

Trace metal and organic biosignatures in digitate stromatolites from terrestrial siliceous hot spring deposits: Implications for the exploration of martian life

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ABSTRACT

Novel biosignatures of laminated, microbial, digitate sedimentary structures – stromatolites – from modern geothermal fields of the Taupō Volcanic Zone, New Zealand, and from El Tatio, Chile, provide an opportunity to investigate evidence of extremophile life preserved in siliceous hot spring deposits, or sinters, interpreted as analogs for early life on Earth and possibly Mars. Synchrotron-μXRF, electron microprobe analysis, Raman spectroscopy, and optical microscopy are used in a coordinated approach to identify corroborating textural and chemical (organic, inorganic) evidence of life in these modern, opaline (amorphous) siliceous materials. Fluid mobile elements, such as As and Sr, track the growth history of the digitate structures. Trace element enrichments of Ca, Al, Ga, +/- Fe, Mn, As, Rb, Cs, and Sr, are identified in silicified sheaths of microbial filaments embedded within the sinter. In contrast, silicified diatoms in some sinter samples show no trace element enrichment. Gallium enrichments have also been observed in other 16 ka and Jurassic (150 Ma) microbial palisade sinter textures, suggesting the potential for preservation through geologic time, even after recrystallization to quartz. Raman analysis reveals spectra of organics, consistent with pigments for UV protection in cyanobacteria, in silicified sheaths around microbial filaments and are co-located with trace metal enrichments in digitate structures. Due to spectral bands, the location of these molecules (i.e., in the sheaths), and the sampling locations, we ascribe the spectra to scytonemin and carotenoid class molecules. The combined analytical approach outlined here provides a robust means to assess the validity of novel biosignatures, with application to the exploration of Mars, where preservation of opaline silica in >3.6 Ga deposits has the potential to preserve a range of microbial biosignatures.

1. Introduction

Terrestrial hydrothermal settings are increasingly recognized as key targets in the search for extra-terrestrial life, as well as early life on Earth (Farmer, 2000; Cavicchioli, 2002; Konhauser et al., 2003; Westall et al., 2015; Ruff and Farmer, 2016; Djokic et al., 2017; Hays et al., 2017; Cady

et al., 2018; Finkel et al., 2023). In 2007, the Mars Exploration Rover *Spirit* detected deposits of opaline silica (opal-A; SiO₂•nH₂O) and sulfate-rich soils near Home Plate in the Columbia Hills of Gusev Crater, Mars (Squyres et al., 2008). Initially, the co-occurrence of silica and sulfate was interpreted to be the product of destructive fumarolic acid-sulfate leaching rather than constructive silica deposition (Squyres

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et al., 2008). However, subsequent comparison to terrestrial hot spring analogs has indicated a more likely origin for the martian siliceous materials as silica sinter (Ruff et al., 2011; Ruff and Farmer, 2016; Ruff et al., 2020). Additional evidence for sinter formation in Gusev Crater is the potential hot spring vent mound Pioneer Mound, ~80 m to the west of Home Plate, which is also composed of opaline silica, and resembles subaerial hot spring vent mounds at El Tatio and Puchuldiza in Chile (Ruff et al., 2020). The martian opaline silica outcrops as nodular masses with a mm-scale digitate morphology. The digitate silica structures at Home Plate are unlikely to have formed by more recent wind erosion during their exhumation and exposure on the present-day martian surface, as evident from wind abrasion experiments undertaken on modern siliceous sinter deposits (Ruff et al., 2020). Moreover, in modern New Zealand digitate sinter, the internal morphology of laminated silica and microbes (Nersezova et al., 2023), and their microbiological characterization (Sriaporn et al., 2020), confirms more broadly that terrestrial hot spring digitate textures are stromatolites. We define stromatolite here as biosedimentary constructions sensu Grey and Awramik (2020, p. 4): "...a laminated organosedimentary structure produced by sediment trapping, binding and/or precipitation as a result of the growth, behavior, and metabolic activity of micro-organisms." Thus, similar morphological and compositional features on Mars are a key target in the search for evidence of extra-terrestrial life (Ruff et al., 2011, 2020; Nersezova et al., 2023). Nonetheless, morphology/texture alone is not adequate to demonstrate that a depositional feature is a stromatolite, and multiple lines of evidence are required to verify biogenicity (e.g., Cady et al., 2003), particularly as it is well known that many of Earth's stromatolites in the geological record do not preserve microfossils (e.g., Awramik and Grey, 2005; Grey and Awramik, 2020; Lepot, 2020), and that abiotic mimics may exist (Grotzinger and Rothman, 1996; Grotzinger and Knoll, 1999; McLoughlin et al., 2008; Neveu et al., 2018). In the present study, we examine trace metal distributions as potential inorganic chemical biosignatures in the context of spatially co-associated, organic microbial and well-characterized morphological biosignatures in digitate stromatolitic sinter from modern terrestrial hot springs, which are morphologically analogous to the siliceous digitate features discovered by *Spirit* on Mars.

