Written by Tomas Richardson-Sanchez (last updated March 2021)

Instructions on R analysis of ICP-MS data

About: This document was originally made to support sample analysis and data analysis with the PerkinElmer NexION 300D ICP-MS in the Murphy lab in the Department of Microbiology & Immunology at the University of British Columbia. The methods and R scripts described within are available on Github and can be used more broadly for ICP-MS data analysis with other machines and in other lab settings.

All data analysis scripts: https://github.com/Tomas-RS/ICPMS-data-analysis-scripts.git
'ICPMSheavymetals' R package: https://github.com/Tomas-RS/ICPMSheavymetals.git

Document contents:

General ICP-MS data analysis protocol:

1. Prepare samples
2. Analyze samples
3. Prepare data files (Sample_run and Sample_digest) for analysis
4. Set up Rstudio with data files and scripts
5. Run appropriate data analysis scripts
6. Copy Results files and back up all files on lab drive

*This document describes steps 3, 4, 5, and 6. For steps 1 and 2, consult 'ICP-MS notes and protocols' Word document in Murphy lab server (ICPMS > ICP-MS notes and protocols) and consider the following notes to ensure the R scripts work correctly:

- Sample names should not contain any special characters (%, /, !) and should have underscores instead of spaces where possible (e.g. 'Fe_media_TRS1', not 'TRS media (Fe)')
- In the ICP-MS sample run, make sure all blanks are named "Blank", all standards for standard curves are named "__ ppb metal standard" (e.g. '10 ppb metal standard' or '1 ppb metal standard'), and all standards used throughout to check for drift are named "__ ppb standard" (e.g. '1 ppb standard'). The exact spelling (including upper/lower case) is important for the R script to recognise each of these types of samples. Often the 1 ppb standard solution will be used for both drift detection and standard curve construction, so take care to use the appropriate names ('1 ppb standard' vs '1 ppb metal standard') at different instances.
- Use last lines of script (clear console/environment/plots) at any stage to restart data analysis.

<u>Set up of data files</u> (Sample_run_date and Sample_digest_date):

1. ICP-MS data analysis on the PerkinElmer NexION 300D has a table of values as the main output (Report view window → Intensities tab). This should be exported as an Excel file, making sure 'Show RSDs' box is checked before clicking 'Export all'. Save the file with the name "Sample_run_date" (e.g. Sample_run_201211). Also copy this file to the Murphy lab server as raw data.
   *Figure 1 shows table in NexION software prior to exporting

2. The Sample_run_date file should be modified in the Intensities' worksheet accordingly:
   A) Delete 'Daily performance check' rows along with any other rows that should not be included in the analysis (e.g. early blanks, tubes that the autosampler missed, samples with signals affected by high concentration of previous samples)
   B) Add column titles 'Sequence_position' and 'Sample_name', and add underscores to metals (e.g. Fe 56 (cps) becomes Fe_56).
   C) Delete columns corresponding to acquisition time, dataset file, and method file.
   D) Delete all other worksheets in the Excel file (keep only 'Intensities')
   E) Save as a .csv file (Save as > CSV (Comma Delimited))
   *Figures 2 and 3 represent a before and after for preparing a 'Sample_run_date' file.

3. The "ICPMS_sample_digest_date(yymmdd)_date (R compatible)" file in the Murphy lab drive should be copied (do not overwrite file in lab drive) and then edited accordingly:
   A) Enter sample digestion values using the provided 'Wet Ash' and 'Dry Ash' formulae - 'standard_factor_digestion', and 'Sample_dilution_factor' columns will autofill. Sample_name must EXACTLY MATCH those entered in the NexION software during the ICP-MS run – if they do not match then modify them in either of the .csv documents prior to R analysis. Add details on right side of document (lab, name, dates) and save Excel file for record keeping.
   B) Make a copy of this file and delete peripheral rows (wet/dry ash examples, instructions) and columns (lab/name/dates).
   C) Save as a .csv file (Save as > CSV (Comma Delimited)) and rename to 'Sample_digest_date' (e.g. Sample_digest_201211).
   D) Open .csv file and delete all columns except 'Sample_name', 'standard_factor_digestion', and 'Sample_dilution_factor'. Save file.
   * Figures 4 and 5 represent a before and after for preparing a 'Sample_digest_date' file.

Figure 1: Screenshot of NexION 300D software output for ICP-MS data analysis:

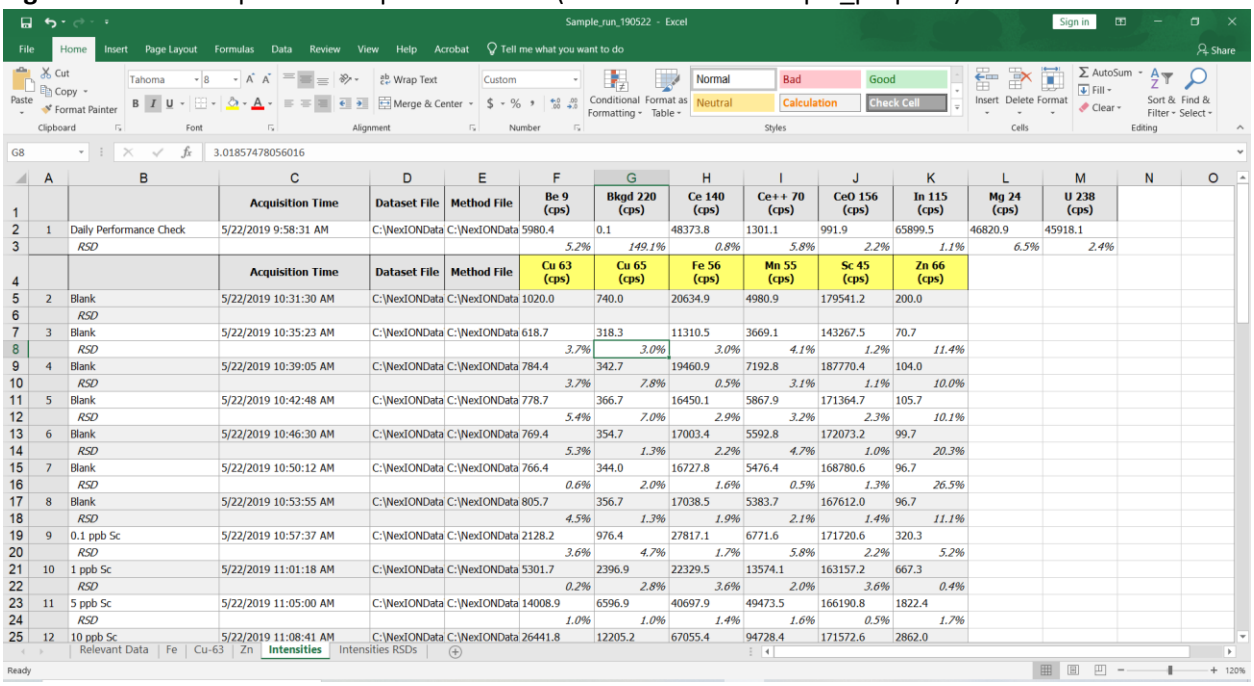
Report View

Current Sample | Intensities | Concentrations | Unfactored Concentrations | Internal Standards

