Standard Operating Procedure for the analysis of trace elements in hydrothermal fluids by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Monica Correggia[1], Luciano Di Iorio[1], Alessia Bastianoni[1], Mustafa Yücel[2], Angelina Cordone[1], Donato Giovannelli[1,3,4,5,6,*]

[1] *Department of Biology, University of Naples "Federico II", Naples, Italy*
[2]*Institute of Marine Sciences, Middle East Technical University, Turkey*
[3] *Marine Chemistry & Geochemistry Department - Woods Hole Oceanographic Institution, MA, USA*
[4] *Istituto per le Risorse Biologiche e Biotecnologiche Marine, Consiglio Nazionale delle Ricerche, CNR-IRBIM, Ancona, Italy*
[5] *Earth-Life Science Institute, Tokyo Institute of Technology, Ookayama, Tokyo, Japan*
[6] *Department of Marine and Coastal Science, Rutgers University, New Brunswick, NJ, USA*

**Corresponding author: donato.giovannelli@unina.it*

ABSTRACT

This SOP validates an inductively coupled plasma mass spectrometry (ICP-MS) procedure for the trace element determinations in hydrothermal fluids. Hydrothermal fluids are aqueous solutions with a wide range of temperature, salinity, pH and trace elements that can be used by a set of microbial proteins containing redox-sensitive transition metals as their catalytic core. Due to the high variability of these samples, we have developed this protocol taking into account the special features of the matrices analyzed. An ICP-MS 7900 Agilent system was used. Calibration curves are linear in the 0.01 to 100 μg/L concentration range.

Keywords: trace element, hydrothermal fluids, inductively coupled plasma mass spectrometry, Standard Operating Procedure, aqueous geochemistry

SCOPE AND APPLICATION

This Standard Operating Procedure (SOP) was developed in the Giovannelli Lab at the Department of Biology of the University of Naples Federico II and outlines the analysis of trace elements involved in biogeochemical cycles from solid and liquid matrices using inductively coupled plasma mass spectrometry (ICP-MS). Although trace metals are recognized as important for microbial metabolism, they are not classically considered as the main factors that drive functional microbial diversity (Giovannelli 2022). Generally, microbial diversity is linked to environmental main physical-chemical parameters, such as temperature, pH, pressure, salinity, O_2 availability or substrates (Delgado-Baquerizo et al. 2018). The role of metals in influencing biodiversity has been studied classically in the context of their toxicity. In contrast, information on minimum concentration requirements for

specific trace elements is severely limited and very few studies have investigated their role in microbial functional diversity control (Morel and Price 2003; Bertrand et al. 2007; Giovannelli 2022). Microorganisms play a crucial role in biogeochemical cycles as they are involved in redox reaction, processes that clearly alter the composition of biosphere and geosphere, which have evolved over time, influencing each other. Biogeochemical cycles play a key role in controlling the interaction between geosphere and biosphere (Falkowski, Fenchel, and Delong 2008; Moore et al. 2017; Jelen, Giovannelli, and Falkowski 2016). Trace metals such as Fe, Co, Ni, Mo, W, V and Cu are used in specific sites of protein or enzymes, a set of microbial proteins containing redox-sensitive transition metals as their core catalytic but, despite the importance of this process, this relation has not been investigated in detail. Moreover, the availability of transition metal and substrates has changed over the course of Earth's history as a result of changing redox conditions, particularly global oxygenation (Anbar 2008). This evolution has allowed microbes to access a larger number of redox couples (Jelen, Giovannelli, and Falkowski 2016). Thus, an additional level of explanatory power could be provided by the availability of metals required for the key enzymes required for the different variants of each pathway, as demonstrated by recent work in convergent margins (Fullerton et al. 2021; Rogers et al. 2022).

Deeply-sourced seeps, encompassing a wide variety of diverse secondary geothermal features (Giovannelli et al. 2022), have a wide range of temperature, pH and salinity. Our dataset routinely includes samples with pH varying between 0.5 to 11.2, temperatures ranging from 2 °C to 375 °C and salinity from 0 % to 35 %. Traditional trace elements analysis of geothermal waters focuses on elements that are used to understand geochemical processes mainly linked to the source of the fluids (Féraud et al. 2009), the extent of water-rock interactions (Grandell et al. 2016) or the presence of anomalies of economically valuable metals also in the future market of clean energy technology such as gold, lithium, cobalt (Gianelli and Grassi 2001). Comparatively less attention has been devoted to the biologically relevant elements (Giovannelli 2022), and very often datasets lack information on rock composition, fluid geochemistry involving the key biological elements such as molybdenum, vanadium or cobalt. Here we describe the standard operating procedures (SOP) developed to routinely analyze such a wide range of fluid and sediments obtained from deeply-sourced springs. The described SOP aims at measuring a wide range of elements, including the classically determined elements of geological interest as well as the complete set of biological metals.