Biosignatures in sinters on Earth, and potentially Mars, can be summarized in three main categories related to the morphological, organic chemical, and inorganic chemical characteristics of the microorganisms inhabiting hydrothermal environments (cf. Westall et al., 2015; Neveu et al., 2018). They are preserved and expressed in the geological record through different mechanisms (Farmer and Des Marais, 1999; Campbell et al., 2015; Westall et al., 2015). Morphological biosignatures include features that are part of, or produced or constructed by microorganisms, which in siliceous sinters can include bacterial, algal, or plant cells (Jones et al., 1997, 2000; Barbieri et al., 2014); cellular products such as exopolymeric substances [EPS] (Handley et al., 2005, 2008); microbial colonies, mats, and biofilms (e.g., Cady and Farmer, 1996); or sedimentary bioconstructions, such as stromatolites (e.g., Walter et al., 1972; Djokic et al., 2017) and textural sinter microfacies (e.g., Walter et al., 1996; Campbell et al., 2001; Schinteie et al., 2007; Lynne, 2012). Using Raman spectroscopy, organic chemical biomolecules (degraded or intact) comprising cells and/or EPS can be detected within mineralized sedimentary deposits throughout the geologic record (e.g., Westall et al., 2015; Teece et al., 2023). Previous Raman investigations have found kerogenous organic material inter-laminated with silica in Jurassic sinters, interpreted as fossilized microbial biofilm (e.g., Guido et al., 2019). Moreover, intact UV-protective microbial pigments have been reported from recently fossilized cyanobacteria (e.g., Wynn-Williams et al., 1999; Edwards et al., 2005, 2013; Jehlička et al., 2014a). More broadly, gas chromatography-mass spectrometry (GC-MS) results have highlighted carotenoids co-preserved with geologically altered γ -carotene and β -carotene in Jurassic-aged sinters (Teece et al., 2022). Geologically preserved lipids, alkanes and polyaromatic hydrocarbons have also been detected by GC-MS and

characterized in Holocene (e.g., Kaur et al., 2015; Teece et al., 2020, 2023), Pleistocene (Reinhardt et al., 2019), and Jurassic (Teece et al., 2022) sinters. Additionally, two of these studies also detected fossilized organic carbon by use of Raman spectroscopy (Reinhardt et al., 2019; Teece et al., 2023).

There are many different signals of microbial metabolism preserved in sinters, including carbon, nitrogen, and sulfur isotopic fractionation (Havig et al., 2011), as well as mineralogical effects, such as the active/passive microbial sequestration of metals (Phoenix et al., 2005; Gangidine et al., 2020, 2021; Murphy et al., 2021), and microbial precipitation of silica and iron minerals (Konhauser and Ferris, 1996; Phoenix et al., 2000; Phoenix and Konhauser, 2008). Many reports of trace metal enrichments in sinter involve Fe and Ga around silicified microbes and inferred EPS (Table 1; Gangidine et al., 2020, 2021; Churchill et al., 2021; Murphy et al., 2021). Recent investigation into the variation of trace metal content during microbially mediated sinter formation indicates that trace metals such as Ga and As are retained through the diagenetic transition from living mat to rock, while in contrast there is significant loss of the major biologically essential elements (C, N, P; Havig et al., 2021). Thus, some trace metals may serve as robust inorganic chemical biosignatures through geologic time. It is important to note that trace elements within siliceous sinter could also be sourced from deep fluids during silica precipitation (e.g., Krupp and Seward, 1990; Jones et al., 2001), or enter the silica matrix during late hydrothermal alteration (e.g., Hampton et al., 2004; Campbell et al., 2019). However, with a careful paragenetic petrographic approach, combined with high-resolution geochemical analysis, these other sources of trace metals in sinter can be assessed.

Quality preservation of biosignatures requires rapid lithification or cementation processes such as silicification to prevent further degradation from the environment and to minimize later diagenesis or alteration (e.g., Farmer and Des Marais, 1999; Trewin, 2007). Although trace elements spatially co-associated with microbial features show potential for use as inorganic chemical biosignatures, existing research is sparse (Gangidine et al., 2020, 2021). At present, the most robust and well-characterized biosignatures of microbial activity in sinter deposits are: (1) macro- and micro-textures of morphologically distinct sinter microfacies (i.e. silica templating of temperature/pH-influenced microbial features), along with Raman-identified carbon (Campbell et al., 2015; Guido et al., 2019; Teece et al., 2023), and (2) lipid biomarker preservation of microbial community components (e.g., Pancost et al., 2005; Parenteau et al., 2014; Kaur et al., 2015; Reinhardt et al., 2019; Williams et al., 2021; Teece et al., 2022; Sánchez-García et al., 2023). Because of the poor preservation potential of molecular biomarkers into the geologic record, microbiological characterization of sinters is only possible for modern samples (e.g., Sriaporn et al., 2020). An individual biosignature generally cannot constitute successful identification of life on its own, as robust verification of ancient or extra-terrestrial life involves the corroboration of morphological and chemical biosignatures (Westall et al., 2015; McMahon and Cosmidis, 2022), in addition to careful characterization of their geological context and fluid-mineral-microbe paragenesis.