☑ Show RSDs

		Acquisition Time	Be 9 (cps)	Bkgd 220 (cps)	Ce 140 (cps)	Ce++ 70 (cps)	CeO 156 (cps)	In 115 (cps)	Mg 24 (cps)	U 238 (cps)
1	Daily Performance Check	9/28/2020 11:46:20 AM	5065.7	0.1	28259.8	953.3	471.9	58184.0	29136.8	31560.4
	RSD		*3.6%*	*136.9%*	*3.8%*	*8.8%*	*6.2%*	*2.5%*	*4.5%*	*2.3%*
2	Daily Performance Check	9/28/2020 11:54:42 AM	4456.7	3.3E-2	23106.5	758.6	401.3	55695.8	25327.9	28532.0
	RSD		*3.4%*	*223.6%*	*2.6%*	*8.7%*	*5.8%*	*2.4%*	*4.3%*	*1.6%*

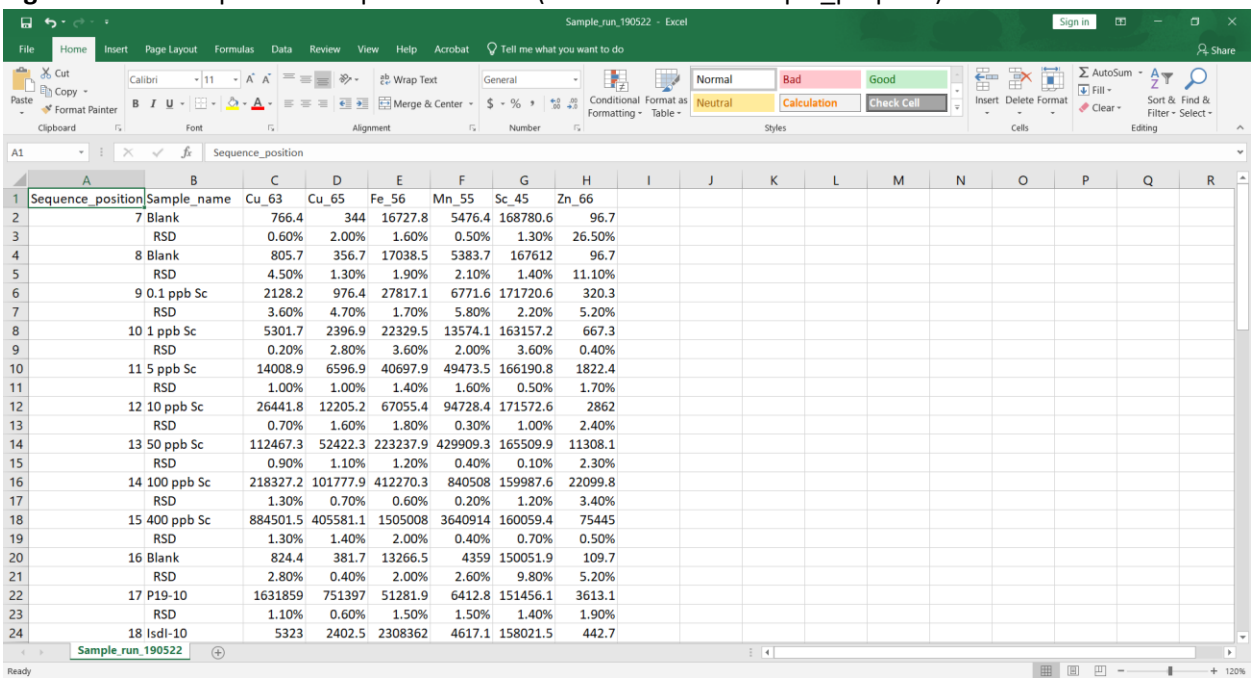
Figure 2: Before Step 2 of 'Set up of data files' (modification of Sample_prep file)