SUMMARY OF METHOD

ICP-MS is a technique that combines mass spectrometry to inductively coupled plasma, useful for determining trace metallic and non-metallic inorganic substances present in a sample. Liquid samples are taken through a peristaltic pump and nebulized, and the resulting aerosol is transported to the plasma torch (generally based on Argon gas) where the elements are ionized. The ions pass through a vacuum chamber where they are separated into photons,

neutrons and interfering ions by an ion lens and a collision chamber (based on diverse inert or reactive gasses depending on the final application). Analytes arrive at the quadrupole mass filter, and are kept in a state of vacuum to minimize interferences. Finally, each element passes through the detector to be counted. In a few minutes ICP-MS is capable of giving quantitative information on a large swath of elements with theoretical limits of detection in the range of 1 ppt to 10 ppb depending on the specific element (Thomas 2003).

We have developed this protocol using an Agilent ICP-MS 7900. The ICP-MS 7900 is a single quadrupole mass spectrometer that filters ions by mass to charge ratio (m/z). Consists of two pairs of rods connected to separate electrical supplies and the applied rod voltage can change rapidly, so the quadrupole can scan a wide mass range more than 10 times per second. This SOP, with minimal instrument specific modifications, can be carried out using other similar instruments. As previously mentioned, hydrothermal fluids are aqueous solutions with a wide range of temperature, salinity and pH values. So, compared to the routine analysis of drinking water or seawater we have developed specific adjustments because the biggest problem during this type of analysis is the amount of total dissolved solid and the salinity. Therefore it is advisable to dilute the sample, to keep total dissolved solids (TDS) below 0.2 %. Some matrix components may deposit around the sampling and the skimmer cones and can lead to long-term signal instability and potentially nebuliser blockage. For example, matrices high in NaCl can form volatile oxides which can settle on cones and be released later on during the run, compromising subsequent samples. As hydrothermal fluids and hydrothermal sediment digestates tend to have high salinities (>3% and up to 30%), care must be taken in diluting the sample while considering the effects of dilution on the detection limits of each element. For comparison, with a 50 % dilution of seawater (1.5 % NaCl final concentrations) there is no significant cone blockage and signal stability remains good.

A common problem in ICP-MS analysis is the possible formation of polyatomic interferences that occur when two or more elements combine and have the same mass as an element of interest. For example, when using HCl for solid sample digestion or cleaning procedures, this can ionize in the plasma to form $^{35}Cl^{16}O^{+}$ and $^{40}Ar^{35}Cl^{+}$, which have the same mass-to charge ratio as $^{51}V^{+}$ and $^{75}As^{+}$ respectively. Another type of common interference is the isobaric interference produced by different isotopes of other elements in the sample creating interference at the same mass as the analyte of interest. For example, vanadium has two isotopes at 50 and 51 amu. Mass 50 is the practical isotope to use in the presence of a chloride, because of the large contribution from the $^{16}O^{35}Cl^{+}$ interference at mass 51. Unfortunately, mass 50 amu coincides with isotopes of titanium and chromium. This makes the determination of vanadium in the presence of titanium and chromium difficult unless mathematical corrections are applied (Thomas 2003).

Finally, there are many factors that can affect the final trace metal analysis results. Given the high sensitivity of the instrument (up to a few ppt for most metals), great care must be taken to minimize external contamination to the used equipment. To minimize possible contamination during field sampling and laboratory preparation procedures we always use specifically selected materials that have been treated to reduce the amount of contaminating metals (see below for details). For example, non-colored plastic tips and vials are preferred to

colored ones, as the dye used for coloring the plastic can leach elements such as Cu, Fe, Zn and Cd, and all plastic and glass vials are acid washed to prevent leaching of Sb, Zn, Mn, Fe, Ba (Moody 1983; Thomas 2003). Indeed, it is preferable not to keep the samples in glass vials for a long period especially if the concentration of these elements are extremely low. The ICP-MS laboratory has restricted access to external personnel, and specific personal protective devices are always used to minimize common contamination from skin, hair, nails or jewelry.

PRINCIPLE OF FUNCTIONING OF ICP-MS

The sample is placed in the autosampler, and a peristaltic pump transports it to the nebulizer, where the liquid sample is converted into aerosol using argon gas. The aerosol passes through a spray chamber, where the larger droplets are removed. The fine droplets are carried by the argon gas flow to the ICP plasma torch. The energy is provided by a radio frequency (RF) generator operating at about 1.5 KW. The RF energy is transferred to the argon gas flow by inductive coupling from a load coil wrapped in the quartz tube. The RF field causes free electrons to oscillate causing them to collide with argon atoms with enough energy to remove an electron, ionizing the argon atoms. The energy density in the ionized argon gas is very high, so the instrument reaches a temperature of 10,000 degrees Celsius. The argon gas passing through the outer quartz tube flows at a rate of around 15 l/min. Two additional smaller quartz tubes are positioned concentrically inside the outer tube. The middle quartz tube carries an auxiliary gas flow which pushes the base of the plasma away from the inner quartz tubes to prevent them from melting. The smallest tube carries the aerosol droplets from the spray chamber to the plasma at a flow rate of around 1 l/min. The aerosol droplets are carried through the center of the plasma, where the droplets are dried, decomposed, dissociated, atomized and finally ionized. The sample passes through a vacuum interface, interface cones that provide optimum vacuum conditions for operation of the quadrupole mass filter and detector, and ion lens with the aim of separating ions from neutral particles and photons. This is important since uncharged particles would cause a high background signal, so they must be prevented from passing through the vacuum system and reaching the detector, this is usually achieved by deflecting the ions off axis, while the photons and neutrals, being uncharged, continue in a straight line and so are removed from the ion beam. The ions pass through a collision cell to resolve the spectral overlaps caused by the unwanted ions (polyatomic), which appear at the same mass as the ions of the analyte being measured.

