspartic acid 1** for run-in's and aspartic acid's, and **soil** for soil samples).

- 2.3. Set up the first 3 rows to run blanks.

- Under Weight [mg], manually type 1.00 (this is an arbitrary value just for the software to accept the sample, the crucible remains empty)

No.	Hole Pos.	Weight [mg]	Name	Method
1	1	1.00	Blnk	blank [O2]
2	2	0.00		
3				

- Under Name, select "Blnk"

No.	Hole Pos.	Weight [mg]	Name	Me
1	1	0.00	Blnk	
2			Windsor	
3			G3-7	
4			G3-6	
5			G3-5	
6			G3-3	
7			G3-2	
8			aspartic acid	
9			RunIn	
10			Blnk	
11			Test	
			glutamic acid	
			uric acid	

- Under Method, select “blank [O2]”

No.	Hole Pos.	Weight [mg]	Name	Method
1	1	0.00	Blnk	blank [O2]
2	2	0.00		blank [O2]
3				butter
4				cereals
5				cheese
6				coal
7				fishmeal
8				glutamic acid
9				milk
10				milk powder
11				oil
12				plant
				sausage

2.4. Set up the next 3 rows for RunIn

- Type the weight in mg of each corresponding run-in sample (~50 mg)
- Under Name, select “RunIn”
- Under Method, select “aspartic acid 1”

Note that there are three different aspartic acid methods (aspartic acid 1, aspartic acid 2, and aspartic acid 3). The differentiation between these is based on the weight [mg] of aspartic acid to use (aspartic acid 1 for 0-120 mg, aspartic acid 2 for 120-600 mg, and aspartic acid 3 for 600-1100 mg). In our case, as we are using ~50 mg of aspartic acid, the corresponding method is **aspartic acid 1.*

2.5. Set up the next 3 rows for aspartic acid

- Type the weight in mg of aspartic acid (~50 mg) in each corresponding sample
- Under Name, select “aspartic acid”
- Under Method, select “aspartic acid 1”

2.6. Set up the following rows for soils

- Type the weight in mg of soils in each corresponding sample (~250-500 mg, recommended)
- Under Name, type the name ID of each soil sample
- Under Method, select "soil"

At the end of these steps, the overall data sheet must look like this:

Untitled (varioMAXcube) - EAS varioMAXcube CN

File Edit View System Math. Statistics Calibration Options Windows Help

No.	Hole Pos.	Weight [mg]	Name	Method	N Area	C Area	N [%]	C [%]	N Factor	C Factor	N Blank	C Blank	C/N ratio	Protein [%]	Protein Factor	Moisture [%]	Memo
1	1	1.00	Blank	blank [O2]	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
2	2	1.00	Blank	blank [O2]	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
3	3	1.00	Blank	blank [O2]	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
4	4	50.00	RunIn	aspartic acid 1	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
5	5	50.00	RunIn	aspartic acid 1	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
6	6	50.00	RunIn	aspartic acid 1	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
7	7	50.00	aspartic acid	aspartic acid 1	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
8	8	50.00	aspartic acid	aspartic acid 1	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
9	9	50.00	aspartic acid	aspartic acid 1	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
10	10	250.00	S1	soil	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
11	11	250.00	S2	soil	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
12	12	250.00	S3	soil	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
13	13	250.00	S4	soil	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
14	14	250.00	S5	soil	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
15	15	250.00	S6	soil	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
16	16	250.00	S7	soil	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
17	17	250.00	S8	soil	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
18	18	250.00	S9	soil	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
19	19	250.00	S10	soil	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	

**Note that every 20-25 samples, must run one or two blanks (empty) and an aspartic acid sample:*

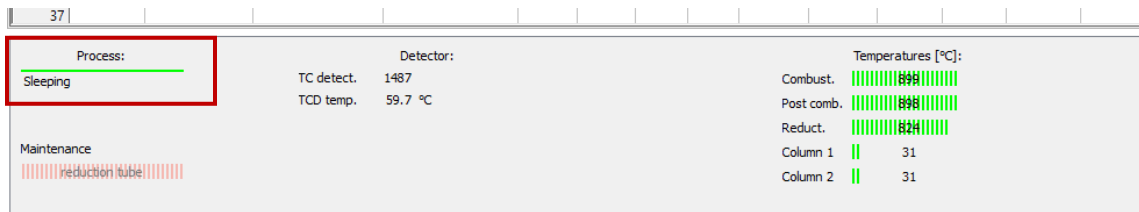
Untitled (varioMAXcube) - EAS varioMAXcube CN

File Edit View System Math. Statistics Calibration Options Windows Help

No.	Hole Pos.	Weight [mg]	Name	Method	N Area	C Area	N [%]	C [%]	N Factor	C Factor	N Blank	C Blank	C/N ratio	Protein [%]	Protein Factor	Moisture [%]	Memo
1	1	1.00	Blank	blank [O2]	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
2	2	1.00	Blank	blank [O2]	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
3	3	1.00	Blank	blank [O2]	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
4	4	50.00	RunIn	aspartic acid 1	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
5	5	50.00	RunIn	aspartic acid 1	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
6	6	50.00	RunIn	aspartic acid 1	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
7	7	50.00	aspartic acid	aspartic acid 1	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
8	8	50.00	aspartic acid	aspartic acid 1	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
9	9	50.00	aspartic acid	aspartic acid 1	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
10	10	250.00	S1	soil	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
11	11	250.00	S2	soil	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
12	12	250.00	S3	soil	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
13	13	250.00	S4	soil	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
14	14	250.00	S5	soil	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
15	15	250.00	S6	soil	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
16	16	250.00	S7	soil	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
17	17	250.00	S8	soil	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
18	18	250.00	S9	soil	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
19	19	250.00	S10	soil	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
20	20	250.00	S11	soil	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
21	21	1.00	Blank	blank [O2]	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
22	22	1.00	Blank	blank [O2]	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
23	23	50.00	aspartic acid	aspartic acid 1	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
24	24	250.00	S12	soil	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
25	25	250.00	S13	soil	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	
26	26	250.00	S14	soil	0	0	0.000	0.000	1.0000	1.0000	0	0	0.0000	0.000	6.2500	0.00	