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1			**Acquisition Time**	**Dataset File**	**Method File**	**Be 9 (cps)**	**Bkgd 220 (cps)**	**Ce 140 (cps)**	**Ce++ 70 (cps)**	**CeO 156 (cps)**	**In 115 (cps)**	**Mg 24 (cps)**	**U 238 (cps)**		
2	1	Daily Performance Check	5/22/2019 9:58:31 AM	C:\NexIONData	C:\NexIONData	5980.4	0.1	48373.8	1301.1	991.9	65899.5	46820.9	45918.1		
3		*RSD*				*5.2%*	*149.1%*	*0.8%*	*5.8%*	*2.2%*	*1.1%*	*6.5%*	*2.4%*		
4			**Acquisition Time**	**Dataset File**	**Method File**	**Cu 63 (cps)**	**Cu 65 (cps)**	**Fe 56 (cps)**	**Mn 55 (cps)**	**Sc 45 (cps)**	**Zn 66 (cps)**				
5	2	Blank	5/22/2019 10:31:30 AM	C:\NexIONData	C:\NexIONData	1020.0	740.0	20634.9	4980.9	179541.2	200.0				
6		*RSD*													
7	3	Blank	5/22/2019 10:35:23 AM	C:\NexIONData	C:\NexIONData	618.7	318.3	11310.5	3669.1	143267.5	70.7				
8		*RSD*				*3.7%*	*3.0%*	*3.0%*	*4.1%*	*1.2%*	*11.4%*				
9	4	Blank	5/22/2019 10:39:05 AM	C:\NexIONData	C:\NexIONData	784.4	342.7	19460.9	7192.8	187770.4	104.0				
10		*RSD*				*3.7%*	*7.8%*	*0.5%*	*3.1%*	*1.1%*	*10.0%*				
11	5	Blank	5/22/2019 10:42:48 AM	C:\NexIONData	C:\NexIONData	778.7	366.7	16450.1	5867.9	171364.7	105.7				
12		*RSD*				*5.4%*	*7.0%*	*2.9%*	*3.2%*	*2.3%*	*10.1%*				
13	6	Blank	5/22/2019 10:46:30 AM	C:\NexIONData	C:\NexIONData	769.4	354.7	17003.4	5592.8	172073.2	99.7				
14		*RSD*				*5.3%*	*1.3%*	*2.2%*	*4.7%*	*1.0%*	*20.3%*				
15	7	Blank	5/22/2019 10:50:12 AM	C:\NexIONData	C:\NexIONData	766.4	344.0	16727.8	5476.4	168780.6	96.7				
16		*RSD*				*0.6%*	*2.0%*	*1.6%*	*0.5%*	*1.3%*	*26.5%*				
17	8	Blank	5/22/2019 10:53:55 AM	C:\NexIONData	C:\NexIONData	805.7	356.7	17038.5	5383.7	167612.0	96.7				
18		*RSD*				*4.5%*	*1.3%*	*1.9%*	*2.1%*	*1.4%*	*11.1%*				
19	9	0.1 ppb Sc	5/22/2019 10:57:37 AM	C:\NexIONData	C:\NexIONData	2128.2	976.4	27817.1	6771.6	171720.6	320.3				
20		*RSD*				*3.6%*	*4.7%*	*1.7%*	*5.8%*	*2.2%*	*5.2%*				
21	10	1 ppb Sc	5/22/2019 11:01:18 AM	C:\NexIONData	C:\NexIONData	5301.7	2396.9	22329.5	13574.1	163157.2	667.3				
22		*RSD*				*0.2%*	*2.8%*	*3.6%*	*2.0%*	*3.6%*	*0.4%*				
23	11	5 ppb Sc	5/22/2019 11:05:00 AM	C:\NexIONData	C:\NexIONData	14008.9	6596.9	40697.9	49473.5	166190.8	1822.4				
24		*RSD*				*1.0%*	*1.0%*	*1.4%*	*1.6%*	*0.5%*	*1.7%*				
25	12	10 ppb Sc	5/22/2019 11:08:41 AM	C:\NexIONData	C:\NexIONData	26441.8	12205.2	67055.4	94728.4	171572.6	2862.0				

Relevant Data | Fe | Cu-63 | Zn | **Intensities** | Intensities RSDs

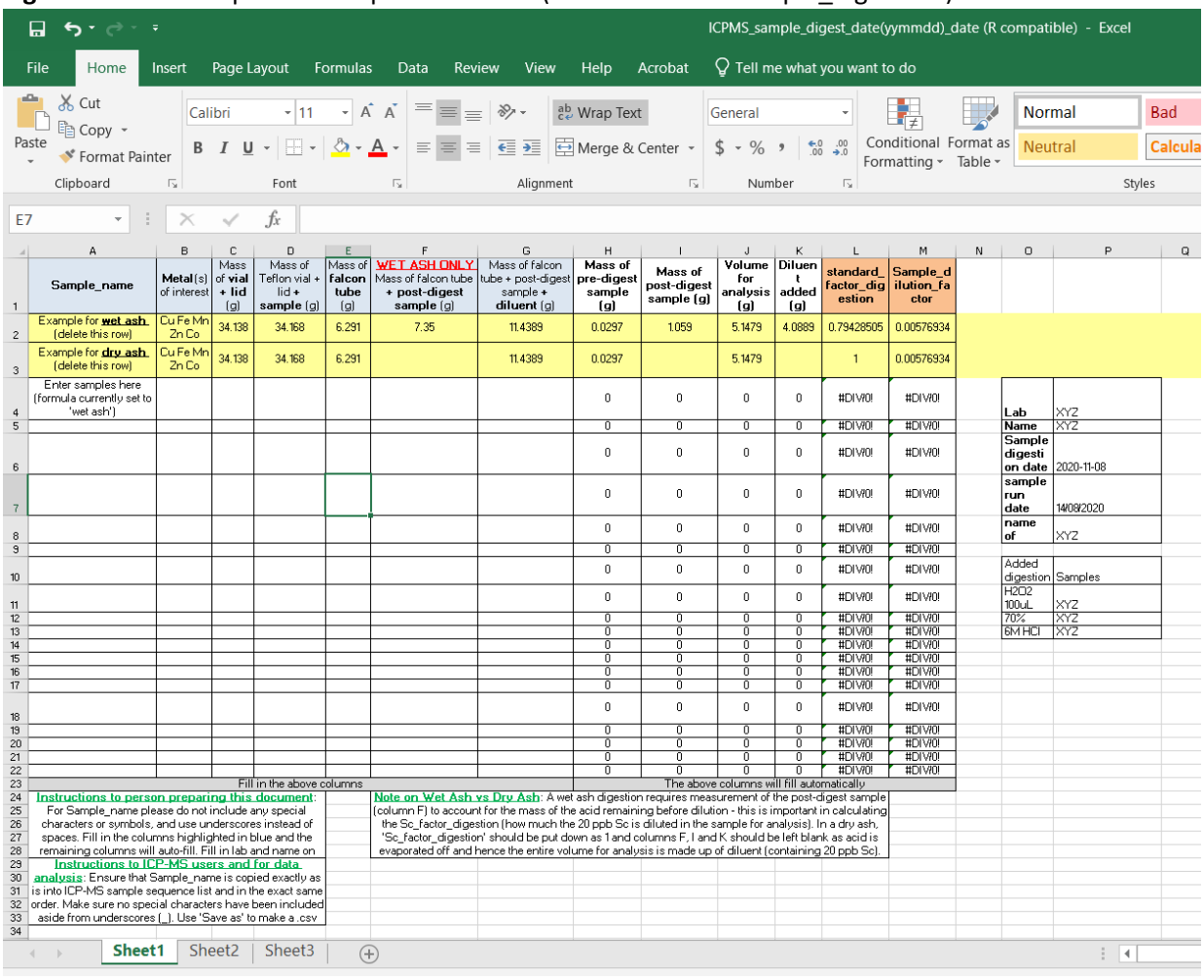
Figure 3: After Step 2 of 'Set up of data files' (modification of Sample_prep file)