The cell is pressurized with helium (He), a non-reactive gas with a flow of 4.7 ml/min. The ions collide with the atoms of He, and larger ions such as polyatomic ones are preferentially removed. The ions arrive at the quadrupole mass spectrometer to filter the ions according to the mass-charge ratio (m/z). The mass spectrometer consists of two bar pairs to which an electric field is applied. Alternating electric fields destabilize the trajectories of all ions above and below the set mass, then ions at any mass other than the set mass are repelled by the ion

beam. At the end, ions arrive at the electron multiplier that uses a high voltage electrode positioned so that ions that emerge from the quadrupole strike the dynode. The electron multiplier detector can detect individual ions, so ultralow concentrations can be detected. For each mass measured, the counts registered by the detector are processed by the data analysis software. For quantitative analysis, the signal measured by the detector is in units of counts per second (CPS) that corresponds to the number of ions striking the detector every second.

STANDARD OPERATING PRACTICE IN THE GIOVANNELLI LAB

The method was developed using inductively coupled plasma mass spectrometry (ICP-MS). In order to reduce the dissolved solid, all samples are filtered in the field through a 0.22 μm filter and diluted prior to analysis. If samples are not processed immediately, they can be stored at 4 °C. All plastic materials used during analysis are washed overnight in an acid bath afterwards materials are rinsed five times with Type I water (18 MΩ/cm) and left to dry under the chemical hood. Glassware is also acid washed to prevent leaching with 1 % HNO_3 for 24 h and washed five times with Type I water (18 MΩ/cm). All samples are diluted gravimetrically with HNO_3 1 % (final concentration), which is also used for blanks. Using HNO_3 as a solvent for the dilutions is preferred to water because some elements are unstable and can co-precipitate. Prior to analysis, sediment samples are digested using microwave assisted digestions following the EPA3051A method, and filtered using laboratory filter paper (pre-treated with a 1:5 HNO_3: H_2O solution). A certified reference material is used to verify the digestion efficiency. Data acquisition and analysis is carried out through MassHunter 4.6 software provided with the instrument. To convert data into a concentration, calibration standards containing known concentrations of elements are used to construct a calibration curve. The mass and the ionization potential, which may be determinants of the matrix effects, are evaluated through an internal standard at the final concentration of 400 μg/L. Internal standard is used to correct for changes in instrument operating conditions and sample-specific matrix effects. The same quantity of internal standard is added to each sample, standard and blank, and results are calculated using the ratio of the analyte and internal standard signal. Calibration curves for the trace element of interest are run in the range of 0.01 and 100 ppb using certified multistandard with $R \geq 0.999$. Calibration is carried out every time the device is switched on and a 10 ppb multistandard is run every 10 samples to check for recovery. Finally, our system is stabilized and connected to an UPS (MISSION 10000) to avoid switching off the instrument in case of power surges.

APPARATUS AND EQUIPMENT

- 7900 ICP-MS (Agilent Technologies, USA)
- SPS4 Autosampler (Agilent Technologies, USA)
- ICP-MS MassHunter 4.6 software Version C.01.06 (Agilent Technologies, USA)
- Multiwave GO Plus, Microwave Digestion System (Anton Paar, Austria)
- MS105DU Analytical balance capable of 0.0001 g sensitivity (Mettler Toledo, USA)
- Class A volumetric flasks
- Graduated Cylinder
- Acid Dispenser
- Pipettes for reagent and standard preparation
- Plastic syringes
- Filters 0.22 µm
- Laboratory filter paper

REAGENTS AND STANDARDS

- Deionized water to wash the plastic material
- Type I water from ultrapure water system with a resistivity of 18 MΩ used for sample and standard preparation (PURIST Ultrapure Water System, Rephile)
- Nitric Acid 67 %, NORMATOM® for trace analysis
- Argon gas high purity (99.999 %)
- Helium gas high purity (99.999 %)
- Hydrochloric Acid 37 % (Reag. USP) for analysis, ACS, ISO
- Stock Multi-element calibration standard-2A, 10 µg/mL of Ag, AI, As, Ba, Be, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, K, Li, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, TI, U, V, Zn; matrix 5 % HNO (8500-6940, Agilent Technologies)
- Stock Multi-element calibration standard-4, 10 µg/mL of B, Ge, Mo, Nb, P, Re, S, Si, Ta, TI, W, Zr; matrix H_2O/0.2 % HF/trace HNO_3 (8500-6942, Agilent Technologies)

- Stock Internal standard mix, 100 mg/L of Bi, Ge, In, Li^6, Lu, Rh, Sc, Tb; matrix 10 % HNO_3 (5188-6525, Agilent Technologies)
- Tuning Solution, 1 µg/L Ce, Co, Li, Mg, TI, Y; matrix 2 wt% HNO_3. This multi element solution is used to check the sensitivity and mass resolution of the instrument (5185-5959, Agilent Technologies)
- ENV-META-12 interlaboratory test (UNICHIM)

CLEANING PROCEDURE

ICP-MS detects elements even in ppt (parts per trillion), so contamination is a very serious issue. The use of glassware is not recommended due to impurities leaching from the glass. Plastic is usually better than glass, however even these materials can contain leachable contaminants, such as phosphorus or barium compounds, so a cleaning procedure must be carried out to minimize possible contamination.