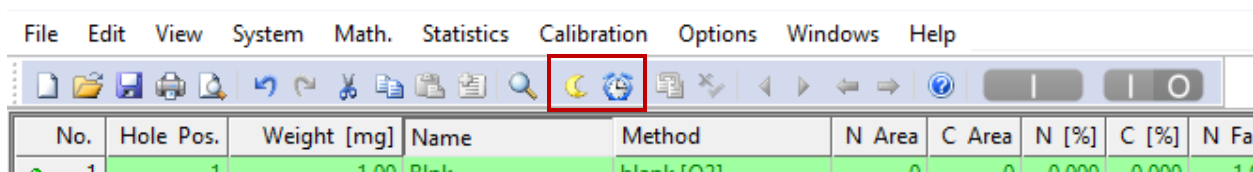
3. Sample running

**Note that when not in operation, the machine stays in a sleeping mode:*

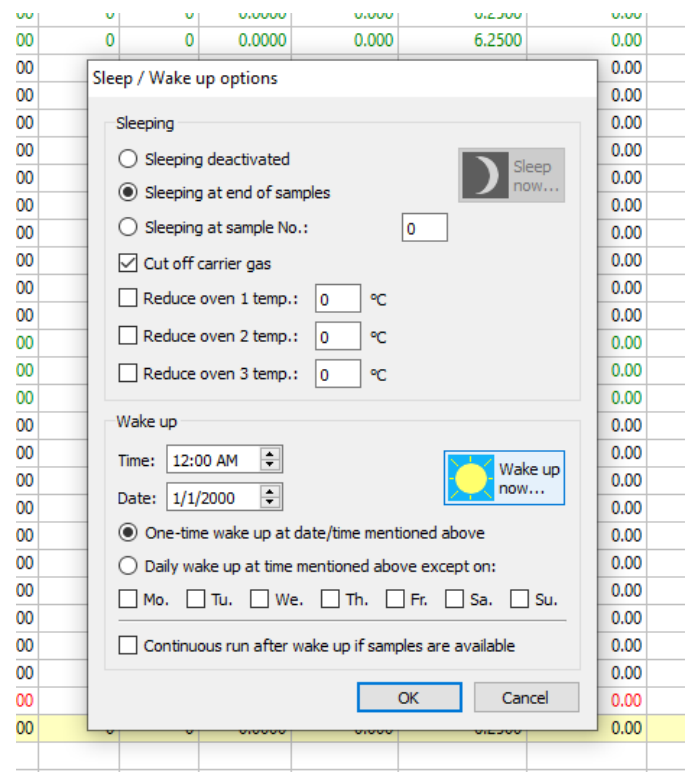


To run samples:

3.1. Click on the moon or the clock symbol to wake up the machine:

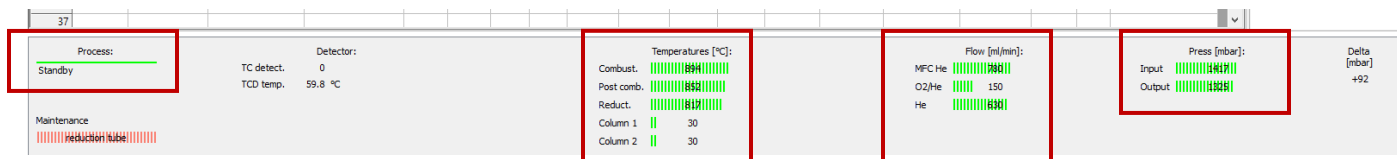


3.2. A new window will appear. Click on the “Wake up now” button.



**Note: before clicking the Wake up now button, make sure that the Sleeping at the end of samples, Cut off carrier gas, and One-time wake up at [...] settings are selected, as displayed above. This way the machine will automatically go to sleep when it finishes running the last samples. This is convenient especially when samples are left running overnight.*

- 3.3. Wait for the process to be on Standby, and the temperature, flow, and pressure bars to be green colored (before running samples).



- 3.4. To start – automatically – running samples (auto-analysis, e.g., setting the machine to run from the first to the last sample), click on the long green button in the toolbar.

File Edit View System Math. Statistics Calibration Options Windows Help

No.	Hole Pos.	Weight [mg]	Name	Method	N Area	C Area	N [%]	C [%]	N Factor	C Factor	N Blank	C F
1	1	1.00	Blank	blank [O2]	0	0	0.000	0.000	1.0000	1.0000	0	
2	2	1.00	Blank	blank [O2]	0	0	0.000	0.000	1.0000	1.0000	0	
3	3	1.00	Blank	blank [O2]	0	0	0.000	0.000	1.0000	1.0000	0	
4	4	50.00	RunIn	aspartic acid 1	0	0	0.000	0.000	1.0000	1.0000	0	
5	5	50.00	RunIn	aspartic acid 1	0	0	0.000	0.000	1.0000	1.0000	0	

OR, if you want to run a single sample, click on the short green button (this will only run the current sample).