	A	B	C	D	E	F	G	H
1	Sequence_position	Sample_name	Cu_63	Cu_65	Fe_56	Mn_55	Sc_45	Zn_66
2	7	Blank	766.4	344	16727.8	5476.4	168780.6	96.7
3		RSD	0.60%	2.00%	1.60%	0.50%	1.30%	26.50%
4	8	Blank	805.7	356.7	17038.5	5383.7	167612	96.7
5		RSD	4.50%	1.30%	1.90%	2.10%	1.40%	11.10%
6	9	0.1 ppb Sc	2128.2	976.4	27817.1	6771.6	171720.6	320.3
7		RSD	3.60%	4.70%	1.70%	5.80%	2.20%	5.20%
8	10	1 ppb Sc	5301.7	2396.9	22329.5	13574.1	163157.2	667.3
9		RSD	0.20%	2.80%	3.60%	2.00%	3.60%	0.40%
10	11	5 ppb Sc	14008.9	6596.9	40697.9	49473.5	166190.8	1822.4
11		RSD	1.00%	1.00%	1.40%	1.60%	0.50%	1.70%
12	12	10 ppb Sc	26441.8	12205.2	67055.4	94728.4	171572.6	2862
13		RSD	0.70%	1.60%	1.80%	0.30%	1.00%	2.40%
14	13	50 ppb Sc	112467.3	52422.3	223237.9	429909.3	165509.9	11308.1
15		RSD	0.90%	1.10%	1.20%	0.40%	0.10%	2.30%
16	14	100 ppb Sc	218327.2	101777.9	412270.3	840508	159987.6	22099.8
17		RSD	1.30%	0.70%	0.60%	0.20%	1.20%	3.40%
18	15	400 ppb Sc	884501.5	405581.1	1505008	3640914	160059.4	75445
19		RSD	1.30%	1.40%	2.00%	0.40%	0.70%	0.50%
20	16	Blank	824.4	381.7	13266.5	4359	150051.9	109.7
21		RSD	2.80%	0.40%	2.00%	2.60%	9.80%	5.20%
22	17	P19-10	1631859	751397	51281.9	6412.8	151456.1	3613.1
23		RSD	1.10%	0.60%	1.50%	1.50%	1.40%	1.90%
24	18	Isdl-10	5323	2402.5	2308362	4617.1	158021.5	442.7

Sample_run_190522

Figure 4: Before Step 3 of 'Set up of data files' (modification of Sample_digest file)



ICPMS_sample_digest_date(yymmdd)_date (R compatible) - Excel

	Sample_name	Metal(s) of interest	Mass of vial + lid (g)	Mass of Teflon vial + lid + sample (g)	Mass of falcon tube (g)	WET ASH ONLY: Mass of falcon tube + post-digest sample (g)	Mass of falcon tube + post-digest sample + diluent (g)	Mass of pre-digest sample (g)	Mass of post-digest sample (g)	Volume for analysis (g)	Diluent added (g)	standard_factor_digestion	Sample_dilution_factor
2	Example for **wet ash** (delete this row)	Cu Fe Mn Zn Co	34.138	34.168	6.291	7.35	11.4389	0.0297	1.059	5.1479	4.0889	0.79428505	0.00576934
3	Example for **dry ash** (delete this row)	Cu Fe Mn Zn Co	34.138	34.168	6.291		11.4389	0.0297		5.1479		1	0.00576934
4	Enter samples here (formula currently set to 'wet ash')							0	0	0	0	#DIV/0!	#DIV/0!
5								0	0	0	0	#DIV/0!	#DIV/0!
6								0	0	0	0	#DIV/0!	#DIV/0!
7								0	0	0	0	#DIV/0!	#DIV/0!
8								0	0	0	0	#DIV/0!	#DIV/0!
9								0	0	0	0	#DIV/0!	#DIV/0!
10								0	0	0	0	#DIV/0!	#DIV/0!
11								0	0	0	0	#DIV/0!	#DIV/0!
12								0	0	0	0	#DIV/0!	#DIV/0!
13								0	0	0	0	#DIV/0!	#DIV/0!
14								0	0	0	0	#DIV/0!	#DIV/0!
15								0	0	0	0	#DIV/0!	#DIV/0!
16								0	0	0	0	#DIV/0!	#DIV/0!
17								0	0	0	0	#DIV/0!	#DIV/0!
18								0	0	0	0	#DIV/0!	#DIV/0!
19								0	0	0	0	#DIV/0!	#DIV/0!
20								0	0	0	0	#DIV/0!	#DIV/0!
21								0	0	0	0	#DIV/0!	#DIV/0!
22								0	0	0	0	#DIV/0!	#DIV/0!
23	Fill in the above columns							The above columns will fill automatically					

Lab	XYZ
Name	XYZ
Sample digestion date	2020-11-08
sample run date	14/09/2020
name of	XYZ
Added digestion	Samples
H2O2 100uL	XYZ
70%	XYZ
6M HCl	XYZ

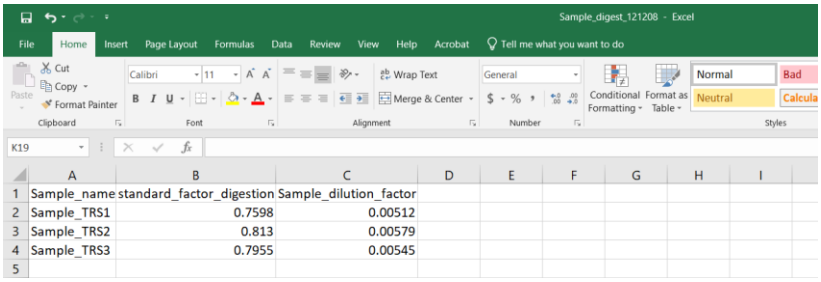
Instructions to person preparing this document: For Sample_name please do not include any special characters or symbols, and use underscores instead of spaces. Fill in the columns highlighted in blue and the remaining columns will auto-fill. Fill in lab and name on

Instructions to ICP-MS users and for data analysis: Ensure that Sample_name is copied exactly as is into ICP-MS sample sequence list and in the exact same order. Make sure no special characters have been included aside from underscores (_). Use 'Save as' to make a .csv

Note on Wet Ash vs Dry Ash: A wet ash digestion requires measurement of the post-digest sample (column F) to account for the mass of the acid remaining before dilution - this is important in calculating the Sc_factor_digestion (how much the 20 ppb Sc is diluted in the sample for analysis). In a dry ash, 'Sc_factor_digestion' should be put down as 1 and columns F, I and K should be left blank as acid is evaporated off and hence the entire volume for analysis is made up of diluent (containing 20 ppb Sc).