All material used for the analysis has been subjected to the following treatment to remove leachable contaminants:

- Rinse tips, tubes and caps with deionized water and soap, let it soak for 24 h. This step is used to remove possible organic contamination.
- Wash with deionized water and place in a 0.5 N Hydrochloridric acid bath, making sure that each material is fully submerged, soak for 12 h. This step is used to remove the possible trace metal contamination.
- Wash with type I water (18 MΩ/cm) five times and shake off excess water. Dry under a chemical hood.

SAMPLE COLLECTION, PRESERVATION AND STORAGE

In the field, samples are collected and filtered through a 0.22 µm membrane filter in metal free plastic tubes and acidified with HNO_3 2 %. If samples are not processed immediately, they can be stored at 4 °C. Store a Nitric acid stock to evaluate the possible metal contamination.

SAMPLE PREPARATION

Liquid samples

1. Gravimetric dilutions are performed sequentially (1:10, 1:100, 1:1000, 1:10000), using an analytical balance, in acid washed 15 mL falcon tubes with 1 % HNO_3, max 2 h before the analysis start.
2. Prepare blanks with the same acid used to prepare the dilution.
3. After the analysis, store the samples along with the blank and an aliquot of nitric acid used in the analysis for future references.

Solid samples

Before running ICP-MS, samples are microwaved digested following the EPA 3051A method for digestion of sediments, sludges, soils and oil. This digestion provides the total trace metal concentration present in the sample.

Microwave digestion of sediment samples

1. Dry the homogenized sample, about 2 g, at 60 °C for 48 h (samples should have been stored and taken using metal-free containers).
2. After, weigh about 0.5 g using the analytical balance in a metal free container.
3. Transfer the sample into a clean PTFE digestion vessel and add 12 mL of aqua regia with concentrated HNO_3 and HCl (3:1). Do this under the chemical hood.
4. Prepare a blank with just 12 mL of aqua regia.
5. Close the vessel and place them inside the rotor considering the recommendations of the manufacturer.
6. Set up the digestion method by selecting “EPA3051A”.
6. Lower the chemical hood glass when the digestion system starts the cooling stage and DO NOT OPEN IT until it finishes.
7. Once the digestion is finished and the vessels are cooled (approx. 25-30 min), open the digester and the vessels under the chemical hood. Prepare as many 50 mL volumetric flasks (previously rinsed with 1 % HNO_3 for 24 h) as samples to be digested and filter them using laboratory filter paper (pre-treated with the 1:5 HNO_3:H_2O mixture) placed in a long stem funnel.

8. Bring samples to volume with ultrapure water and store the digestates in metal-free falcon tubes.
9. Use the solution obtained for the preparation of the dilutions to be run with ICP-MS.

Digestion efficiency is verified using ENV-META-12 certified test material. Our recovery for As, Cd, Co, Cr, Cu, Ni, Pb, V, Tl from ENV-META-12 certified test material (UNICHIM), used for the analysis of different types of matrices such as soil, sediment, sludge and waste is reported in Table 1.

QUALITY CONTROL OF THE ANALYTICAL SET UP

The full analytical setup consists of a series of QC blanks, samples and QC standard run in a specific order described in Figure 1. The QC blanks are used to verify the absence of elements carryover and confirm low background levels while the QC standard is used to check for peak drift and consistent quantification of the standards.

- Calibration is performed every time the device is switched on and repeated when the QC step fails while using the routine standard 10 ppb.
- Blanks composed of 1 % HNO_3 are run at the beginning of every run.
- Two QC blanks 1 % HNO_3 are run as samples at the beginning of each run and once every 10 samples after the routine QC standard.
- The 10 ppb calibration standard solution is run as a sample for routine QC verification once every 10 samples.

ANALYTICAL PROCEDURE

- Switch on exhaust fume and peristaltic pump;
- Open the gas valve;
- Turn on the Autosampler and Chiller;
- Turn on the Plasma and wait about 40 min for the tuning and the instrument set up. Perform the Autotune step to check the instrument sensitivity;
- Create the Batch and choose the Methods. Usually, we use the “General Purpose method” (high sensitivity) for typical aqueous or acid digested samples (< 0.1 % TDS). Instead, for aqueous or acid digested samples with high TDS content where exceptionally high matrix tolerance is required use the “High Matrix method” (low

sensitivity) in the 50 to 300 μg/L standard concentration range. In "Acquisition Parameters" add or remove the element and choose the gas mode (He or No Gas). In "Acquisition methods" add the standard concentration and levels. In "Sample list" make a sample list analysis with relative information like expedition, sample origin, sample type, position and dilution applied to the sample;

- Validate the Methods and add to "Queue".