File Edit View System Math. Statistics Calibration Options Windows Help

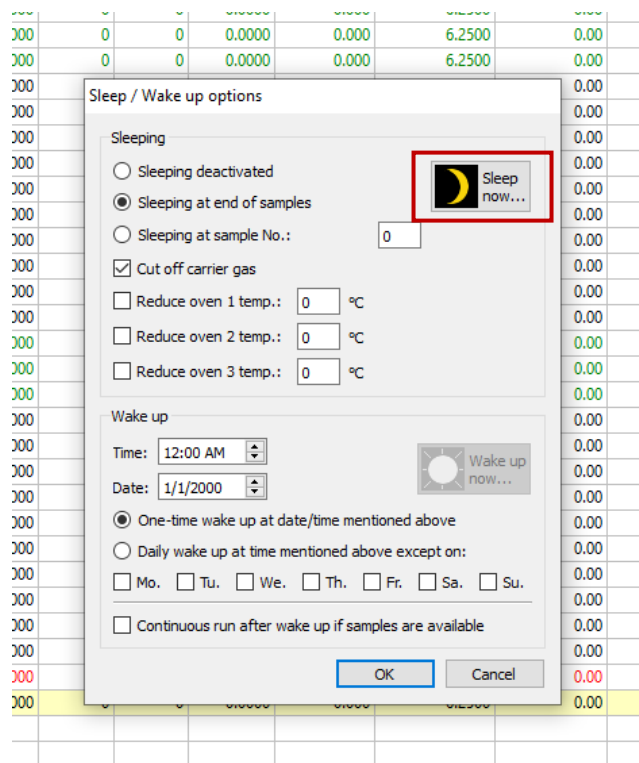
No.	Hole Pos.	Weight [mg]	Name	Method	N Area	C Area	N [%]	C [%]	N Factor	C Factor	N Blank	C F
1	1	1.00	Blank	blank [O2]	0	0	0.000	0.000	1.0000	1.0000	0	
2	2	1.00	Blank	blank [O2]	0	0	0.000	0.000	1.0000	1.0000	0	
3	3	1.00	Blank	blank [O2]	0	0	0.000	0.000	1.0000	1.0000	0	
4	4	50.00	RunIn	aspartic acid 1	0	0	0.000	0.000	1.0000	1.0000	0	
5	5	50.00	RunIn	aspartic acid 1	0	0	0.000	0.000	1.0000	1.0000	0	

**Note: the short red button can be used to stop the auto-analysis, as well as the single-analysis.*

File Edit View System Math. Statistics Calibration Options Windows Help

No.	Hole Pos.	Weight [mg]	Name	Method	N Area	C Area	N [%]	C [%]	N Factor	C Factor	N Blank	C F
1	1	1.00	Blank	blank [O2]	0	0	0.000	0.000	1.0000	1.0000	0	
2	2	1.00	Blank	blank [O2]	0	0	0.000	0.000	1.0000	1.0000	0	
3	3	1.00	Blank	blank [O2]	0	0	0.000	0.000	1.0000	1.0000	0	
4	4	50.00	RunIn	aspartic acid 1	0	0	0.000	0.000	1.0000	1.0000	0	
5	5	50.00	RunIn	aspartic acid 1	0	0	0.000	0.000	1.0000	1.0000	0	

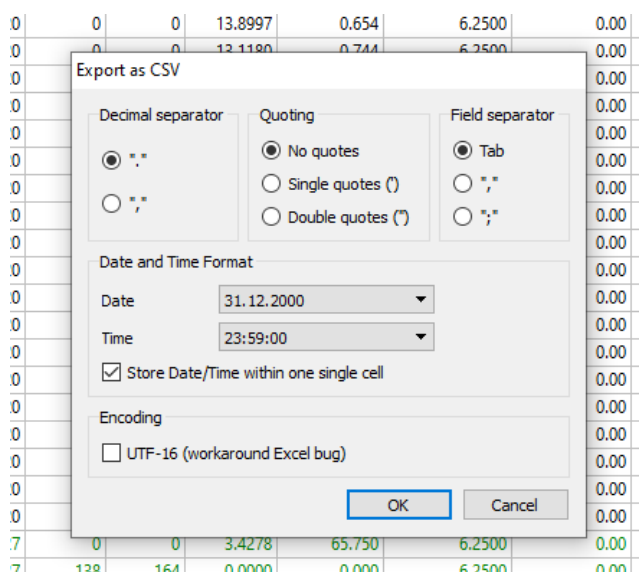
Once the analysis is finished, the machine will go back to sleeping mode if the Sleeping at the end of samples option was selected. If not, one can manually turn on the sleeping mode by clicking one more time in the moon symbol at the toolbar, and then clicking the “Sleep now” button in the appearing window:



**Note: to save the project and retrieve it later for further work directly in the Vario Max Cube ® software: File > Save As > type a name. Then to open it: File > Open.*

4. Exporting data

- 4.1. Export/Import > Export as CVS (for Excel)
- 4.2. A new window will appear. Click on OK.



4.3. Once saved, the Excel sheet must look like this:

AutoSave

FileHomeInsertPage LayoutFormulasDataReviewViewHelpAcrobat

Calibri11A^A

II. Maintenance work

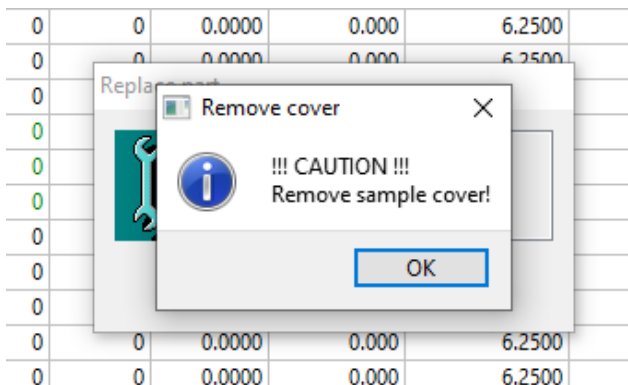
1. Checking/Replacing the reduction tube

The reduction tube must be replaced once all layers of tungsten are expanded and burned (e.g., change of color to black with yellow spotting). For soil analysis, this is thought to happen each ~100-120 samples. Periodic checking of the status of tungsten in the reduction tube, in between a determinate number of samples (~30-50 samples), is recommended.