Sheet1 Sheet2 Sheet3

Figure 5: After Step 3 of 'Set up of data files' (modification of Sample_digest file)



Sample_digest_121208 - Excel

	A	B	C
1	Sample_name	standard_factor_digestion	Sample_dilution_factor
2	Sample_TRS1	0.7598	0.00512
3	Sample_TRS2	0.813	0.00579
4	Sample_TRS3	0.7955	0.00545
5			

Set up of R studio for analysis:

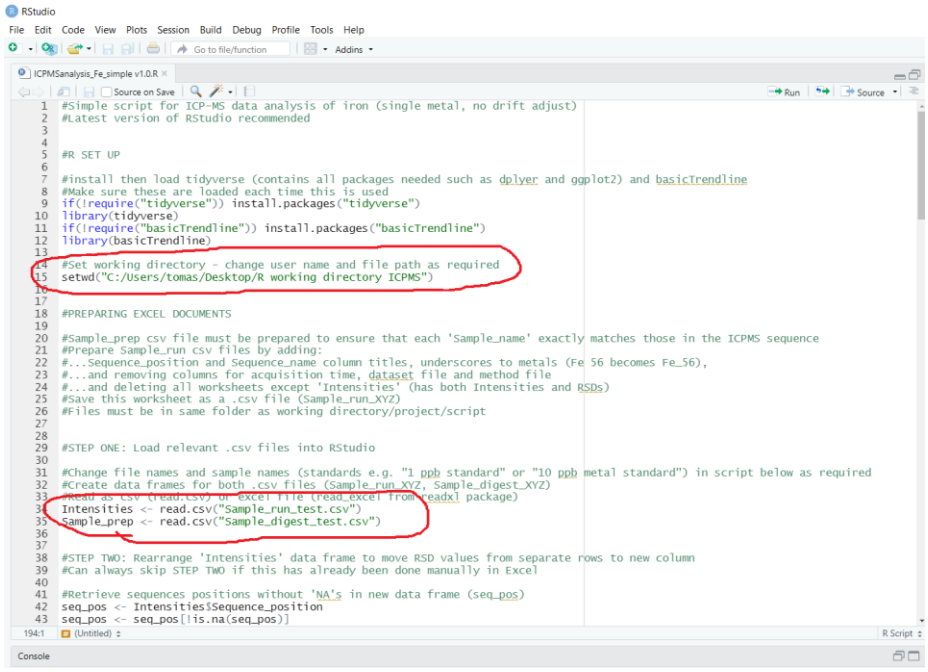
1. Download the newest versions of R (https://www.r-project.org/) and R studio (https://rstudio.com/products/rstudio/download/#download).

2. Create a folder on your computer's desktop named "R working directory ICPMS" and copy the data files (Sample_prep.csv and Sample_run.csv) and relevant R scripts into this folder.

3. Open the relevant R script and modify the following sections in the script:
   A) Change line 15 of the script by modifying the working directory to match that of the 'R working directory ICPMS' folder on your computer. Examples below:
   #Set working directory - change user name and file path as required
   setwd("C:/Users/tomas/Desktop/R working directory ICPMS") → Tom's laptop
   setwd("C:/Users/mikehata/Desktop/R working directory ICPMS") → ICP-MS computer
   B) Change lines 34-35 of the script by modifying the Sample_run and Sample_digest file names to match those given. Examples below:
   #Read as csv (read.csv) or excel file (read_excel from readxl package)
   Intensities <- read.csv("Sample_run_test.csv")
   Sample_prep <- read.csv("Sample_digest_test.csv") → default in script
   Intensities <- read.csv("Sample_run_201211.csv")
   Sample_prep <- read.csv("Sample_digest_201211.csv") → files from Dec 12, 2020
   C) Change lines 111-116 and 134-138 by modifying the standard curve sample concentrations to match those used in the ICP-MS run. Examples below:
   #Group (under Iron_standard curve) then rename all standard curve samples and convert to numeric
   Iron_standardcurve <- filter(Iron_analysis, Sample_name == "0.1 ppb metal standard"
   | Sample_name == "1 ppb metal standard"
   | Sample_name == "5 ppb metal standard"
   | Sample_name == "10 ppb metal standard"
   | Sample_name == "50 ppb metal standard"
   | Sample_name == "100 ppb metal standard"
   | Sample_name == "200 ppb metal standard") → default in script
   Iron_standardcurve <- filter(Iron_analysis, Sample_name == "0.09 ppb metal standard"
   | Sample_name == "1.22 ppb metal standard"
   | Sample_name == "4.99 ppb metal standard"
   | Sample_name == "10.05 ppb metal standard"
   | Sample_name == "50.10 ppb metal standard"
   | Sample_name == "101.38 ppb metal standard"
   | Sample_name == "200.95 ppb metal standard") → modified