INSTRUMENT CALIBRATION

In our laboratory we routinely quantify biometal such as V, Mn, Fe, Co, Ni, Cu, As, Se, Mo, Cd and W (Giovannelli 2022), and other elements such as Ti, Cr, Ga, Rb, Sr, Zr, Nb, Ag, Cs, Ba, Ta, Re, Tl, Pb, U. Moreover, in the next future we will also quantify rare earth elements such as Ce, Dy, Er, Eu, Gd, Ho, La, Lu, Nd, Pr, Sm, Th, Tm, Y, Yb. The signal measured by ICP-MS is in count per second, in order to convert this into a concentration value, external calibration standards containing known concentration of elements are used to produce a calibration curve. Calibration is carried out every time the device is switched on and in case of failed QC step while using the routine 10 ppb standard. The correlation coefficient resulting from analysis is considered acceptable when $r^2 \geq 0.999$ (Figure 2).

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

Limit of detection (LOD) is the smallest measure that can be detected with reasonable certainty for an analytical procedure, while the Limit of quantification (LOQ) is the smallest concentration that can be determined with accuracy and precision. There are several methods to calculate LOD and LOQ. We use the method based on the calibration curves of low concentration of target analyte (Indrayanto 2018).

Limit of detection (LOD) is expressed as:

$$\mathrm{LOD} = 3.3 * \sigma/S$$

and Limit of quantification (LOQ) is expressed as:

$$\mathrm{LOQ} = 10 * \sigma/S$$

where σ is the standard deviation of the regression and S is the slope of the calibration curve (Tietje and Brouder 2010). The LOD and LOQ values obtained for our ICP-MS SOP are given in Table 2 and Table 3.

APPLICATIONS

The proposed procedure was applied to investigate the concentration of trace elements biologically significant in hydrothermal fluid. Examples of the application of this procedure are presented in Figure 4 that shows a PCA (Principal component analysis), extremely useful when working with data sets that have a lot of features, on the samples retrieved on the AEO19 expedition to the islands of the Aeolian archipelago and in the Gulf of Naples during the FEAMP expedition. Depending on the length of the arrow, we have a smaller or larger contribution of that specific variable on the sample.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the member of the Giovannelli Lab for help in setting up the geobiochemistry laboratory.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this manuscript.

FUNDING INFORMATION

Support to this manuscript and for the setup of the geobiochemistry laboratory came from funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program Grant Agreement No. 948972—COEVOLVE—ERC-2020-STG to DG.

COMPETING INTERESTS

The authors declare no competing interests.

DATA AND SOFTWARE AVAILABILITY

The data produced for this manuscript is available at https://github.com/giovannellilab/GiovannelliLab_SOPs and released a a permanent repository from ZENODO with DOI 10.5281/zenodo.7614720.

REFERENCES

Anbar, Ariel D. 2008. “Elements and Evolution.” *Science* 322 (5907): 1481–83. https://doi.org/10.1126/science.1163100.

Bertrand, Mak A., Julie M. Rose, Christina R. Riesselman, Maeve C. Lohan, Abigail E. Noble, Peter A. Lee, Giacomo R. DiTullio, and Erin M. Bertrand. 2007. “Vitamin B_{12} and Iron Colimitation of Phytoplankton Growth in the Ross Sea.” *Limnology and Oceanography* 52 (3): 1079–93. https://doi.org/10.4319/lo.2007.52.3.1079.

Delgado-Baquerizo, Manuel, Angela M. Oliverio, Tess E. Brewer, Alberto Benavent-González, David J. Eldridge, Richard D. Bardgett, Fernando T. Maestre, Brajesh K. Singh, and Noah Fierer. 2018. “A Global Atlas of the Dominant Bacteria Found in Soil.” *Science* 359 (6373): 320–25. https://doi.org/10.1126/science.aap9516.

Falkowski, Paul G., Tom Fenchel, and Edward F. Delong. 2008. “The Microbial Engines That Drive Earth’s Biogeochemical Cycles.” *Science* 320 (5879): 1034–39. https://doi.org/10.1126/science.1153213.

Féraud, Gilbert, Cécile Potot, Jean-François Fabretti, Yves Guglielmi, Marc Fiquet, Vittorio Barci, and Pierre-Charles Maria. 2009. “Trace Elements as Geochemical Markers for Surface Waters and Groundwaters of the Var River Catchment (Alpes Maritimes, France).” *Comptes Rendus Chimie* 12 (8): 922–32. https://doi.org/10.1016/j.crci.2009.02.002.

Fullerton, Katherine M., Matthew O. Schrenk, Mustafa Yücel, Elena Manini, Marco Basili, Timothy J. Rogers, Daniele Fattorini, et al. 2021. “Effect of Tectonic Processes on Biosphere–Geosphere Feedbacks across a Convergent Margin.” *Nature Geoscience* 14 (April): 301–6. https://doi.org/10.1038/s41561-021-00725-0.

Gianelli, Giovanni, and Sergio Grassi. 2001. “Water–Rock Interaction in the Active Geothermal System of Pantelleria, Italy.” *Chemical Geology* 181 (1–4): 113–30. https://doi.org/10.1016/S0009-2541(01)00276-5.