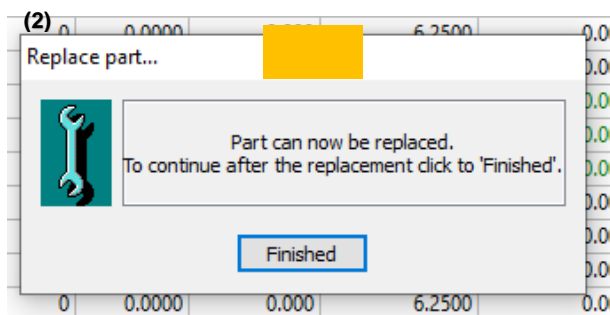
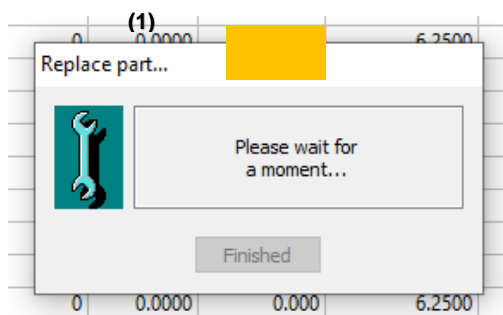
- 1.1. Options > Maintenance > Replace parts
- 1.2. A new window will appear. Read the caution notice and click on Next.



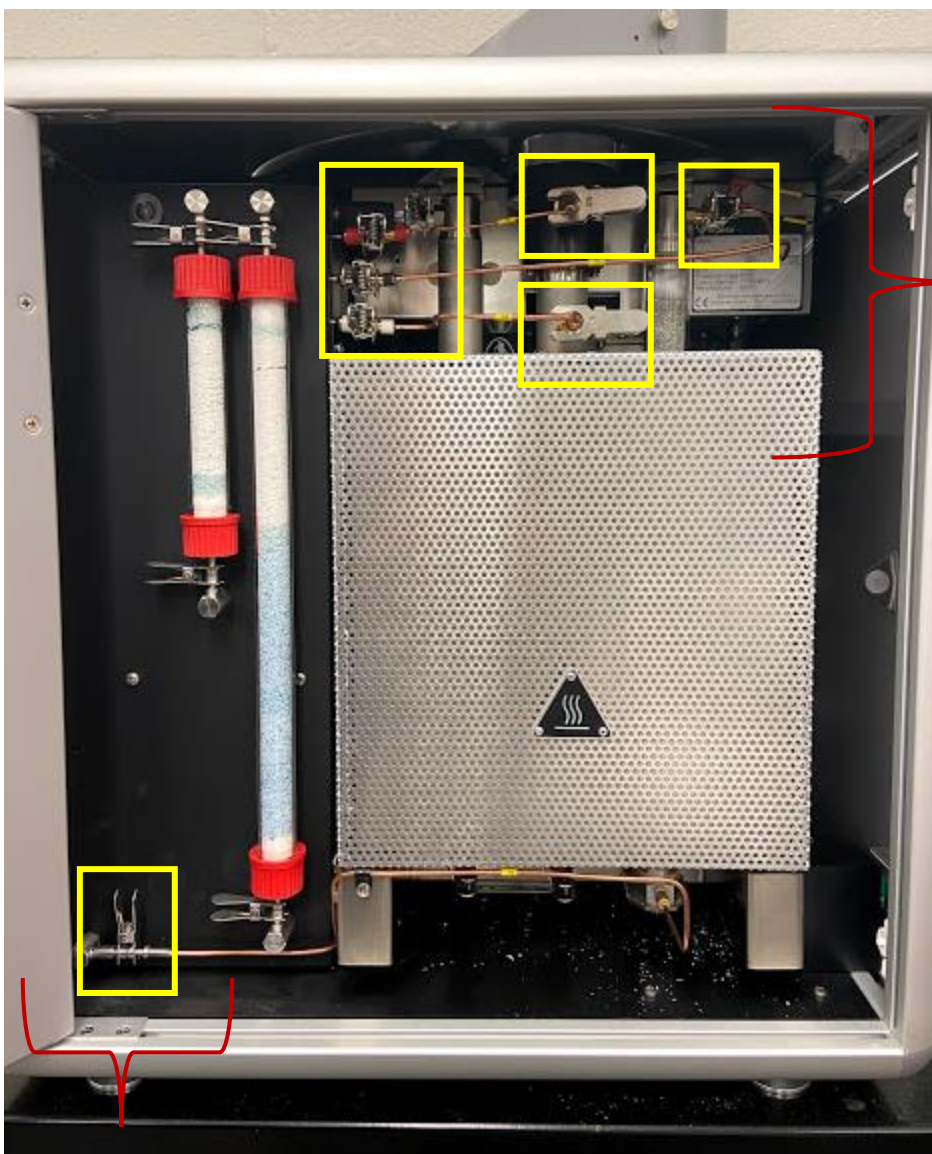
- 1.3. A new window will appear. Remove the sample cover and click on OK.



- 1.4. A new window will appear. Please wait for a moment (1) until the next window (2) shows up. **DO NOT** click on finished **until** you have placed the reduction tube back, re-assembled the core and placed back the sample cover (see details for this below).



