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METHOD ARTICLE

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

[version 2; peer review: 1 approved, 2 approved with reservations]

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Abstract

This standard operating procedure (SOP) validates an inductively coupled plasma mass spectrometry (ICP-MS) procedure for the determination of trace elements 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.

Plain language summary

This manuscript outlines the standard operating procedure used to determine trace elements in hydrothermal fluids, which are characterized by a high variability of physical-chemical parameters. Due to the high variability of our samples, we customized the method to compensate for the variability on the physico-chemical parameters. The obtained data on the distribution and abundance of trace elements in sediments and fluids can be used to track geochemical processes mobilizing the metals as well as their influence on the microbial diversity in geothermal systems.

Open Peer Review

Approval Status ? ✓ ?

	1	2	3
version 2 (revision) 24 Jul 2024		✓ view	
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Any reports and responses or comments on the article can be found at the end of the article.

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

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REVISED **Amendments from Version 1**

As suggested by the reviewers, we have made substantial changes compared to the previously published version:

We have changed the title of the manuscript

We have significantly reduced the methods and operational principles section

We have added the raw data used for Figure 4 in a new Table named Table 5 (we have added this table in the text and we also uploaded it as a new table)

We added a new Table with all the operational conditions as Table 1 (we have added this table in the text and we also uploaded it as a new table)

We modified Table 2.

We have added several citations in the main text and we included them at the end of the reference list, as indicated by the guidelines.

Any further responses from the reviewers can be found at the end of the article

Introduction

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 determination 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, 2023a). Generally, microbial diversity is linked toa number of physical and chemical parameters including, but not limited to, 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. By 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 (Bertrand *et al.*, 2007; Giovannelli, 2023a; Morel & Price, 2003). 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 *et al.*, 2008; Jelen *et al.*, 2016; Moore *et al.*, 2017). Trace metals such as Fe, Co, Ni, Mo, W, V and Cu are used in a set of microbial proteins containing redox-sensitive transition metals as their core catalytic (Hay Mele *et al.*, 2023) 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 *et al.*, 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 determination 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 & Grassi, 2001). Comparatively less attention has been devoted to the biologically relevant elements (Giovannelli, 2023a), 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 an 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.

Methods

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, neutral particles 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 ion strikes 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 (G8403A). 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 suspended particulate matter and the salinity. 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 that 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, it was reported that with a 50 % dilution of seawater (1.5 % NaCl final concentrations) there is no significant cone blockage and signal stability remains good (Thomas, 2003).

A common problem in ICP-MS analysis is the possible formation of interferences (Olesik, 2014), like polyatomic interference that occur when two or more elements combine and have the same mass as an element of interest (for example $^{35}Cl^{16}O^{+}$ an $^{51}V^{+}$), fortunately most of the spectral overlaps can be reduced by the collision cell.

Finally, there are many factors that can affect the final trace metal determination 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 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. 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. 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. 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 (Wilschefski & Baxter, 2019). ICP-MS operational conditions of this SOP are described in Table 1.

Standard operating practice in the giovannelli lab

The method was developed using inductively coupled plasma mass spectrometry (ICP-MS 7900, G8403A). In order to reduce the suspended particulate, all samples are filtered in the field through a 0.22 µm filter, this has the benefit of removing all the particles that might dissolve after acidification and removing all the microbial cells. All plastic and glassware materials used during analysis are washed in an acid bath (see below). 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 (Figure 1). Internal standard is used to correct for changes in

Table 1. ICP-MS operation conditions.

RF	1.5 KW
Plasma Gas	15 L/min
Auxiliary Gas	0.9 L/min
Nebulizer Gas	1.09 L/min
He Flow	4.7 L/min
Spray Chamber Temperature	2 °C
Replicate	3
Nebulizer pump	0.1 rps

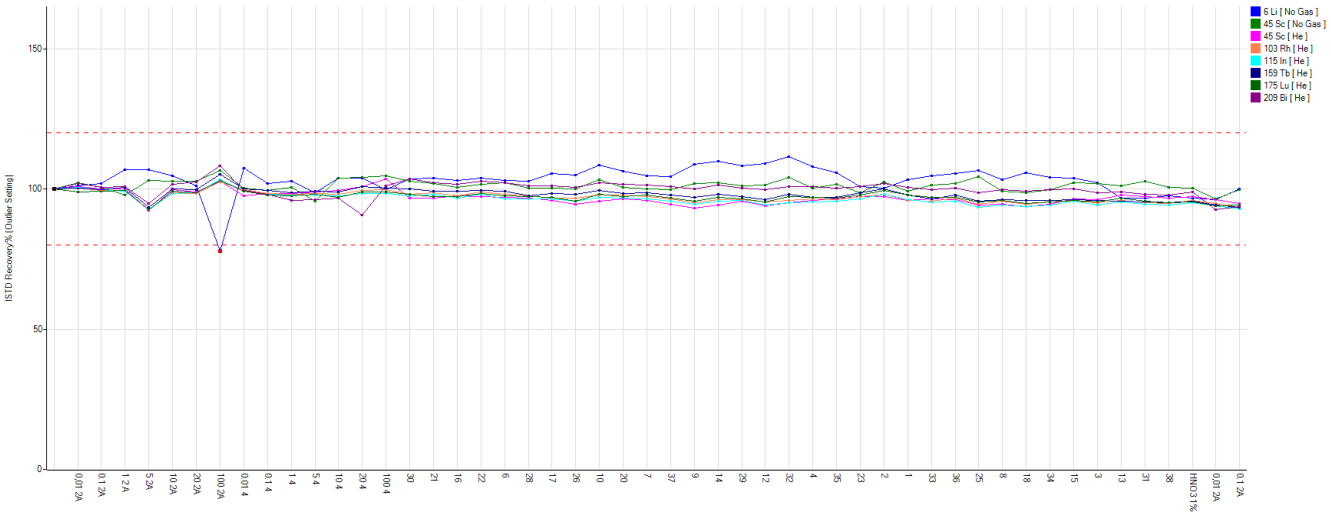

Figure 1. Internal standard recovery during an analytical run.

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 (G8403A Agilent Technologies, USA)
- SPS4 Autosampler (AU204810646, Agilent Technologies, USA)
- ICP-MS MassHunter 4.6 software Version C.01.06 (Agilent Technologies, USA) (free alternative, OpenChrom)
- Multiwave GO Plus, Microwave Digestion System (Anton Paar, Austria)
- Analytical balance capable of 0.0001 g sensitivity (MS105DU Mettler Toledo, USA)
- Graduated Cylinder
- Acid Dispenser
- Pipettes for reagent and standard preparation
- Plastic syringes
- PP syringe filters 0.22 µm (TS-900-063 Perlabo)
- Laboratory filter paper
- PTFE spatula

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)
- ENVIR-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 hydrochloric 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 to HNO_3 2 %. Instead, solid samples are collected with a PRFE spatula in a metal free tube. If liquid samples are not processed immediately, they can be stored at 4 °C, solid samples are stored at -20 °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, 1:1,000, 1:10,000) based on the salinity of the samples, 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 overnight dried and 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 that could be available for microbes.