Giovannelli, Donato. 2022. “Geosphere and Biosphere Coevolution: The Role of Trace Metals Availability in the Evolution of Biogeochemistry.” EarthArXiv. https://doi.org/10.31223/X5QH1G.

Giovannelli, Donato, Peter H. Barry, J. Maarten de Moor, Gerdhard L. Jessen, Matthew O. Schrenk, and Karen G. Lloyd. 2022. “Sampling across Large-Scale Geological Gradients to Study Geosphere–Biosphere Interactions.” *Frontiers in Microbiology* 13. https://doi.org/10.3389/fmicb.2022.998133.

Grandell, Leena, Antti Lehtilä, Mari Kivinen, Tiina Koljonen, Susanna Kihlman, and Laura S. Lauri. 2016. “Role of Critical Metals in the Future Markets of Clean Energy Technologies.” *Renewable Energy* 95 (September): 53–62. https://doi.org/10.1016/j.renene.2016.03.102.

Indrayanto, Gunawan. 2018. “Validation of Chromatographic Methods of Analysis: Application for Drugs That Derived From Herbs.” In *Profiles of Drug Substances, Excipients and Related Methodology*, 43:359–92. Elsevier. https://doi.org/10.1016/bs.podrm.2018.01.003.

Jelen, Benjamin I., Donato Giovannelli, and Paul G. Falkowski. 2016. “The Role of Microbial

Electron Transfer in the Coevolution of the Biosphere and Geosphere." *Annual Review of Microbiology* 70 (1): 45–62. https://doi.org/10.1146/annurev-micro-102215-095521.

Moody, John R. 1983. "Sampling and Storage of Materials for Trace Elemental Analysis." *TrAC Trends in Analytical Chemistry* 2 (5): 116–18. https://doi.org/10.1016/0165-9936(83)88011-X.

Moore, Eli K., Benjamin I. Jelen, Donato Giovannelli, Hagai Raanan, and Paul G. Falkowski. 2017. "Metal Availability and the Expanding Network of Microbial Metabolisms in the Archaean Eon." *Nature Geoscience* 10 (9): 629–36. https://doi.org/10.1038/ngeo3006.

Morel, F. M. M., and N. M. Price. 2003. "The Biogeochemical Cycles of Trace Metals in the Oceans." *Science* 300 (5621): 944–47. https://doi.org/10.1126/science.1083545.

Rogers, Timothy J., Joy Buongiorno, Gerdhard L. Jessen, Matthew O. Schrenk, James A. Fordyce, J. Maarten de Moor, Carlos J. Ramírez, et al. 2022. "Chemolithoautotroph Distributions across the Subsurface of a Convergent Margin." *The ISME Journal*, October, 1–11. https://doi.org/10.1038/s41396-022-01331-7.

Thomas, Robert. 2003. *Practical Guide to ICP-MS*. 0 ed. CRC Press. https://doi.org/10.1201/9780203027073.

Tietje, Christian, and Alan Brouder, eds. 2010. "International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use." In *Handbook of Transnational Economic Governance Regimes*, 1041–53. Brill | Nijhoff. https://doi.org/10.1163/ej.9789004163300.i-1081.897.

Figures and Tables

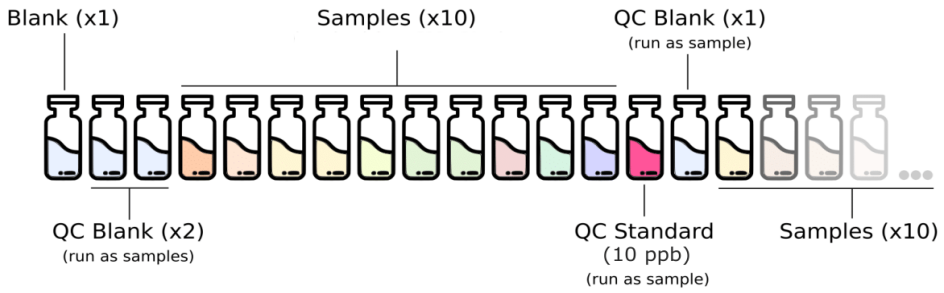

Figure 1. Analytical set up for the routine analysis of trace metal in deeply-sourced seeps fluid samples.