Digitate sinters on Earth are siliceous microstromatolites that form as subaerial, finger-like silica protrusions growing upward from the substrate at the air-water interface of terrestrial hot springs, specifically around spring-vent pool rims and in shallow outflow channels (e.g., Schinteie et al., 2007; Handley et al., 2005, 2008; Ruff et al., 2020; Sriaporn et al., 2020; Nersezova et al., 2023). Digitate sinter forms in fluids of all temperature ranges and a wide range of chemistries (e.g., Sriaporn et al., 2020). The use of the term 'digitate' to describe finger-like vertical protrusions in sinter deposits has been adopted from Ruff and Farmer (2016), and is utilized here in conjunction with the morphological subtypes sensu Sriaporn et al. (2020): height/width aspect ratios of height > width termed 'spicular', height $\approx$ width termed 'bulgy', and width > height termed 'knobby'. Notwithstanding the morphological comparisons made here, we make no claims as to the role

Table 1

Literature data with evidence for microbial and organic enrichment or sequestration of metals in sinters.

Microbial enrichment of metals in sinters	Analytical method	Mg	Al	Mn	Fe	Ga	As	Sr	Sb	Hg	Tl
Devonian microfossils from Drummond Basin, Australia ¹	SIMS (in situ)	●	●	●	●			●			
Silicified modern microbes from YNP ²	SIMS (in situ)			●	●	●					
~5.15 ka geysirite spicules from Te Kopia, TVZ ³	μXRF (in situ)					●					
Modern microbes from Champagne Pool, TVZ ⁴	EDS & TEM (in situ)						●		●		
Modern, organic-rich YNP sinter ⁵	XRF (bulk)				●	●					
Organic fraction of modern Tokaanu geysirite, TVZ ⁶	XRF (bulk)									●	●

SIMS = secondary ion mass spectrometry; (μ)XRF = (micro) x-ray fluorescence; EDS = energy dispersive spectroscopy; TEM = transmission electron microscopy; YNP = Yellowstone National Park; TVZ = Taupō Volcanic Zone. 1Gangidine et al., (2021); 2Gangidine et al., (2020); 3Murphy et al., (2021); 4Phoenix et al., (2005); 5Churchill et al., (2021); 6McKenzie et al., (2001).

of microbes in the generation of morphologically similar structures outcropping on Mars because without sample return, their critical internal features cannot be characterized. We note that most silica precipitation in geothermal settings is facilitated through abiotic chemical mechanisms and influenced by environmental controls. In all known cases, high evaporation settings of cyclical, aperiodic wetting and drying are required to produce the laminated digitate morphology (Nersezova et al., 2023), such as evaporative capillary rise ('wicking') (Campbell et al., 2002; Mountain et al., 2003; Schinteie et al., 2007), and wave action or splashing (Jones et al., 1997; Mountain et al., 2003; Handley et al., 2005) of silica super-saturated fluids. Silica-saturated fluids from deep geothermal reservoirs are discharged and cooled at Earth's surface to precipitate amorphous opal-A silica, as silica solubility at surface temperatures and pressures is much lower than at depth, which triggers extensive surface silica deposition (Iler, 1979; Mountain et al., 2003). The internal laminae in sinters are created by mat-forming and biofilm-producing micro-organisms such as bacteria and algae, which act as a substrate for the precipitation of silica (Doemel and Brock, 1974; Cady and Farmer, 1996; Walter et al., 1996; Jones and Renaut, 2003). Although digitate sinters have thus far been found to host and silicify a variety of microorganisms, the active/passive role of biology in the formation of sinter, in general, has yet to be robustly established (Konhauser et al., 2001; Benning et al., 2004; Phoenix et al., 2003; Sriaporn et al., 2020). This study investigates the correlation and co-occurrence among trace elements, morphology, and organics preserved in digitate sinters from modern geothermal settings in the Taupō Volcanic Zone (TVZ) of New Zealand and the Central Andes of Chile (Fig. 1A, B) to determine the potential of trace elements to serve as inorganic chemical biosignatures. To evaluate this integrated approach deeper into geologic time, we also include a Jurassic palisade sinter (~150 Ma, non-digitate) from Patagonia, Argentina (Fig. 1C) for comparison, which yields similar preserved microbial textures.