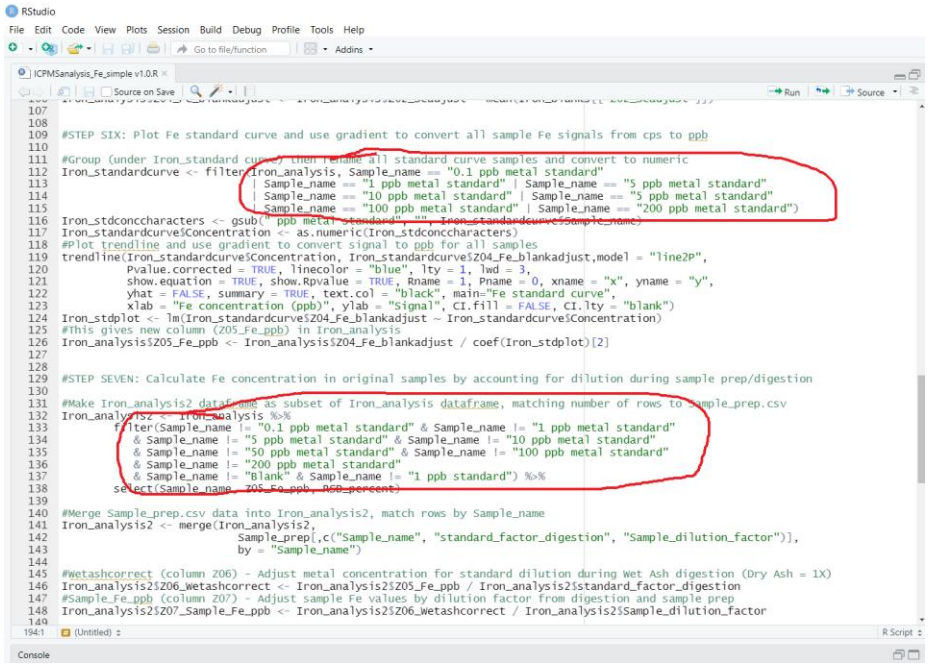
4. Run R script by highlighting lines 1-181 (until ###END DATA ANALYSIS###) and then hitting Ctrl+Enter. This runs the highlighted script with output in the console, and data frames and plots appearing on the right side of the Rstudio window. Look in newly generated 'Results' folder in R working directory for the results of the analysis (Desktop > R working directory ICPMS > Results).

Figure 6: Sections to edit in script from steps 3A (working directory) and 3B (file names)



```
#Simple script for ICP-MS data analysis of iron (single metal, no drift adjust)
#Latest version of RStudio recommended


#R SET UP

#install then load tidyverse (contains all packages needed such as dplyer and ggplot2) and basicTrendline
#Make sure these are loaded each time this is used
if(!require("tidyverse")) install.packages("tidyverse")
library(tidyverse)
if(!require("basicTrendline")) install.packages("basicTrendline")
library(basicTrendline)

#Set working directory - change user name and file path as required
setwd("C:/Users/tomas/Desktop/R working directory ICPMS")


#PREPARING EXCEL DOCUMENTS

#Sample_prep csv file must be prepared to ensure that each 'Sample_name' exactly matches those in the ICPMS sequence
#Prepare Sample_run csv files by adding:
#...Sequence_position and Sequence_name column titles, underscores to metals (Fe 56 becomes Fe_56),
#...and removing columns for acquisition time, dataset file and method file
#...and deleting all worksheets except 'Intensities' (has both Intensities and RSDs)
#Save this worksheet as a .csv file (Sample_run_XYZ)
#Files must be in same folder as working directory/project/script


#STEP ONE: Load relevant .csv files into RStudio

#Change file names and sample names (standards e.g. "1 ppb standard" or "10 ppb metal standard") in script below as required
#Create data frames for both .csv files (Sample_run_XYZ, Sample_digest_XYZ)
#Read as csv (read.csv) or Excel file (read_excel from readxl package)
Intensities <- read.csv("Sample_run_test.csv")
Sample_prep <- read.csv("Sample_digest_test.csv")


#STEP TWO: Rearrange 'Intensities' data frame to move RSD values from separate rows to new column
#Can always skip STEP TWO if this has already been done manually in Excel

#Retrieve sequences positions without 'NA's in new data frame (seq_pos)
seq_pos <- Intensities$Sequence_position
seq_pos <- seq_pos[!is.na(seq_pos)]
```

Figure 7: Sections to edit in script from step 3C (standard concentrations)



```
#STEP SIX: Plot Fe standard curve and use gradient to convert all sample Fe signals from cps to ppb

#Group (under Iron_standard curve) then rename all standard curve samples and convert to numeric
Iron_standardcurve <- filter(Iron_analysis, Sample_name == "0.1 ppb metal standard"
                             | Sample_name == "1 ppb metal standard" | Sample_name == "5 ppb metal standard"
                             | Sample_name == "10 ppb metal standard" | Sample_name == "5 ppb metal standard"
                             | Sample_name == "100 ppb metal standard" | Sample_name == "200 ppb metal standard")
Iron_stdconccharacters <- gsub(" ppb metal standard", "", Iron_standardcurve$Sample_name)
Iron_standardcurve$Concentration <- as.numeric(Iron_stdconccharacters)
#Plot trendline and use gradient to convert signal to ppb for all samples
trendline(Iron_standardcurve$Concentration, Iron_standardcurve$Z04_Fe_blankadjust,model = "line2P",
          Pvalue.corrected = TRUE, linecolor = "blue", lty = 1, lwd = 3,
          show.equation = TRUE, show.Rpvalue = TRUE, Rname = 1, Pname = 0, xname = "x", yname = "y",
          yhat = FALSE, summary = TRUE, text.col = "black", main="Fe standard curve",
          xlab = "Fe concentration (ppb)", ylab = "Signal", CI.fill = FALSE, CI.lty = "blank")
Iron_stdplot <- lm(Iron_standardcurve$Z04_Fe_blankadjust ~ Iron_standardcurve$Concentration)
#This gives new column (Z05_Fe_ppb) in Iron_analysis
Iron_analysis$Z05_Fe_ppb <- Iron_analysis$Z04_Fe_blankadjust / coef(Iron_stdplot)[2]


#STEP SEVEN: Calculate Fe concentration in original samples by accounting for dilution during sample prep/digestion

#Make Iron_analysis2 dataframe as subset of Iron_analysis dataframe, matching number of rows to Sample_prep.csv
Iron_analysis2 <- Iron_analysis %>%
        filter(Sample_name != "0.1 ppb metal standard" & Sample_name != "1 ppb metal standard"
         & Sample_name != "5 ppb metal standard" & Sample_name != "10 ppb metal standard"
         & Sample_name != "50 ppb metal standard" & Sample_name != "100 ppb metal standard"
         & Sample_name != "200 ppb metal standard"
         & Sample_name != "Blank" & Sample_name != "1 ppb standard") %>%
        select(Sample_name, Z05_Fe_ppb, RSD_percent)

#Merge Sample_prep.csv data into Iron_analysis2, match rows by Sample_name
Iron_analysis2 <- merge(Iron_analysis2,
                        Sample_prep[,c("Sample_name", "standard_factor_digestion", "Sample_dilution_factor")],
                        by = "Sample_name")

#Wetashcorrect (column Z06) - Adjust metal concentration for standard dilution during Wet Ash digestion (Dry Ash = 1X)
Iron_analysis2$Z06_Wetashcorrect <- Iron_analysis2$Z05_Fe_ppb / Iron_analysis2$standard_factor_digestion
#Sample_Fe_ppb (column Z07) - Adjust sample Fe values by dilution factor from digestion and sample prep
Iron_analysis2$Z07_Sample_Fe_ppb <- Iron_analysis2$Z06_Wetashcorrect / Iron_analysis2$Sample_dilution_factor
```