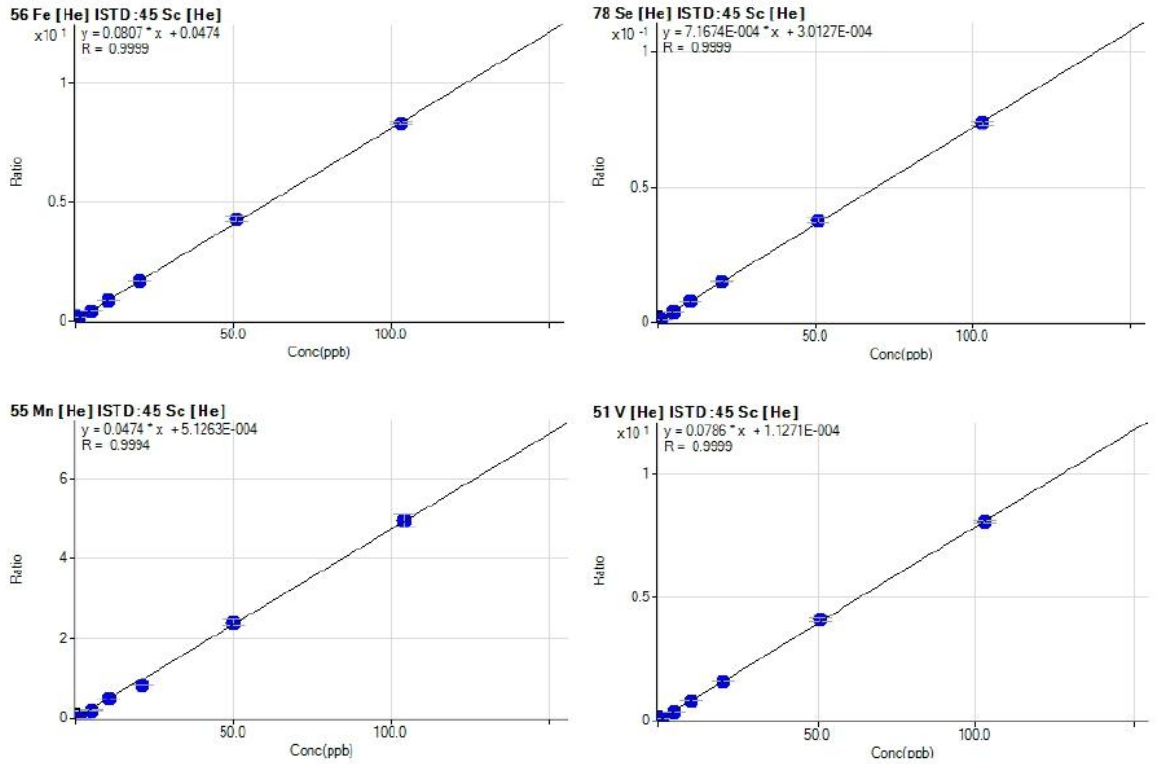

Figure 2. Example of calibration curves for Fe, Se, Mn and V with a final concentration of 0.01, 0.1, 1, 5, 10, 20, 50, 100 µg/L determined following the described SOP.

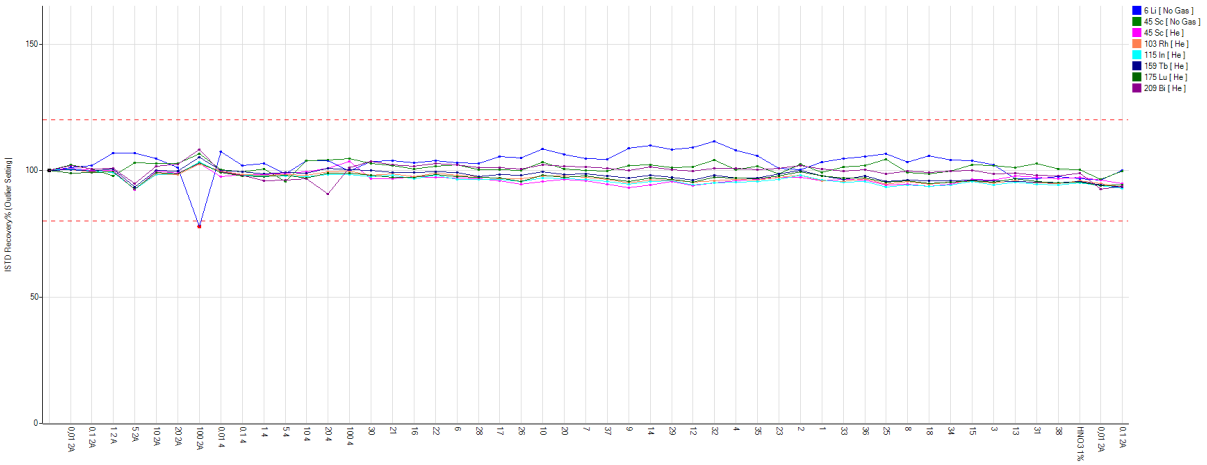

Figure 3. Internal standard recovery during an analytical run.