- 1.5. Once the "Part can now be replaced [...]" window is up, you may open the front door of the hardware and start disassembling the core.
- 1.6. Remove all clamps and metal connections from the core (see picture below, yellow marked = remove).



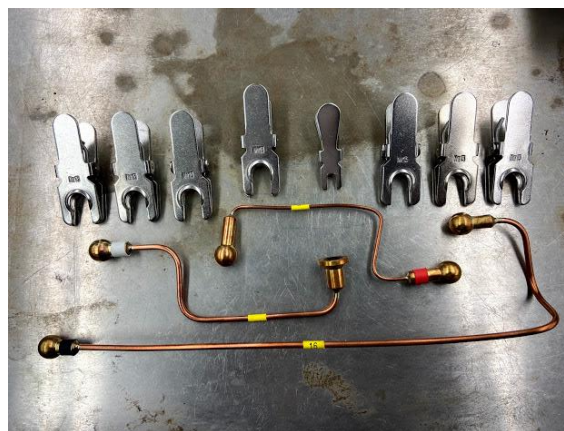
TOP

Remove all clamps and metal connections.

BOTTOM

Remove this clamp **only**.

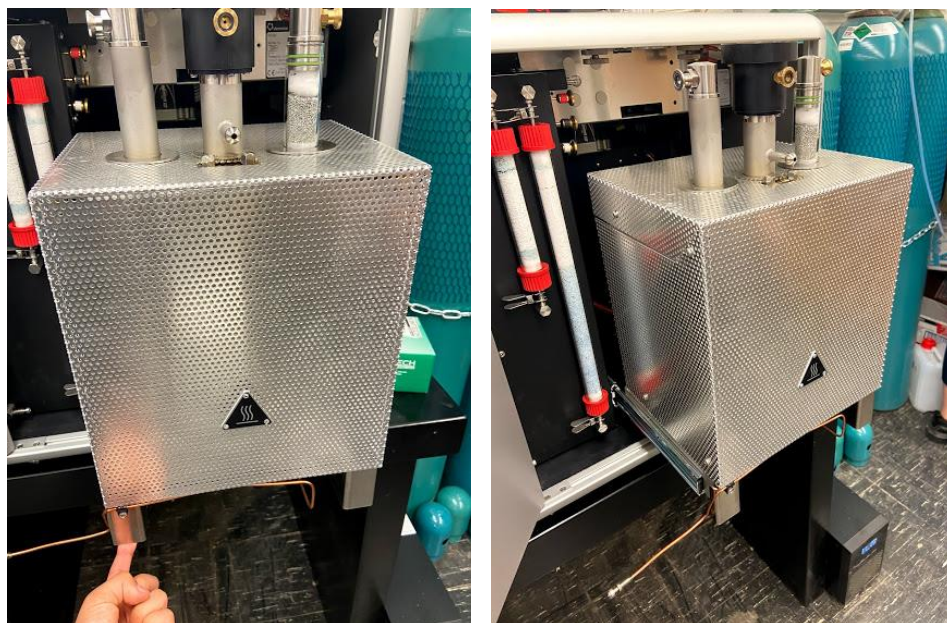
**Note: a total of 8 clamps (7 regular, 1 small) and 3 metal connections must be removed (see picture on the right).*



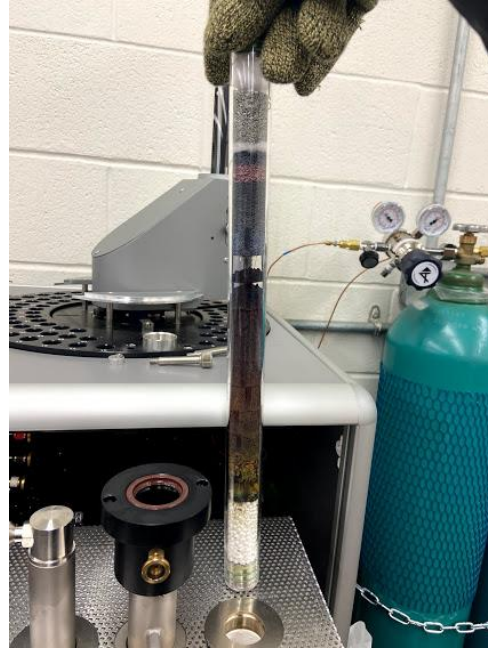
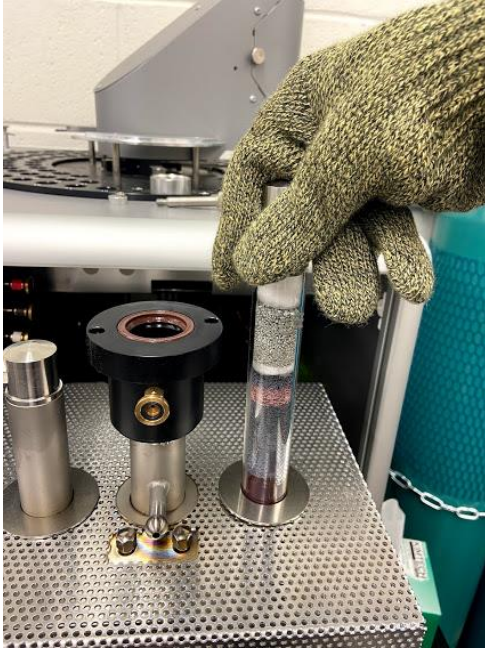
- 1.7. Careful not to drop anything into the combustion tube, remove the screws at the sample plate.



- 1.8. Carefully pull the core towards the front by grabbing it from the bottom part.