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”.
7. Lower the chemical hood glass when the digestion system starts the cooling stage and do not open it until it finishes.
8. 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 fil-ter them using laboratory filter paper (pre-treated with the 1:5 HNO_3:H_2O mixture) placed in a long stem funnel.
9. Bring samples to volume with ultrapure water and store the digestates in metal-free falcon tubes.
10. Use the solution obtained for the preparation of the dilutions to be run with ICP-MS.

Digestion efficiency is verified using ENVIR-META-12 certified test material. Digestion is performed in triplicates; data are expressed as mean ± SD. 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 2.

Table 2. Results of the analysis of the microwave assessed acid digested reference materials.

Analyte	%Recovery	mg/L ± SD
^{75}As	95.9	24.46 ± 0.40
^{111}Cd	92.7	2.55 ± 0.04
^{59}Co	97	13.1 ± 0.32
^{52}Cr	75.2	115.8 ± 1.09
^{63}Cu	97.6	89.22 ± 1.72
^{60}Ni	98.6	77.09 ± 1.60
^{208}Pb	91.6	181.36 ± 2.15
^{51}V	75.2	63.21 ± 0.13
^{205}Tl	93.5	3.92 ± 0.04
^{66}Zn	90.7	241.27 ± 1.45
^{9}Be	93.8	1.21 ± 0.03

Quality control (QC) of the analytical setup

The full analytical setup consists of a series of quality control (QC) blanks, samples and QC standard run in a specific order described in Figure 2. 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.

Creation of the method

- 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;
- 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;
- Perform the Autotune step to check the instrument sensitivity;
- 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. Calibration curves are linear in the 0.01 to 100 µg/L concentration range. The calibration points are: 0.01, 0.1, 0.5, 1, 5, 10, 20, 50, 100 µg/L. The correlation coefficient resulting from analysis is considered acceptable when $r^2 \geq 0.999$ (Figure 3).

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).

LOD is expressed as:

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

and 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 (Al-Hakkani, 2019; Lister, 2005; Tietje & Brouder, 2010). The LOD and LOQ values obtained for our ICP-MS SOP are given in Table 3 and Table 4.

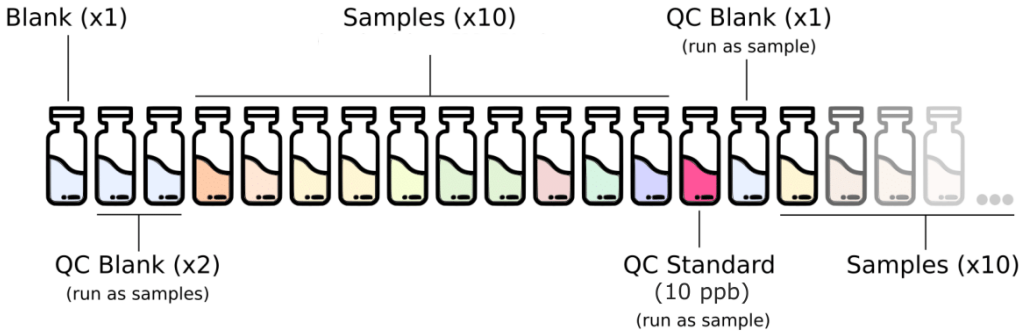

Figure 2. Analytical setup for the routine analysis of trace metal in deeply-sourced seeps fluid samples. QC, quality control.

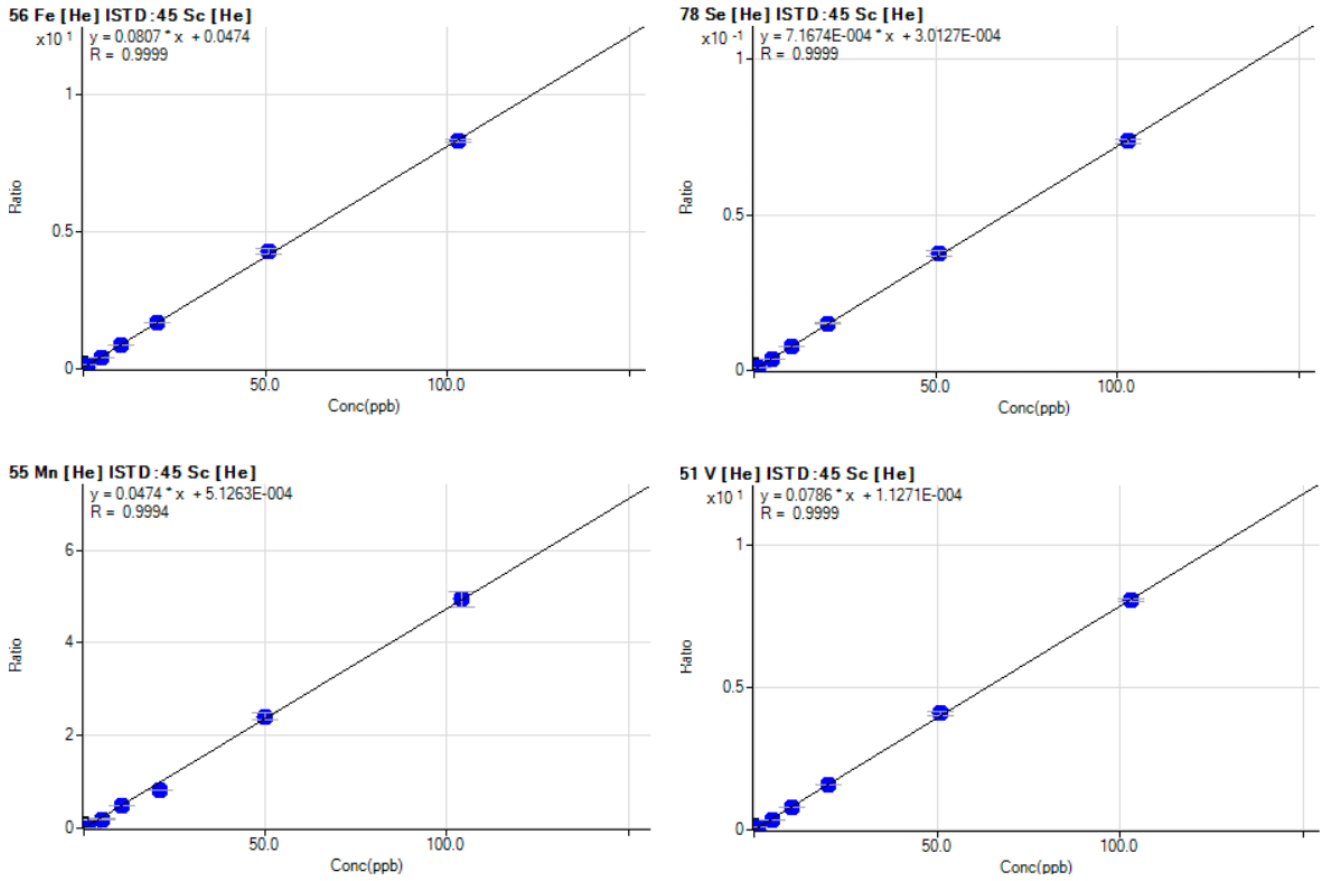

Figure 3. Example of calibration curves for Fe, Se, Mn and V with a final concentration of 0.01, 0.1, 1, 5, 10, 20, 50, 100 µg/L determined following the described SOP. SOP, standard operating procedure.