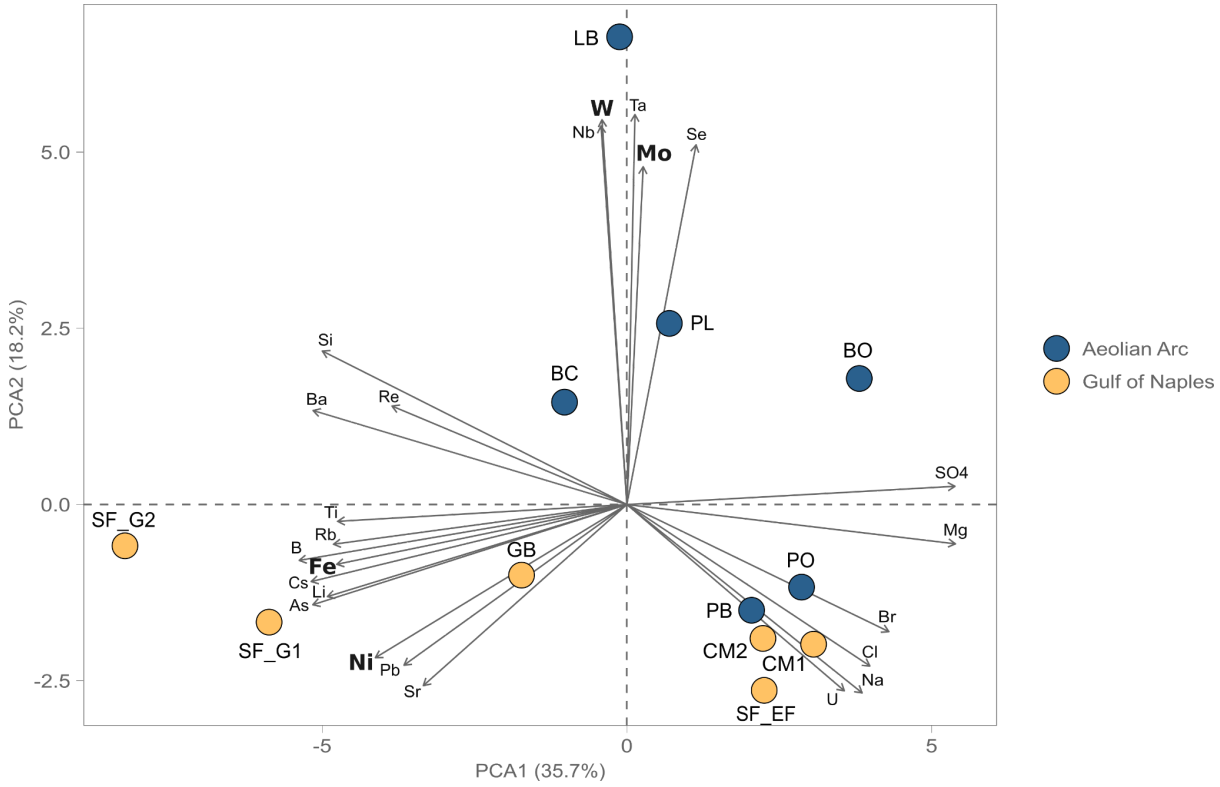

Figure 4. PCA analysis of hydrothermal fluids from the Aeolian Arc and the Gulf of Naples (Italy, unpublished data) based on trace metal concentration (trace metals with important biological functions are highlighted in bold).

Table 1. Results of the analysis of the microwave assessed acid digested reference materials.

Analyte	% Recovery
^{75}As	99.9
^{111}Cd	99.5
^{59}Co	95.8
^{52}Cr	92.4
^{63}Cu	97.8
^{60}Ni	87.2
^{208}Pb	89.7
^{51}V	100
^{205}Tl	92.4
^{66}Zn	81.1
^{9}Be	23.9

Table 2. LOD and LOQ for the ICP-MS SOP for the determination of biometals in hydrothermal fluids and sediments in the Giovannelli Lab.

	LOD		LOQ	
Analyte	**µg/L**	**nM**	**µg/L**	**nM**
^{51}V	0.0227	0.4460	0.0688	1.3514
^{55}Mn	0.0231	0.4203	0.0700	1.2736
^{56}Fe	0.0318	0.5690	0.0963	1.7242
^{59}Co	0.0203	0.3436	0.0614	1.0413
^{60}Ni	0.0365	0.6227	0.1108	1.8870
^{63}Cu	0.0207	0.3253	0.0626	0.9857
^{75}As	0.0252	0.3370	0.0765	1.0213
^{78}Se	0.0322	0.4080	0.0976	1.2364
^{95}Mo	0.0158	0.1647	0.0479	0.4991
^{111}Cd	0.0187	0.1666	0.0567	0.5047
^{182}W	0.0157	0.0854	0.0476	0.2587

Table 3. LOD and LOQ for the ICP-MS SOP.

	LOD		LOQ	
Analyte	**µg/L**	**nM**	**µg/L**	**nM**
^{47}Ti	0.0536	1.1194	0.1624	3.3920
^{52}Cr	0.0216	0.4156	0.0655	1.2595
^{71}Ga	0.0200	0.2873	0.0607	0.8705
^{85}Rb	0.0135	0.1580	0.0409	0.4789
^{88}Sr	0.0156	0.1780	0.0473	0.5394
^{90}Zr	0.0146	0.1598	0.0442	0.4843
^{93}Nb	0.0069	0.0742	0.0209	0.2248
^{107}Ag	0.0086	0.0793	0.0259	0.2404
^{133}Cs	0.0134	0.1007	0.0405	0.3050
^{137}Ba	0.0127	0.0922	0.0384	0.2795
^{181}Ta	0.0130	0.0721	0.0395	0.2185
^{185}Re	0.0154	0.0827	0.0467	0.2506
^{205}Tl	0.0043	0.0209	0.0129	0.0632
^{208}Pb	0.0155	0.0750	0.0471	0.2273
^{238}U	0.0099	0.0415	0.0300	0.1259