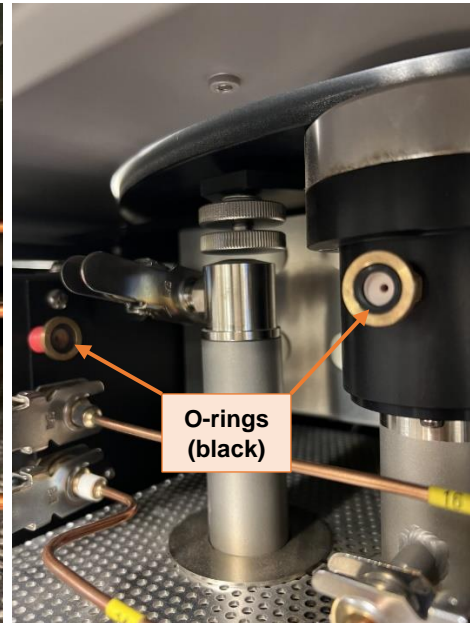


- 1.9. Carefully take out the reduction tube (third from left to right) and place it in a steel rack (use of heat protective gloves is mandatory, the tube is at $> 900^{\circ}\text{C}$).

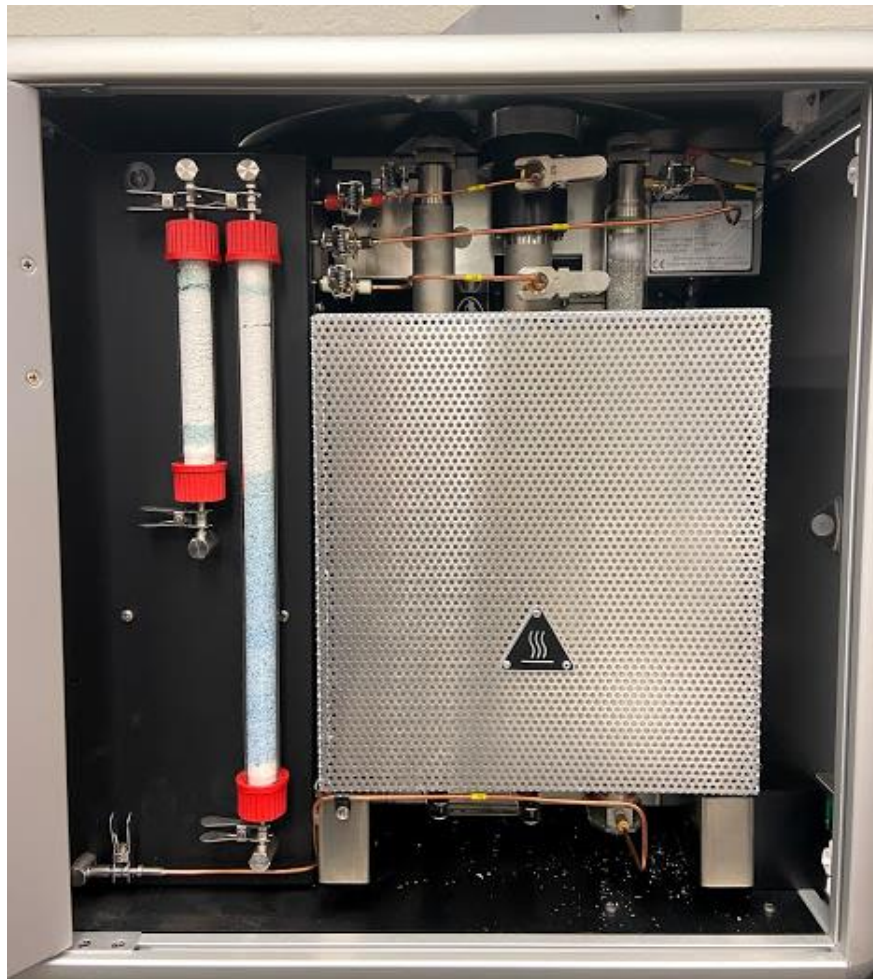


- 1.10. Insert back the new (if replacing) or current (if just checking) reduction tube, and re-assemble all parts (clamps, metal connections and screws). Make sure to check that the O-rings of every connection are still in place.

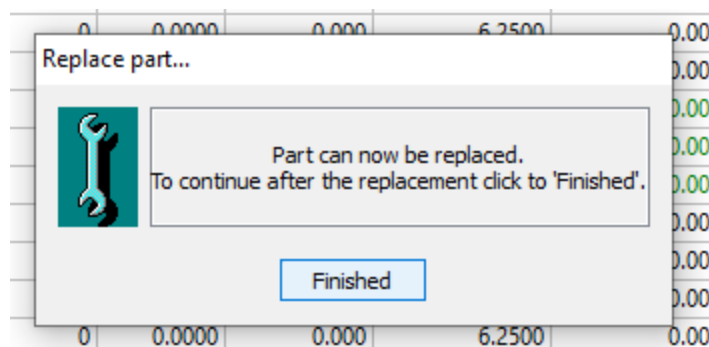
Note: the metal connections are **color-coded corresponding to their position (red, black, white).*



After re-assembling, the core must look like this:



- 1.11. Once finished checking/replacing the reduction tube and re-assembled all parts, put back the sample cover (the light should be on once the cover is back to place) and click on finished.