Table 3. LOD and LOQ for the ICP-MS SOP for the determination of biometals in hydrothermal fluids and sediments in the Giovannelli Lab. LOD, limit of detection; LOQ, limit of quantification; ICP-MS, inductively coupled plasma mass spectrometry; SOP, standard operating procedure.

	LOD		LOQ	
Analyte	µg/L	nM	µg/L	nM
^{51}V	0.0227	0.446	0.0688	1.3514
^{55}Mn	0.0231	0.4203	0.07	1.2736
^{56}Fe	0.0318	0.569	0.0963	1.7242
^{59}Co	0.0203	0.3436	0.0614	1.0413
^{60}Ni	0.0365	0.6227	0.1108	1.887
^{63}Cu	0.0207	0.3253	0.0626	0.9857
^{75}As	0.0252	0.337	0.0765	1.0213
^{78}Se	0.0322	0.408	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 4. LOD and LOQ for the ICP-MS SOP. LOD, limit of detection; LOQ, limit of quantification; ICP-MS, inductively coupled plasma mass spectrometry; SOP, standard operating procedure.

	LOD		LOQ	
Analyte	**µg/L**	**nM**	**µg/L**	**nM**
^{47}Ti	0.054	1.12	0.162	3.39
^{52}Cr	0.022	0.42	0.066	1.26
^{71}Ga	0.020	0.29	0.061	0.87
^{85}Rb	0.014	0.16	0.041	0.48
^{88}Sr	0.016	0.18	0.047	0.54
^{90}Zr	0.015	0.16	0.044	0.48
^{93}Nb	0.007	0.07	0.021	0.22
^{107}Ag	0.009	0.08	0.026	0.24
^{133}Cs	0.013	0.10	0.041	0.31
^{137}Ba	0.013	0.09	0.038	0.28
^{181}Ta	0.013	0.07	0.040	0.22
^{185}Re	0.015	0.08	0.047	0.25
^{205}Tl	0.004	0.02	0.013	0.06
^{208}Pb	0.016	0.08	0.047	0.23
^{238}U	0.010	0.04	0.030	0.13

Case study

The proposed procedure was applied to investigate the concentration of trace elements biologically significant (*i.e.*, Fe, Co, Ni, Mo, Mn, W, As) in hydrothermal fluids collected on the AEO19 expedition to the islands of the Aeolian archipelago and in the Gulf of Naples during the FEAMP expedition. The data (number of determinations = 3) were used to conduct a principal component analysis (PCA) in order to cluster the samples based on the trace element profiles. As can be seen from Figure 4, the samples taken from the two active volcano islands of Vulcano and Panarea (BC, BO, PL and LB) are greatly influenced by higher concentrations of W and Mo. The samples from the Gulf of Naples and the samples from the inactive volcanic islands of the Aeolian Arquipelago cluster together on the lower half of the the PCA, with the high temperature sites of SF_G1, SF_G2 and GB that are strongly influenced by the concentrations of Ni and Fe. This suggests a differential mobility of the different trace metals in response to the diverse volcanic settings and the different temperatures of the investigated areas. Row data and corresponding precision are given in Table 5. The underlying data are available in GitHub (Giovannelli, 2023b).

Ethics and consent statement

Ethical approval and consent were not required.

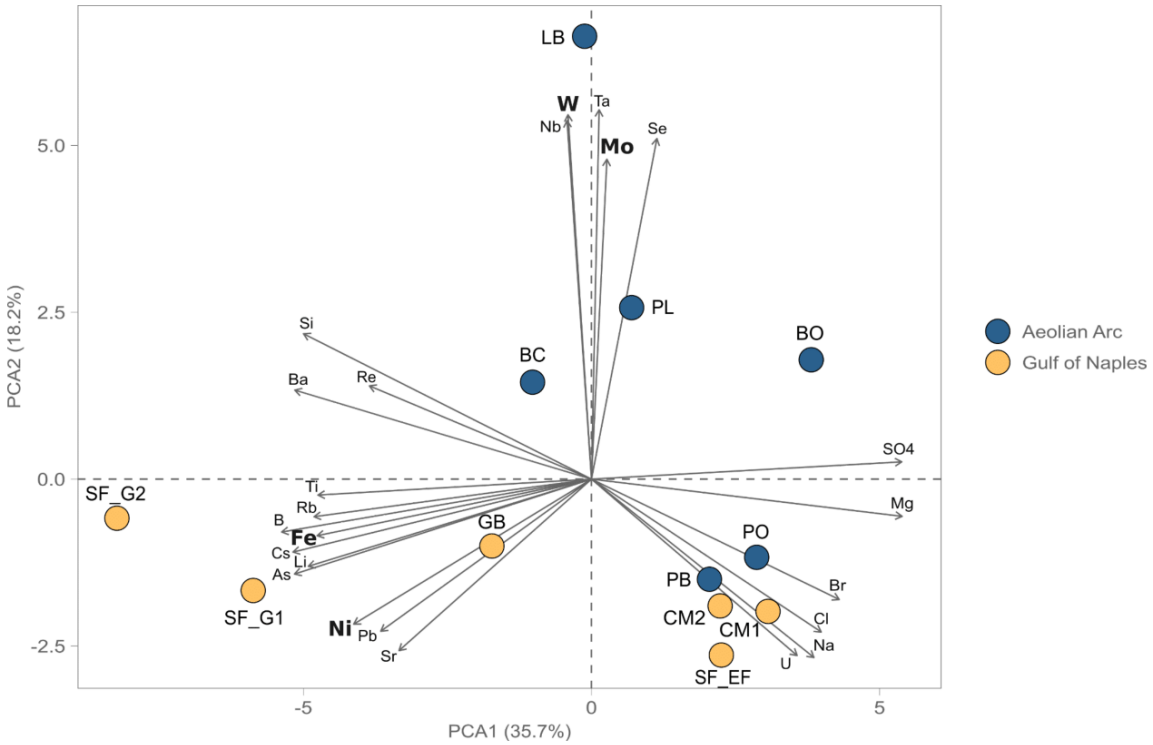

Figure 4. PCA analysis of hydrothermal fluids from the Aeolian Arc and the Gulf of Naples (Italy, unpublished data) based on trace metal concentration (trace metals with important biological functions are highlighted in bold). PCA, Principal component analysis.

Table 5. Row data used for PCA analysis (expressed in ppb). Zeros represent values below detection limits.