Figure 8: Appearance of Rstudio window before running analysis

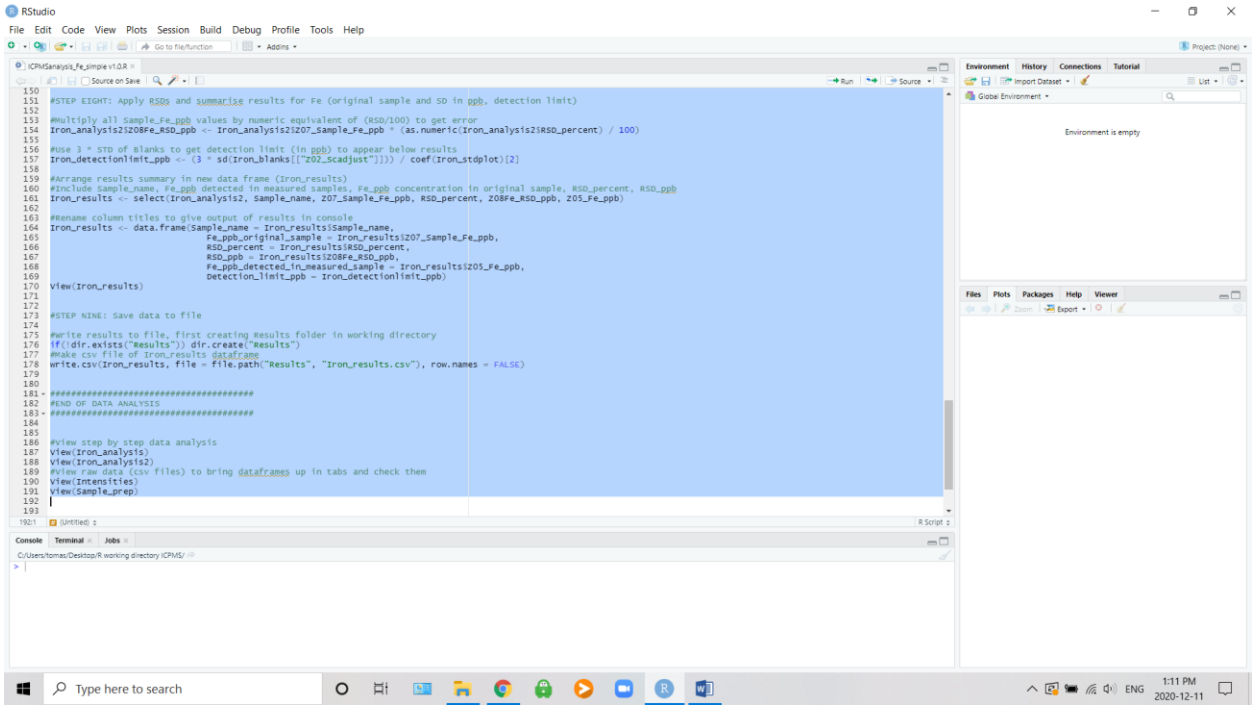
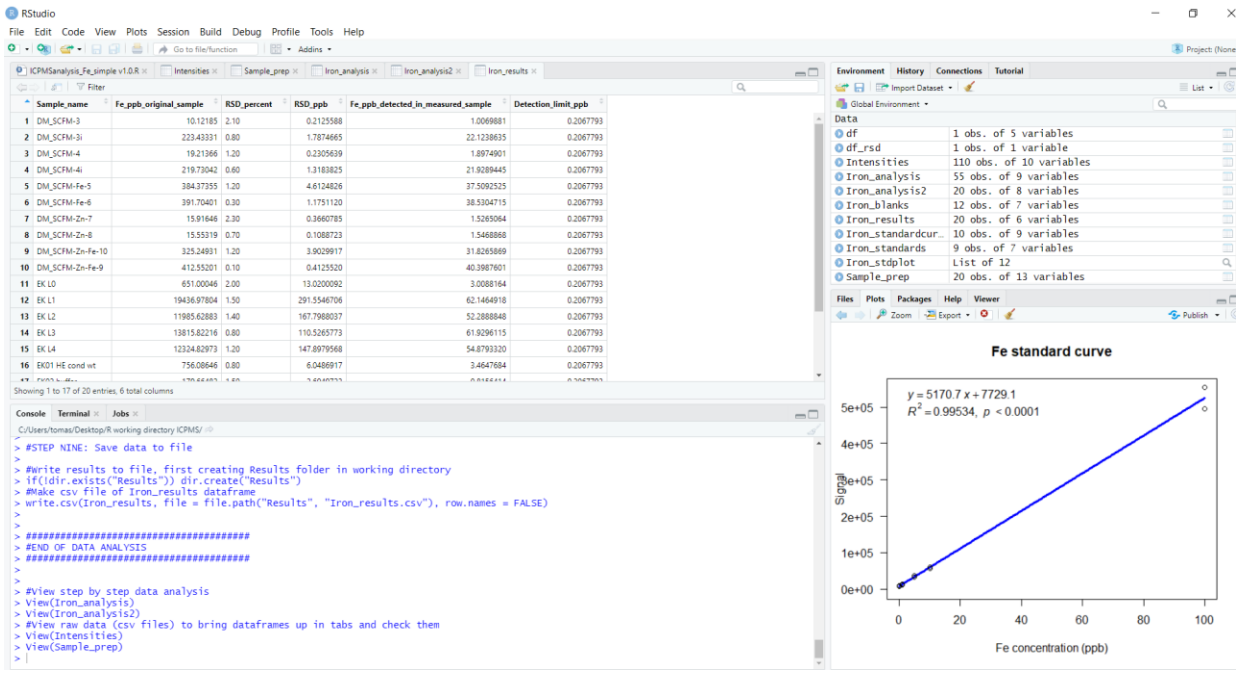
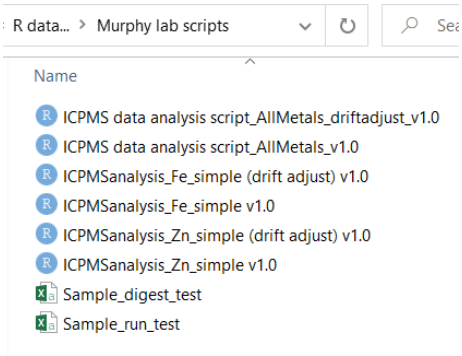