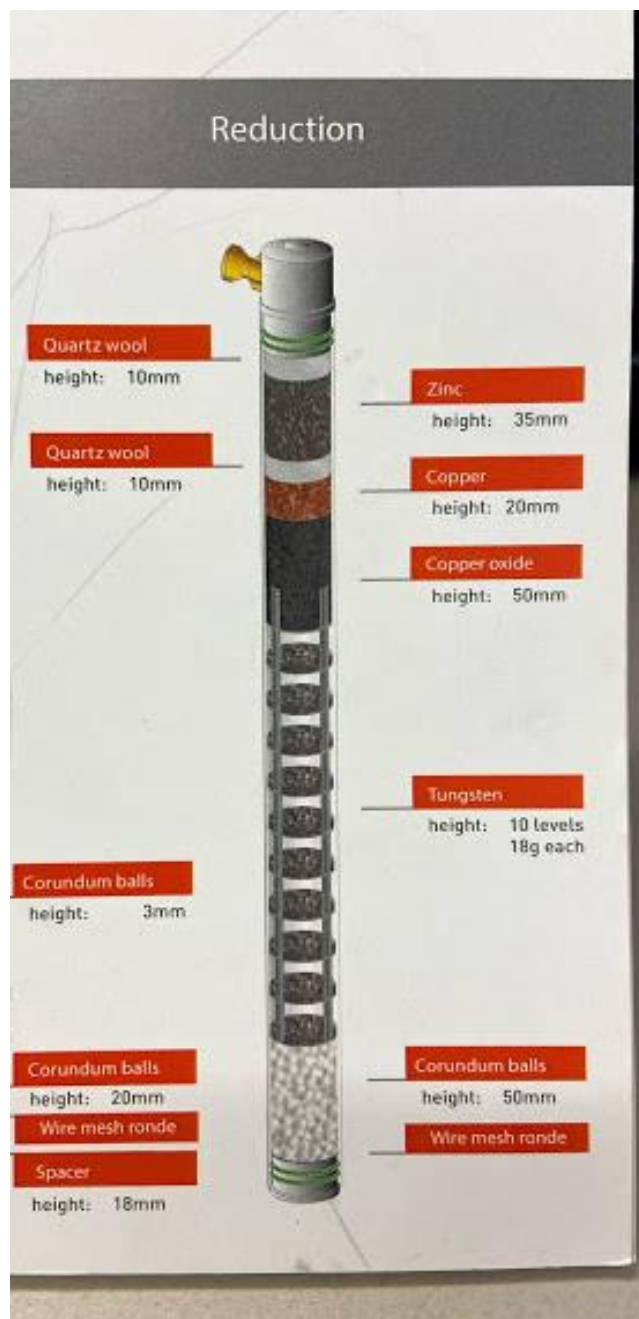


- 1.12. After replacing (e.g. inserting a **new** reduction tube)
 - Options > Maintenance > Intervals
 - A new window will appear. Manually type 0 (zero) in the “Standing” text box. This will reset the count of the samples analyzed by the current (in-use) reduction tube.

**Note that this window also serves to check how many samples have been run with the current reduction tube. This serves as a notice to be aware if - and when - checking/replacing may be needed.*

2. Packing a new reduction tube

To prepare a new reduction tube, a series of compounds must be added into the corresponding tube, using a funnel and a metered ruler (follow the instructions depicted below, these may also be found in the short manual and in a separate sheet near the machine).



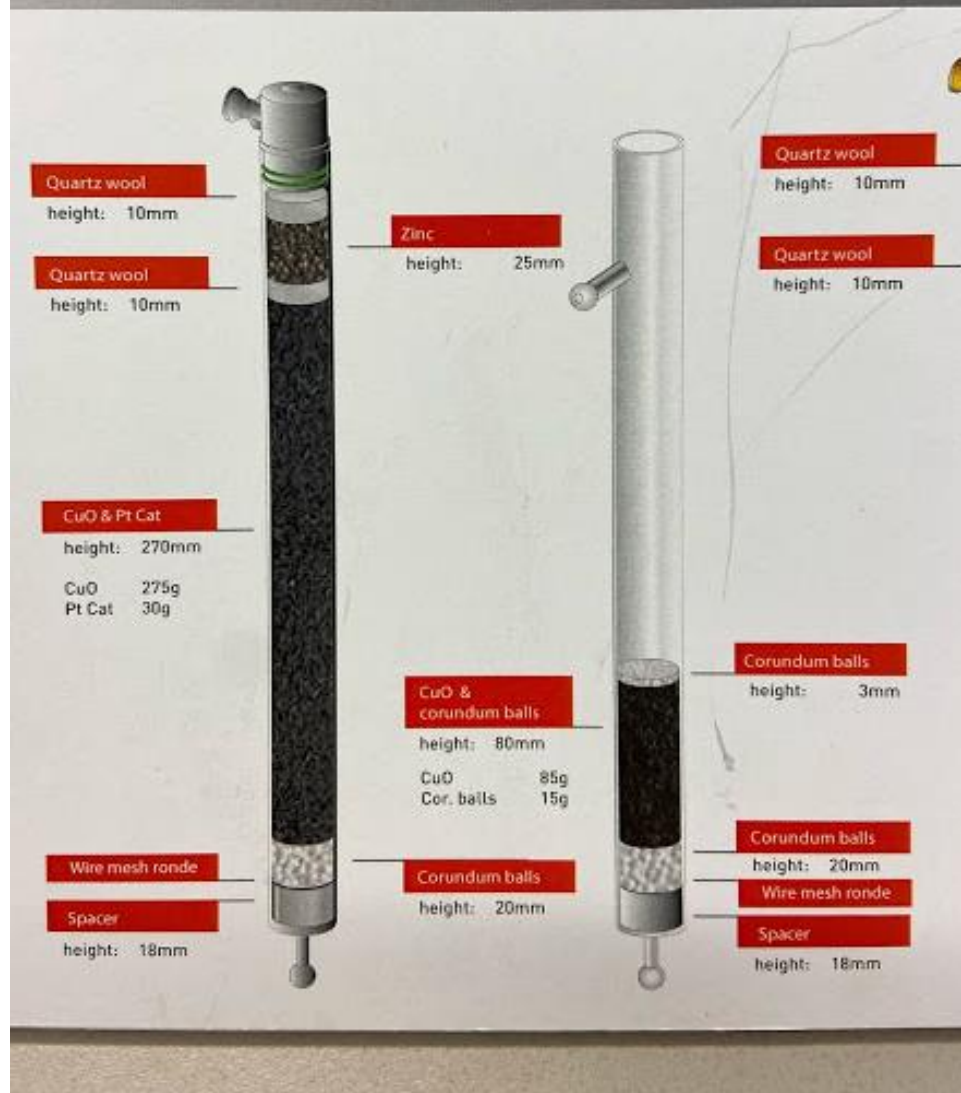
**Note that the combustion, post-combustion, and drying tubes may also need replacing. However, their lifetime is considerably greater than the one of the reduction tube. Therefore, their replacement will not happen very often. If needed, please see the instructions depicted below (these may also be found in the short manual and in a separate sheet near the machine).*