SiteID	Li	Be	B	Al	Si	P	Ti	V	Cr	Fe	Co	Ni	Cu	Zn	As	Se	Rb	Sr	Zr	Nb	Mo	Ag	Cd	Cs	Ba	Ta	W	Re	Tl	Pb	U
LB	0.386 ± 0.027	0.001 ± 0.001	11.525 ± 0.092	0.425 ± 0.010	29.893 ± 1.746	0	0.003 ± 0.010	0	0	0	0	0	0.103 ± 0.002	0	0.151 ± 0.002	0.023 ± 0.021	0.696 ± 0.022	6.245 ± 0.076	0	0.05 ± 0.010	0.181 ± 0.013	0.001 ± 0.001	0	0.034 ± 0.003	0.115 ± 0.045	0.029 ± 0.002	0.177 ± 0.018	0.003 ± 0.001	0	0	0
PL	0.24 ± 0.018	0.002 ± 0.001	8.455 ± 0.214	0.358 ± 0.014	26.456 ± 1.253	0.342 ± 0.524	0	0.003 ± 0.001	0.014 ± 0.004	0	0.001 ± 0.001	0	0.06 ± 0.006	1.144 ± 0.087	0.15 ± 0.002	0.008 ± 0.009	0.223 ± 0.012	7.252 ± 0.114	0	0.02 ± 0.004	0.126 ± 0.003	0.004 ± 0.001	0	0.012 ± 0.002	0.052 ± 0.014	0.02 ± 0.001	0.065 ± 0.003	0.001 ± 0	0	0.003 ± 0	0.002 ± 0.001
BC	0.532 ± 0.026	0	7.623 ± 0.041	0.719 ± 0.009	23.413 ± 0.645	24.499 ± 0.514	0.012 ± 0.018	0.003 ± 0.001	0.024 ± 0.003	0	0	0	0.236 ± 0.002	2.797 ± 0.108	0.159 ± 0.004	0	0.38 ± 0.011	8.15 ± 0.047	0	0.014 ± 0.002	0.118 ± 0.002	0.002 ± 0.001	0.001 ± 0.001	0.095 ± 0.002	0.122 ± 0.006	0.019 ± 0.002	0.039 ± 0.002	0.001 ± 0.001	0.006 ± 0.001	0.004 ± 0.001	0.002 ± 0
BO	0.124 ± 0.013	0	1.329 ± 0.026	0.677 ± 0.011	9.16 ± 0.509	7.837 ± 0.417	0	0	0.019 ± 0.001	0	0	0	0.274 ± 0.002	0.746 ± 0.055	0.15 ± 0.001	0.016 ± 0.015	0.1 ± 0.003	1.443 ± 0.024	0.013 ± 0.003	0.011 ± 0.003	0.105 ± 0.004	0.002 ± 0.001	0.001 ± 0.001	0.024 ± 0.002	0.015 ± 0.020	0.018 ± 0.002	0.027 ± 0.001	0.001 ± 0	0	0	0
PO	0.224 ± 0.010	0	4.978 ± 0.051	0.007 ± 0.003	0.693 ± 0.557	0	0	0.001 ± 0.000	0	0	0	0	0.072 ± 0.005	0.118 ± 0.051	0.148 ± 0.002	0	0.146 ± 0.011	8.265 ± 0.061	0	0.005 ± 0.001	0.115 ± 0.001	0.001 ± 0.001	0	0.004 ± 0.001	0.001 ± 0.009	0.011 ± 0.002	0.011 ± 0.001	0	0.001 ± 0.001	0	0.003 ± 0.001
PB	0.25 ± 0.068	0	5.395 ± 1.023	0.372 ± 0.123	10.982 ± 9.247	0	0	0.001 ± 0.000	0	0	0	0	0.059 ± 0.004	0.764 ± 0.028	0.149 ± 0.002	0	0.138 ± 0.006	8.015 ± 0.056	0	0.004 ± 0.002	0.111 ± 0.001	0.007 ± 0.002	0.001 ± 0.001	0.003 ± 0.001	0.054 ± 0.004	0.01 ± 0.002	0.01 ± 0.002	0	0.001 ± 0.001	0.003 ± 0.001	0.003 ± 0
SF_G2	4.2 ± 0.081	0	33.273 ± 0.603	0.754 ± 0.021	35.889 ± 1.655	534.797 ± 11.878	0.056 ± 0.019	0.002 ± 0.001	0.022 ± 0.002	0.362 ± 0.018	0	0.057 ± 0.018	0.028 ± 0.003	1.637 ± 0.060	1.29 ± 0.033	0	1.869 ± 0.028	8.739 ± 0.097	0	0.011 ± 0.001	0.112 ± 0.003	0.006 ± 0.001	0	1.359 ± 0.019	0.162 ± 0.053	0.012 ± 0.002	0.024 ± 0.001	0.009 ± 0.001	0.001 ± 0.001	0.01 ± 0.001	0
CM2	0.186 ± 0.037	0	5.439 ± 0.065	0.096 ± 0.008	0	4.468 ± 0.353	0	0.002 ± 0.001	0	0	0	0.033 ± 0.015	0	0	0.151 ± 0.002	0	0.126 ± 0.009	8.03 ± 0.058	0	0.003 ± 0.001	0.111 ± 0.004	0.001 ± 0.001	0	0.001 ± 0.001	0	0.009 ± 0.002	0.007 ± 0.002	0	0	0.006 ± 0.001	0.003 ± 0.001
SF_G1	4.647 ± 0.015	0	36.822 ± 0.393	0.349 ± 0.010	40.46 ± 1.569	0	0.013 ± 0.027	0.001 ± 0.001	0.013 ± 0.006	0.078 ± 0.047	0	0.082 ± 0.038	0.011 ± 0.003	1.178 ± 0.133	1.541 ± 0.103	0	2.109 ± 0.110	9.717 ± 0.601	0	0.002 ± 0.002	0.101 ± 0.002	0.002 ± 0.001	0.001 ± 0.001	1.523 ± 0.080	0.204 ± 0.014	0.006 ± 0.002	0.015 ± 0	0	0.001 ± 0	0.009 ± 0.001	0
CM1	0.229 ± 0.042	0	5.551 ± 0.058	0	0	0	0	0.002 ± 0.002	0	0	0	0	0.015 ± 0.002	0	0.15 ± 0.001	0	0.121 ± 0.007	7.717 ± 0.084	0	0.001 ± 0	0.11 ± 0.003	0.002 ± 0.001	0	0.001 ± 0.001	0	0.006 ± 0.002	0.002 ± 0.001	0	0	0	0.003 ± 0.001
GB	4.777 ± 0.033	0	26.883 ± 0.324	0.103 ± 0.003	27.988 ± 0.848	0	0	0.001 ± 0.001	0	0.132 ± 0.098	0	0	0.117 ± 0.005	0	0.961 ± 0.117	0	2.482 ± 0.022	7.793 ± 0.055	0	0.001 ± 0.001	0.097 ± 0.001	0.002 ± 0	0.001 ± 0.001	0.148 ± 0.007	0.045 ± 0.011	0.005 ± 0.001	0.005 ± 0.001	0	0	0	0
SF_EF	0.242 ± 0.020	0	6.202 ± 0.112	0.699 ± 0.020	0	0	0	0.005 ± 0.001	0.007 ± 0.003	0	0	0.033 ± 0.025	0.018 ± 0.002	0.105 ± 0.041	0.153 ± 0.002	0	0.132 ± 0.012	7.74 ± 0.139	0	0.002 ± 0.001	0.111 ± 0.002	0.003 ± 0.001	0	0.006 ± 0.001	0.004 ± 0.021	0.005 ± 0.002	0.003 ± 0.001	0	0	0.009 ± 0.001	0.003 ± 0

Open Research Europe 2024, 3:90 Last updated: 26 JUN 2025

Data availability

Underlying data

Data available from: https://github.com/giovannellilab/GiovannelliLab_SOPs. The data for this manuscript are included in the folder ICP-MS_SOP. All the SOPs from the lab are released as a single repository to improve findability and reproducibility of the methods used in the lab.

Archived data at the time of publication: https://doi.org/10.5281/zenodo.7614719 (Giovannelli, 2023a).