Figure 9: Appearance of Rstudio window after running analysis

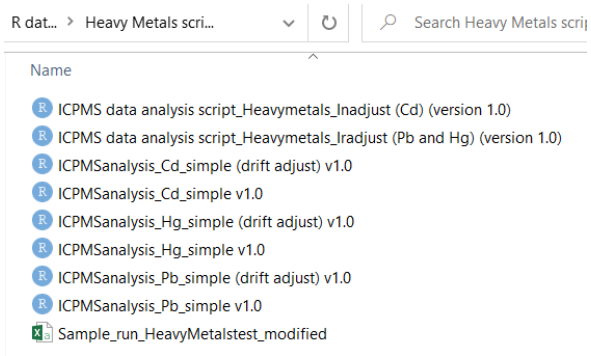


List of R scripts

Murphy lab scripts:



Heavy Metals scripts:



*Simple scripts are recommended if edits need to be made or if only a few metals are being analyzed.
*Complex scripts are recommended when many metals are being analyzed at once.

Murphy lab…
ICPMSanalysis_Fe_simple: Simple script for analyzing iron concentration in samples
ICPMSanalysis_Fe_simple (drift adjust): same as above but with drift adjustment applied

ICPMSanalysis_Zn_simple: Simple script for analyzing zinc concentration in samples
ICPMSanalysis_Zn_simple (drift adjust): same as above but with drift adjustment applied

ICPMS data analysis script_AllMetals: More complex script for analyzing many metals at once. Currently set to use Sc_45 as internal standard so will have to edit script if other internal standard is used.
ICPMS data analysis script_AllMetals_driftadjust: same as above but with drift adjustment applied

Heavy Metals project…
ICPMSanalysis_Cd_simple: Simple script for analyzing cadmium concentration in samples
ICPMSanalysis_Cd_simple (drift adjust): same as above but with drift adjustment applied

ICPMSanalysis_Hg_simple: Simple script for analyzing mercury concentration in samples
ICPMSanalysis_Hg_simple (drift adjust): same as above but with drift adjustment applied

ICPMSanalysis_Pb_simple: Simple script for analyzing lead concentration in samples
ICPMSanalysis_Pb_simple (drift adjust): same as above but with drift adjustment applied

ICPMS data analysis script_HeavyMetals_Inadjust (Cd): Complex script to analyze cadmium concentrations in sample using indium internal standard. It is recommended to use ICPMSanalysis_Cd_simple script instead.
ICPMS data analysis script_HeavyMetals_Iradjust (Pb and Hg): Complex script to analyze lead and mercury concentrations in sample using iridium internal standard. It is recommended to use ICPMSanalysis_Pb_simple and ICPMSanalysis_Hg_simple scripts instead.

*HEAVY METALS NOTE: Scripts for Heavy Metals project have already been modified to remove the need for the Sample_digest file and instead apply a 1/50 dilution factor directly. These scripts only require the Sample_run file to be prepared according to above instructions.

R package and Github repositories

Github repository 1: ICPMS data analysis scripts
https://github.com/Tomas-RS/ICPMS-data-analysis-scripts.git
This repository supplements the information in this document, containing files for all of the described R scripts, and supplementary .csv files for testing. The R scripts all contain annotation to explain each step of the data analysis method. Files are available for download from the repository.

Github repository 2: ICPMSheavymetals R package
https://github.com/Tomas-RS/ICPMSheavymetals.git
This repository contains an R package that was developed to facilitate ICP-MS data analysis using R. This was specifically made for the Heavy Metals Project collaboration with CBR at UBC. R scripts contained in Github repository 1 (ICPMS data analysis scripts) were adapted into functions and made into an R package that can be installed directly from github into R studio. Each data analysis process is split into three separate functions which must be called one at a time.
For example, the functions to use if analyzing cadmium concentration without drift adjustment:
setup_all1 – This first function takes the ICP-MS raw results (.csv file) and arranges the relevant data into a data frame named 'Intensities'. Input into the function is a .csv file name (e.g. "Sample_run_test").
setup_cadmium2 – This second function takes the 'Intensities' data frame and rearranges the data for just the cadmium results, giving the 'Cadmium_analysis' data frame. Alternatively, there are other functions (**setup_lead2** and **setup_mercury2**) available when other metals are being analyzed. Input into the functions are "Cd_111", "Pb_208", or "Hg_202", along with 'Intensities' to use the data frame generated by the first function.
analyse_cadmium3 – This last function takes the 'Cadmium_analysis' data frame and carries out the remainder of the data analysis using the In_115 internal standard, including plotting baseline drift (but not adjusting accordingly), subtracting blank values, plotting a standard curve, converting cps to ppb, then adjusting for dilution factor (1/50) to give the cadmium concentration in the original blood sample. If you want to adjust according to baseline drift, instead use the **analyse_cadmium_withdrift3** function. Similar functions exist for lead (**analyse_lead3**, **analyse_lead_withdrift3**) and mercury (**analyse_mercury3**, **analyse_mercury_withdrift3**) which use the Ir_193 internal standard. Inputs into the functions include 'Cadmium_analysis', 'Mercury_analysis', or 'Lead_analysis' to use the data frame generated by the second function, followed by the standard that was used to monitor baseline drift (e.g. "1 ppb standard"), then the standards used for a standard curve (e.g. "10 ppb metal standard").

For example, run this script in R to determine lead concentration with drift adjustment:

```
setup_all1("Sample_run_HeavyMetalstest_modified.csv")
setup_lead2("Pb_208", Intensities)
analyse_lead_withdrift3(Lead_analysis, "1 ppb standard", "0.0004 ppb metal standard", "0.002 ppb metal standard", "0.02 ppb metal standard", "0.1 ppb metal standard", "0.5 ppb metal standard", "1 ppb metal standard", "5 ppb metal standard", "10 ppb metal standard")
```

To install this package directly in R studio, run the following code:

```
install.packages("devtools")
devtools::install_github("Tomas-RS/ICPMSheavymetals")
library(ICPMSheavymetals)
```