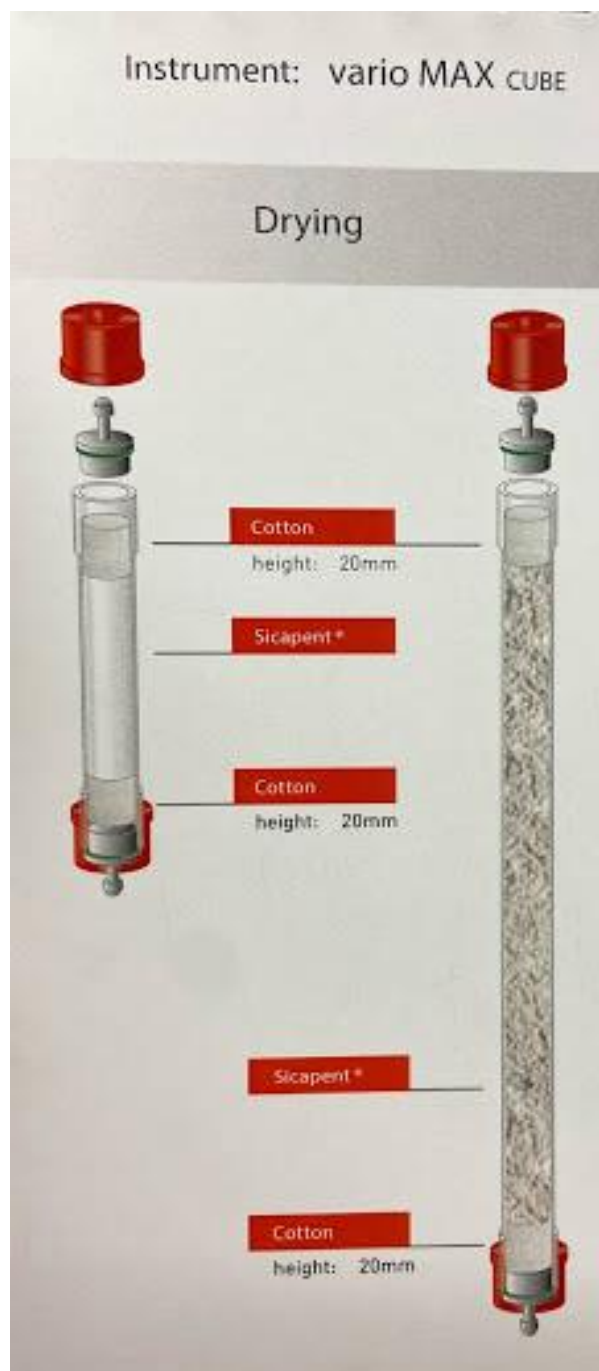
Mode of operation: CN

Post Combustion

Combustion

Re





3. Leak check

3.1. When should a leak check be performed:

- Significant increase/decrease of the daily factor (the value of daily factor should be around 1 ± 0.01)
- Blanks with high nitrogen values
- Decrease of the pressure values "Inlet" and "Outlet".

SOP: C/N determination by high-temperature combustion | [UIUC Soils Lab](#) | Last revised 21 July 2021

- Too high helium flow at the “Inlet”.
 - Too low helium flow at the “Outlet”.
- 3.2. Performing a leak test to find the leak
- Click Options > Diagnostics > Leak test.
 - If the leak test did not pass, the dialog leads you step by step through the leak test and helps you to localize the leak.
 - The leak test components kits (supplied with the analyzer) is required for this option.

References:

Short manual of vario Max cube. Elementar.

Suggested reading:

Tabatabai, M. A. and Bremner, J. M. 1990. “Automated instruments for determination of total carbon, total nitrogen, and total sulfur in soils by combustion techniques”. In *Soil Analysis: Modern Instrumental Techniques*, 2nd Edn., Edited by: Smith, K. A. 174–203. New York, NY: Marcel Dekker. [\[Google Scholar\]](#)

Yeomans, J. C., and J. M. Bremner. "Carbon and nitrogen analysis of soils by automated combustion techniques." *Communications in Soil Science and Plant Analysis* 22.9-10 (1991): 843-850. <https://doi.org/10.1080/00103629109368458>

Citation:

SOP: Elementar C/N Analysis. 2021. Soils Lab, University of Illinois Urbana-Champaign. Urbana, IL. Accessed at: <https://margenot.cropsciences.illinois.edu/methods-sops/>

Questions can be directed to Andrew Margenot at margenot@illinois.edu