This project contains the following underlying data:

- Table1.csv. Contains the data for Table 1 of the manuscript.
- Table2.csv. Contains the data for Table 2 of the manuscript.
- Table3.csv. Contains the data for Table 3 of the manuscript.
- Table4.csv. Contains the data for Table 4 of the manuscript.
- Table5.csv. Contains the data for Table 5 of the manuscript.

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

Author contributions

All authors contributed equally to this manuscript.

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The authors wish to acknowledge the members of the Giovannelli Lab for help in setting up the Geo biochemistry laboratory.

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Open Peer Review

Current Peer Review Status: ? ✓ ?

Version 2

Reviewer Report 09 August 2024

https://doi.org/10.21956/openreseurope.19125.r42437

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✓ **Maria Carmen Barciela-Alonso**
University of Santiago de Compostela, Coruña, Spain

The authors have made some changes taking into account the comments I have made.
1. Regarding LODs and LOQs, the authors should indicate in the text that the values presented in Tables 3 and 4 are instrumental limits of detection and quantification.
2. For the study of the accuracy of the method, the authors use the reference material ENV-META-12, for 11 elements. An alternative way to evaluate the accuracy of the method is to perform analytical recovery studies. The authors can use spiked samples. That is, samples spiked with known concentrations of the studied elements. In my opinion the work could be completed by including analytical recovery studies.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Spectrometry, Trace element determination, envisonmental and biological samples, speciation

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 16 October 2023

https://doi.org/10.21956/openreseurope.16961.r35307

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

Introduction:

"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..."

“Analysis of trace elements” is not correct terminology. Samples are analyzed for determination of .. So it should be determination of trace elements. Please correct in the title and entire manuscript.

“Generally, microbial diversity is linked to environmental main physical-chemical parameters,”

A bit confusing statement. Maybe better “ linked to a number of physical and chemical parameters, including, but not limited to,...”

"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."

This statement is not clear. Please revise and simplify..

"Here we describe the SOP developed to routinely analyze such a wide range..."

It’s better this way - 'Here we describe an SOP developed to routinely analyze such a wide range..'

Methods

"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. ICP-MS can also measure major and minor elements not only trace elements. ICP-MS cannot measure substances. Please use “species” instead. 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."

What do mean by neutrons? Neutral species! I don’t think plasma produces neutrons!

"Finally, each element passes through the detector to be counted."

This not a correct statement. Elements do not pass through detector. Ions hit the detector. What

passes through quadrupole are ions not elements. And separation is based on m/z ratio that occurs sequentially – called hopping mode. Please revise this section and describe corrected operation of quadruple mass filter and ion detection process.

"We have developed this protocol using an Agilent ICP-MS 7900 (G8403A). The ICP-MS 7900 is a single quadrupole mass spectrometer that filters ions by mass to charge ratio (m/z). It 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."

Delete this section. Just provide instrument model and operating conditions.

"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."

Please shorten this section and be concise. Provide recommended sample handling and storage conditions. It is already known that glass containers are not suitable for ICP-MS measurements.

"Principle of functioning of ICP-MS"

Please delete entire section. This is book knowledge. No need to describe operation principles of ICP-MS.

"Standard Operating Practice in the Giovannelli lab. The method was developed using inductively coupled plasma mass spectrometry (ICP-MS)."

Please provide model of ICP-MS instrument here again.

"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."

How do you reduce dissolved solids by filtration? Maybe more appropriate to say suspending particulate matter or colloidal matter. All dissolved species will pass through filter that can only trap particulate matter.

"A certified reference material is used to verify the digestion efficiency."

What sort of SRM was used for sediment QA/QC? Please provide list of used SRMs as well as

recommended SRM. Also, indicate that SRM is used for verifying digestion efficiency and accuracy of ICP-MS analysis. Please provide purity grade for syringe filter and filter papers. What sort of pipettes are used? How did you check pipette calibrations?

Cleaning procedure

Please delete the first paragraph of this sections. “ICP-MS detects elements even in ppt …”

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 HNO3 2 %."

Are samples acidified to 2% HNO3 or acidified with 2%HNO3? How much is used? Better to report final acidity of samples.

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."

Please revise as “Before running ICP-MS, samples are digested by microwave-assisted digestion system using the EPA 3051A method..”

EPA 3051A with HNOe3-HCL is not a total digestion method. It is an extraction method. You won’t be able to digest silicates without HF. Please revise your statement.

"2. After, weigh about 0.5 g using the analytical balance in a metal free container."

Delete steps 5 through 7.

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

There is only one SRM not SRMs. Please revise as “Recoveries for the target elements measured in ENV-META-12 certified referent material. Samples (n=xx) are digested according to EPA 3551A method."

How many samples/replicates are digested? There should be error values in this table.

Quality control (QC) of the analytical setup

Figure 2: Why don’t you run QA-QC SRM during analysis. How do you check actual QA-QC in real samples. 10 ppb QC standard only verifies your calibration!

Need to revise this run sequence and add QA-QC external SRMs after QC Standard. Also you need to verify calibration linearity and accuracy at the end of the analysis once unknowns are run.

Analytical procedure

Please delete this section. This is not analytical procedure but creating a method file that varies from instrument to instrument. Simply mention instrument preparation and daily performance test in this section etc.

Instrument calibration

"In our laboratory we routinely quantify biometal such as V, Mn, Fe, Co, Ni, Cu, As, Se, Mo, Cd and W"

Never heard of biometal! Please use proper and simple terms – trace metals etc.

Delete this section - "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."

Provide how many standards are used and calibration range in this section..

**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, limit of detection; LOQ, limit of quantification; ICP-MS, inductively coupled plasma mass spectrometry; SOP, standard operating procedure.

Values in Table 2 and 3 have too many significant figures. I think is acceptable to report LOD and LOQ for 2 significant figures. Like V 0.022 ug/L and 0.45 nM etc.

Is the rationale for developing the new method (or application) clearly explained?

Partly

Is the description of the method technically sound?

Yes

Are sufficient details provided to allow replication of the method development and its use by others?

Partly

If any results are presented, are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions about the method and its performance adequately supported by the

findings presented in the article?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Analytical chemistry, ICP-MS method development, geological analysis

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 15 Feb 2024

Donato Giovannelli

We want to thank the reviewer for the useful suggestions and comments. We have addressed all the questions raised and significantly improved the manuscript. We provide a point by point response below.

1. We have changed as requested in the text and throughout the manuscript were appropriate.

2. We thank the reviewer for the comment, we have change the sentence.

3. We thank the reviewer for the comment, we have simplify the sentence.

4. We thank the reviewer for the comment, we have change the sentence.

5. We wish to thank the reviewer for catching this typos. We corrected them accordingly.

6. Corrected.

7. We have deleted this section and included a table (new Table.1) that highlights the performance feature of the instrument..

8. We believe that a brief introduction to the concept of contamination and how to minimize these can be helpful for readers who are not familiar with these topics, and have added recommendations as suggested.

9. We have minimize this section, but we think that is useful to give a brief introduction to the principle of functioning of the instrument.

10. Corrected.

11. We wish to thank the reviewer. We corrected them accordingly.

12. ENVIR-META-12 is a reference material recommended by the Italian Ministry for Environments directives (D.Lgs. 152 del 3 aprile 2006) for evaluating heavy metals in

environmental matrices, and it is used for validation of the methods across laboratories in Italy. Syringe filters and paper are pretreated with nitric acid and consideration is given to the possible contamination by metals. Calibration of pipettes and instruments is carried out routinely following manufacturers specification.

13. We believe that a brief section with the cleaning procedure can be useful to described the procedures that routinely adopt in the lab.

14. Corrected.

15. We agree with the reviewer with this sentence. We are interested in the metals available to microbes such as those related to carbonate. For this reason we don't perform a total digestion up to silicate, but only partial digestion.

16. We believe that this steps are important to describe the procedures that routinely adopt in the lab to increase reproducibility of the methods without leaving anything out of the description.

17. Corrected. now Table 2

18. We think that analyzing blank and a SRM (runs as a sample, not as a calibration point) every 10 samples is a good way to check the QA/QC, also for all samples and QA/QC SRM the %RDS is evaluated.

19. The calibration linearity and accuracy are checked every analysis.

20. We agree with the reviewer. We have change the title but we think that is advisable to mention also the basic steps that are routinely carried out during the analysis.

21. Biometals are subsets of metals that have important biological functions. For reference see Giovannelli et al., 2023 Nat Rev Earth and Env (https://doi.org/10.1038/s43017-023-00477-y), Hay Mele et al., 2023 Essay in Biochem (https://doi.org/10.1042/EBC20230012) and the great book by Crichton ¨Biological inorganic chemistry" (edited by Academic press).

22. We think to give an indication that soon will be possible analyze also rare earth elements.

23. We have add this section.

24. Corrected.

25. Corrected.

Competing Interests: No competing interests were disclosed.

Author Response 24 Apr 2024

Donato Giovannelli

We want to thank the reviewer for the useful suggestions and comments. We have addressed all the questions raised and significantly improved the manuscript. We provide a point by point response below. "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..." “Analysis of trace elements” is not correct terminology. Samples are analyzed for determination of .. So it should be determination of trace elements. Please correct in the title and entire manuscript. We have changed as requested in the text and throughout the manuscript were appropriate. “Generally, microbial diversity is linked to environmental main physical-chemical parameters,” A bit confusing statement. Maybe better “ linked to a number of physical and chemical parameters, including, but not limited to,...” We thank the reviewer for the comment, we have change the sentence "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." This statement is not clear. Please revise and simplify.. We thank the reviewer for the comment, we have simplify the sentence. "Here we describe the SOP developed to routinely analyze such a wide range..." It’s better this way - 'Here we describe an SOP developed to routinely analyze such a wide range..' We thank the reviewer for the comment, we have change the sentence "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. ICP-MS can also measure major and minor elements not only trace elements. ICP-MS cannot measure substances. Please use “species” instead. 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." What do mean by neutrons? Neutral species! I don’t think plasma produces neutrons! We wish to thank the reviewer. We corrected them accordingly. "Finally, each element passes through the detector to be counted." This not a correct statement. Elements do not pass through detector. Ions hit the detector. What passes through quadrupole are ions not elements. And separation is based on m/z ratio that occurs sequentially – called hopping mode. Please revise this section and describe corrected operation of quadruple mass filter and ion detection process. Corrected We have developed this protocol using an Agilent ICP-MS 7900 (G8403A). The ICP-MS 7900 is a single quadrupole mass spectrometer that filters ions by mass to charge ratio (m/z). It 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." Delete this section. Just provide instrument model and operating conditions. We have deleted this section and included a table (new Table.1) that highlights the performance feature of the instrument. "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." Please shorten this section and be concise. Provide recommended sample handling and storage conditions. It is already known that glass containers are not suitable for ICP-MS measurements. We believe that a brief introduction to the concept of contamination and how to minimize these can be helpful for readers who are not familiar with these topics. "Principle of functioning of ICP-MS" Please delete entire section. This is book knowledge. No need to describe operation principles of ICP-MS. We have minimize this section, but we think that is useful to give a brief introduction to the principle of functioning of the instrument "Standard Operating Practice in the Giovannelli lab. The method was developed using inductively coupled plasma mass spectrometry (ICP-MS)." Please provide model of ICP-MS instrument here again. Corrected "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." How do you reduce dissolved solids by filtration? Maybe more appropriate to say suspending particulate matter or colloidal matter. All dissolved species will pass through filter that can only trap particulate matter. We wish to thank the reviewer. We corrected them accordingly. "A certified reference material is used to verify the digestion efficiency." What sort of SRM was used for sediment QA/QC? Please provide list of used SRMs as well as recommended SRM. Also, indicate that SRM is used for verifying digestion efficiency and accuracy of ICP-MS analysis. Please provide purity grade for syringe filter and filter papers. What sort of pipettes are used? How did you check pipette calibrations? ENVIR-META-12 is a reference material recommended by the Italian Ministry for Environments directives (D.Lgs. 152 del 3 aprile 2006) for evaluating heavy metals in environmental matrices, and it is used for validation of the methods across laboratories in Italy. Syringe filters and paper are pretreated with nitric acid and consideration is given to the possible contamination by metals. Calibration of pipettes and instruments is carried out routinely following manufacturers specification. Cleaning procedure Please delete the first paragraph of this sections. “ICP-MS detects elements even in ppt …” We believe that a brief section with the cleaning procedure can be useful to described the procedures that routinely adopt in the lab 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 HNO3 2 %." Are samples acidified to 2% HNO3 or acidified with 2%HNO3? How much is used? Better to report final acidity of samples. Corrected 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." Please revise as “Before running ICP-MS, samples are digested by microwave-assisted digestion system using the EPA 3051A method..” EPA 3051A with HNOe3-HCL is not a total digestion method. It is an extraction method. You won’t be able to digest silicates without HF. Please revise your statement. We agree with the reviewer with this sentence. We are interested in the metals available to microbes such as those related to carbonate. For this reason we don’t perform a total digestion up to silicate, but only partial digestion. After, weigh about

0.5 g using the analytical balance in a metal free container." Delete steps 5 through 7. We believe that this steps are important to describe the procedures that routinely adopt in the lab to increase reproducibility of the methods without leaving anything out of the description Table 1. Results of the analysis of the microwave assessed acid digested reference materials. There is only one SRM not SRMs. Please revise as “Recoveries for the target elements measured in ENV-META-12 certified referent material. Samples (n=xx) are digested according to EPA 3551A method." How many samples/replicates are digested? There should be error values in this table. Corrected. now Table2 Quality control (QC) of the analytical setup Figure 2: Why don’t you run QA-QC SRM during analysis. How do you check actual QA-QC in real samples. 10 ppb QC standard only verifies your calibration! We think that analyzing blank and a SRM (runs as a sample, not as a calibration point) every 10 samples is a good way to check the QA/QC, also for all samples and QA/QC SRM the %RDS is evaluated Need to revise this run sequence and add QA-QC external SRMs after QC Standard. Also you need to verify calibration linearity and accuracy at the end of the analysis once unknowns are run. The calibration linearity and accuracy are checked every analysis Analytical procedure Please delete this section. This is not analytical procedure but creating a method file that varies from instrument to instrument. Simply mention instrument preparation and daily performance test in this section etc. We agree with the reviewer. We have change the title but we think that is advisable to mention also the basic steps that are routinely carried out during the analysis Instrument calibration "In our laboratory we routinely quantify biometal such as V, Mn, Fe, Co, Ni, Cu, As, Se, Mo, Cd and W" Never heard of biometal! Please use proper and simple terms – trace metals etc. Biometals are subsets of metals that have important biological functions. For reference see Giovannelli et al., 2023 Nat Rev Earth and Env (https://doi.org/10.1038/s43017-023-00477-y), Hay Mele et al., 2023 Essay in Biochem (https://doi.org/10.1042/EBC20230012) and the great book by Crichton ¨Biological inorganic chemistry" (edited by Academic press). Delete this section - “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.” we think to give an indication that soon will be possible analyze also rare earth elements Provide how many standards are used and calibration range in this section.. We have add this section 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, limit of detection; LOQ, limit of quantification; ICP-MS, inductively coupled plasma mass spectrometry; SOP, standard operating procedure. Values in Table 2 and 3 have too many significant figures. I think is acceptable to report LOD and LOQ for 2 significant figures. Like V 0.022 ug/L and 0.45 nM etc. Corrected

Competing Interests: No competing interests were disclosed.

Reviewer Report 16 October 2023

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Maria Carmen Barciela-Alonso
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[2] University of Santiago de Compostela, Coruña, Spain

The manuscript describes a protocol for trace element analysis in sediments and hydrothermal fluids. The topic is interesting and may be of great use to other researchers. But in my opinion, the protocol needs to be improved to be applied.

The aspects that should be revised are the following:

In the section "Methods" on page 3, the first paragraph should be deleted. This information is published in the analytical instrumentation manuals.

In my opinion the section "Principle of functioning of ICP-MS" should be deleted. I would include only a table with the instrumental measurement conditions.

In the section "Sample collection, preservation and storage" it should be indicated how the sediment sample is collected and how it is preserved.

Regarding calibration, due to the complexity of the sample matrix, I think it would be appropriate to evaluate whether it is necessary to use the standard additions method.

For the study of the accuracy of the method, the authors use the reference material ENV-META-12, for 11 elements. How do you evaluate the accuracy for the hydrothermal fluid and for the other elements in the sediment?

I think there is an error in the LOD expression (LOD = 3.3□ σ/ S). I think it is LOD = 3□ σ / S.

I do not understand the information in tables 2 and 3. It is the same detection limit for hydrothermal fluid and sediment?. In my opinion the LOD and LOQ should be given for the initial sample, considering the whole sample preparation procedure.

The authors should include a table with the concentration values obtained for the samples.

Is the rationale for developing the new method (or application) clearly explained?
Yes

Is the description of the method technically sound?
Yes

Are sufficient details provided to allow replication of the method development and its use by others?

Partly

If any results are presented, are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions about the method and its performance adequately supported by the findings presented in the article?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Spectrometry, Trace element determination, envisonmental and biological samples, speciation

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 15 Feb 2024

Donato Giovannelli

We want to thank the reviewer for the useful suggestions and comments. We have addressed all the questions raised and significantly improved the manuscript. We provide a point by point response below.

1. We believe that a brief introduction to the instrumentation and working principle can be useful for non-expert readers, but we have omitted the interference section and added the citation as suggested.

2. We have reduced this section and included a table (new Table.1) that highlights the performance feature of the instrument.

3. We agree with the reviewer. We have added a brief clarification regarding how sediment are collected and preserved, which was not present in the initial draft

4. We thank the reviewer for the comment, we will take into account the standard addition method. Currently we use internal standards added to the sample.

5. We are seeking reference materials that contain other elements, and we have used this test material as it is the one recommended by the ministerial directives (D.Lgs. 152 del 3 aprile 2006) for evaluating heavy metals in environmental matrices.

6. In literature we have found several ways to calculate limits of detection and quantification, and we added more references in the text. Using 3.3 instead of 3 is anyway more conservative.

7. We have calculated the method LOD and LOQ based on the standard calibration curves. Because of the significant variability in chemical and physical parameters, it's not feasible to use a single sample or a set of samples to determine detection and quantification limits that can be representative of all the samples we analyze. Therefore, we have chosen to assess the instrument's detection and quantification limits separately, while the limits for individual samples will be calculated during sample processing. Now Table 3 and Table 4.

8. As in the previous statement, the detection limits are calculated during the analysis of the samples. Also we have added Table 5 with row data and precision of the same samples.

Competing Interests: No competing interests were disclosed.

Author Response 24 Apr 2024

Donato Giovannelli

We want to thank the reviewer for the useful suggestions and comments. We have addressed all the questions raised and significantly improved the manuscript. We provide a point by point response below. In the section "Methods" on page 3, the first paragraph should be deleted. This information is published in the analytical instrumentation manuals. We believe that a brief introduction to the instrumentation and working principle can be useful for non-expert readers, but we have reduced this section In my opinion the section "Principle of functioning of ICP-MS" should be deleted. I would include only a table with the instrumental measurement conditions. We have reduced this section and included a table (new Table.1) that highlights the performance feature of the instrument. In the section "Sample collection, preservation and storage" it should be indicated how the sediment sample is collected and how it is preserved. We agree with the reviewer. We have added a brief clarification regarding how sediment are collected and preserved, which was not present in the initial draft Regarding calibration, due to the complexity of the sample matrix, I think it would be appropriate to evaluate whether it is necessary to use the standard additions method. We thank the reviewer for the comment, we will take into account the standard addition method. Currently we use internal standards added to the sample. For the study of the accuracy of the method, the authors use the reference material ENV-META-12, for 11 elements. How do you evaluate the accuracy for the hydrothermal fluid and for the other elements in the sediment? We are seeking reference materials that contain other elements, and we have used this test material as it is the one recommended by the ministerial directives (D.Lgs. 152 del 3 aprile 2006) for evaluating heavy metals in environmental matrices I think there is an error in the LOD expression (LOD = 3.3□ σ/ S). I think it is LOD = 3□ σ / S. In literature we have found several ways to calculate limits of detection and quantification, and we added more references in the text. Using 3.3 instead of 3 is anyway more conservative. I do not understand the information in tables 2 and 3. It is the same detection limit for hydrothermal fluid and sediment?. In my opinion the LOD and LOQ should be given for the initial sample, considering the whole sample preparation procedure. We have calculated the method LOD and LOQ based on the standard calibration curves. Because of the significant variability in chemical and physical parameters, it's not feasible to use a single sample or a set of samples to determine detection and

quantification limits that can be representative of all the samples we analyze. Therefore, we have chosen to assess the instrument's detection and quantification limits separately, while the limits for individual samples will be calculated during sample processing. Now Table 3 and Table 4. The authors should include a table with the concentration values obtained for the samples. As in the previous statement, the detection limits are calculated during the analysis of the samples. Also we have added Table 5 with row data and precision of the same samples.

Competing Interests: No competing interests were disclosed.

Reviewer Report 27 September 2023

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Sadhan Bijoy Deb
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[2] RACD, Bhabha Atomic Research Centre Medical Division, Mumbai, Maharashtra, India

This manuscript depicts the development and establishment of a SOP for quantification of trace elements in hydrothermal fluids and sediments by ICP-QMS. The trace metals includes both geological and biological relevant elements. The SOP was applied to analysis of real samples collected on the AE019 expedition to the islands of Aeolian archipelago and in the gulf of Naples during the FEAMP expedition. The sediments were digested by Microwave Digestion and the liquid samples were diluted prior to analysis. The obtained data was employed to construct a PCA of trace metal concentration corresponding to the regions considered.

The data has the potential to be used in future for investigating the role of trace elements on microbial functional diversity control. Microorganisms play a crucial role in biogeochemical cycle that in turn is responsible for controlling the interaction between geosphere and biosphere.

Comments

1. Under the heading “Methods (pg3)”, the instrumentation and working principle (including interferences) of ICP-QMS are explicitly described in the literature. The authors can omit these details from the manuscript and cite the appropriate references. This would reduce the repeat and that the pertinent focus of the present work can blossom out.

2. Under the heading “Methods (pg3)”, the authors had mentioned that “it is advisable to dilute the sample, to keep total dissolved solids (TDS) below 0.2%”. Appropriate reference(s) has to be cited in support of the statement. In the same paragraph, “For comparison with a 50% dilution of seawater (1.5% NaCl final concentration) there is no significant cone blockage and signal stability remains good.” Again reference has to be cited if this sentence

is retained.

The above statements are contradictory to each other. The authors should clearly state the level of dilution they followed in their work and the corresponding TDS achieved in the final solution. The authors can remove the contradicting sentences.

3. The information given under the heading “Principle of functioning of ICP-MS (Pg 4)”, are already available in literature. Hence, I would recommend the removal of text which is available in literature and the authors should cite the relevant references.

   The performance features of the instrument (power, gas flow rate, etc.) should be mentioned in a tabular format and not described in words.

4. There is repeat of same text (cleaning, sample collection, etc.) under the headings – “Standard operating practice in the Giovannelli lab (pg4)”, “cleaning procedure (pg 6)”, and “sample collection, preservation and storage (pg 6)”. The authors should avoid such repetition in the manuscript.

5. Three biologically relevant elements i.e., Fe, Mo & W are absent in certified test material ENV-META-12. The authors should explore other CRMs of sludge/sediment which contains these elements, so that the digestion efficiency of these elements are verified.

6. The accuracy of the SOP for analysis of hydrothermal liquid samples has not been mentioned. The methodology can be validated by using a CRM or standard addition technique.

7. The relevant references for LOD and LOQ had been provided in the text. Hence the definitions of LOD & LOQ need not be given.

8. The raw data [with corresponding precision and number of determinations (N)] prior to PCA analysis should be provided as supporting information

Is the rationale for developing the new method (or application) clearly explained?

Yes

Is the description of the method technically sound?

Yes

Are sufficient details provided to allow replication of the method development and its use by others?

Yes

If any results are presented, are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions about the method and its performance adequately supported by the

findings presented in the article?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Trace metallic analysis, by ICP-MS, in nuclear, geological and environmental samples. Microwave digestion of refractory materials. Gravimetric quantification of major elements in a matrix. Minor element anaysis by UV-Vis spectroscopy

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 15 Feb 2024

Donato Giovannelli

We want to thank the reviewer for the useful suggestions and comments. We have addressed all the questions raised and significantly improved the manuscript. We provide a point by point response below.

1. We believe that a brief introduction to the instrumentation and working principle can be useful for non-expert readers, but we have omitted the interference section and added the citation as suggested.

2. Thank you for pointing this out! We agree and have removed these confusing sentences.

3. We significantly reduced the text and included a table (new Table.1) that highlighted the performance feature of the instrument, as indicated by the reviewer.

4. In the section Standard operating practice we have removed the repetition, present in the section below.

5. Iron, molybdenum, and tungsten are transition metals included among the metals we have examined. We do not anticipate significant deviations in their recoveries. We are seeking reference materials that contain these elements, and we have used this test material as it is the one recommended by the ministerial directives (D.Lgs. 152 del 3 aprile 2006) for evaluating heavy metals in environmental matrices.

6. We have evaluated the accuracy and recovery using the internal standard addition and QC standard for each sample set.

7. As not everyone might be familiar with this concept, we believe that a brief introduction to the concept of limits of detection and quantification can be helpful.

8. We have added the number of determination and a table with row data and precision (Table 5) as requested.

Competing Interests: No competing interests were disclosed.

Author Response 24 Apr 2024

Donato Giovannelli

We want to thank the reviewer for the useful suggestions and comments. We have addressed all the questions raised and significantly improved the manuscript. We provide a point by point response below.

Under the heading “Methods (pg3)”, the instrumentation and working principle (including interferences) of ICP-QMS are explicitly described in the literature. The authors can omit these details from the manuscript and cite the appropriate references. This would reduce the repeat and that the pertinent focus of the present work can blossom out. We believe that a brief introduction to the instrumentation and working principle can be useful for non-expert readers, but we have omitted the interference section and added the citation as suggested. Under the heading “Methods (pg3)”, the authors had mentioned that “it is advisable to dilute the sample, to keep total dissolved solids (TDS) below 0.2%”. Appropriate reference(s) has to be cited in support of the statement. In the same paragraph, “For comparison with a 50% dilution of seawater (1.5% NaCl final concentration) there is no significant cone blockage and signal stability remains good.” Again reference has to be cited if this sentence is retained.The above statements are contradictory to each other. The authors should clearly state the level of dilution they followed in their work and the corresponding TDS achieved in the final solution. The authors can remove the contradicting sentences. Thank you for pointing this out! We agree and have removed these confusing sentences. The information given under the heading “Principle of functioning of ICP-MS (Pg 4)”, are already available in literature. Hence, I would recommend the removal of text which is available in literature and the authors should cite the relevant references. The performance features of the instrument (power, gas flow rate, etc.) should be mentioned in a tabular format and not described in words. We significantly reduced the text and included a table (new Table.1) that highlighted the performance feature of the instrument, as indicated by the reviewer. There is repeat of same text (cleaning, sample collection, etc.) under the headings – “Standard operating practice in the Giovannelli lab (pg4)”, “cleaning procedure (pg 6)”, and “sample collection, preservation and storage (pg 6)”. The authors should avoid such repetition in the manuscript. In the section Standard operating practice we have removed the repetition, present in the section below Three biologically relevant elements i.e., Fe, Mo & W are absent in certified test material ENV-META-12. The authors should explore other CRMs of sludge/sediment which contains these elements, so that the digestion efficiency of these elements are verified. Iron, molybdenum, and tungsten are transition metals included among the metals we have examined. We do not anticipate significant deviations in their recoveries. We are seeking reference materials that contain these elements, and we have used this test material as it is the one recommended by the ministerial directives (D.Lgs. 152 del 3 aprile 2006) for evaluating heavy metals in environmental matrices. The accuracy of the SOP for analysis of hydrothermal liquid samples has not been mentioned. The methodology can be validated by using a CRM or standard addition technique. We have evaluated the accuracy and recovery using the

internal standard addition and QC standard for each sample set. The relevant references for LOD and LOQ had been provided in the text. Hence the definitions of LOD & LOQ need not be given. We believe that a brief introduction to the concept of limits of detection and quantification can be helpful for readers who are not familiar with these topics. The raw data [with corresponding precision and number of determinations (N)] prior to PCA analysis should be provided as supporting information We have added the number of determination and a table with row data and precision (Table5)

Competing Interests: No competing interests were disclosed.