2. Materials and methods

2.1. Sample sites

Digitate sinter from the Rotokawa, Te Kopia, and Atiamuri geothermal systems in the Taupō Volcanic Zone (TVZ) represent acid-sulfate, acid-sulfate-chloride, and alkali chloride fluid chemistries, respectively (Fig. 1A; Nersezova et al., 2023). In the Sinter Flats area of the margin of Lake Rotokawa, a ~0.5 m wide band of spicular, digitate sinter comprises the rim of the currently inactive Mars Pool (~1 m wide pool, Fig. 2A), which is one the many surface expressions of the Rotokawa geothermal field (McNamara et al., 2016). At Mars Pool, the digitate structures are deposited on mud and silicified mud substrates surrounding the pool, and they also are spatio-temporally variably draped in mud from aperiodic inundation of the inactive pool by waters from the Rotokawa lagoon and lake areas (Fig. 3C-E; cf. Jones et al., 2000). Little is known about the age or fluid composition of Mars Pool; however, X-ray diffraction results show that the sinter comprises opal-A, and thus has not undergone diagenesis (Nersezova et al., 2023). The fluids were likely acid-sulfate-chloride, based on the chemistry of the

surrounding springs and the acidophilic biota preserved in the Mars Pool sinter (Nersezova et al., 2023).

The Te Kopia geothermal system (TVZ) straddles the active Paeroa fault and has manifested surface thermal activity for up to 120,000 years (Bignall and Browne, 1994). Initially, deep alkali chloride fluids discharged at the surface as hot pools and geysers and deposited extensive sinter, but due to recent fault movement causing fluctuations in the water table, current thermal activity manifests as steaming ground, fumaroles, and boiling acid-sulfate pools and springs (Bignall and Browne, 1994; Rodgers et al., 2002). Presently, fine spicular digitate sinter is actively depositing onto silica residue derived from steam-altered volcanics, in a small shallow stream ('lower Te Kopia stream') of very hot (~87 °C) acid sulfate fluids (Fig. 2B; 3B).

The Atiamuri geothermal system discharges its deep-origin alkali chloride fluids at the surface at several hot springs, with only two currently depositing sinter (Wood, 1995; Cody et al., 2021). At Whangapaoa Spring, surface thermal activity manifests as a vibrant blue hot pool with two discharge channels, where silica-rich alkali chloride fluids foster thick orange and green microbial mats surrounding the pool and lining the channels to form an extensive sinter apron in the main outflow channel (Cody et al., 2021). In middle to distal apron areas under shallow sheet flow (~48 °C; Sriaporn et al., 2020), bulgy digitate sinter is actively growing on deposits of previously formed sinter (Fig. 2C).

The El Tatio geothermal field is situated in the central Andes mountains in Chile, a region of andesitic arc volcanism associated with the central Chilean Subduction Zone (Fig. 1B). The El Tatio geothermal field is the third largest geyser field in the world and is known for its high evaporation rate and low rainfall, high elevation, and high ultra-violet flux (Nicolau et al., 2014), which together create one of the best analog environments for Mars on Earth (Ruff and Farmer, 2016). Abundant, knobby, digitate sinter forms in the shallow discharge channels (< 40 °C; Barbieri et al., 2014) of near-neutral alkali chloride hot springs (Fig. 2D), with other thermal features including hot and boiling pools, mud pools, and fumaroles (Fernandez-Turiel et al., 2005; Barbieri et al., 2014; Nicolau et al., 2014). These discharge channels typically have shallow flowing waters, which support a diverse assemblage of cyanobacteria and diatoms associated with thin, green microbial mats (Ruff and Farmer, 2016). X-ray diffraction and petrography of modern to Subrecent sinter samples used in this study indicate they are dominantly opal-A, suggesting little to no diagenetic effects (Nersezova et al., 2023).

Within the Jurassic-age (~150 Ma) Deseado Massif of Argentine Patagonia, numerous paleo-hot spring deposits have been located (Fig. 1C; Guido and Campbell, 2011). A palisade sinter sample from the Claudia locality (Guido and Campbell, 2014) is used here to compare trace element geochemistry of fossilized microbial filaments to the studied, modern digitate materials. Raman microscopy has clearly identified preserved kerogen in well-preserved petrographic morphologies of microbial filaments (Guido et al., 2019), and gas chromatography-mass spectrometry analysis reveals exceptional molecular preservation in the distal apron, and a variety of less well-preserved organic biosignatures near the fossilized vent (Teece et al., 2022). However, at Claudia, quartz is the silica phase, indicating

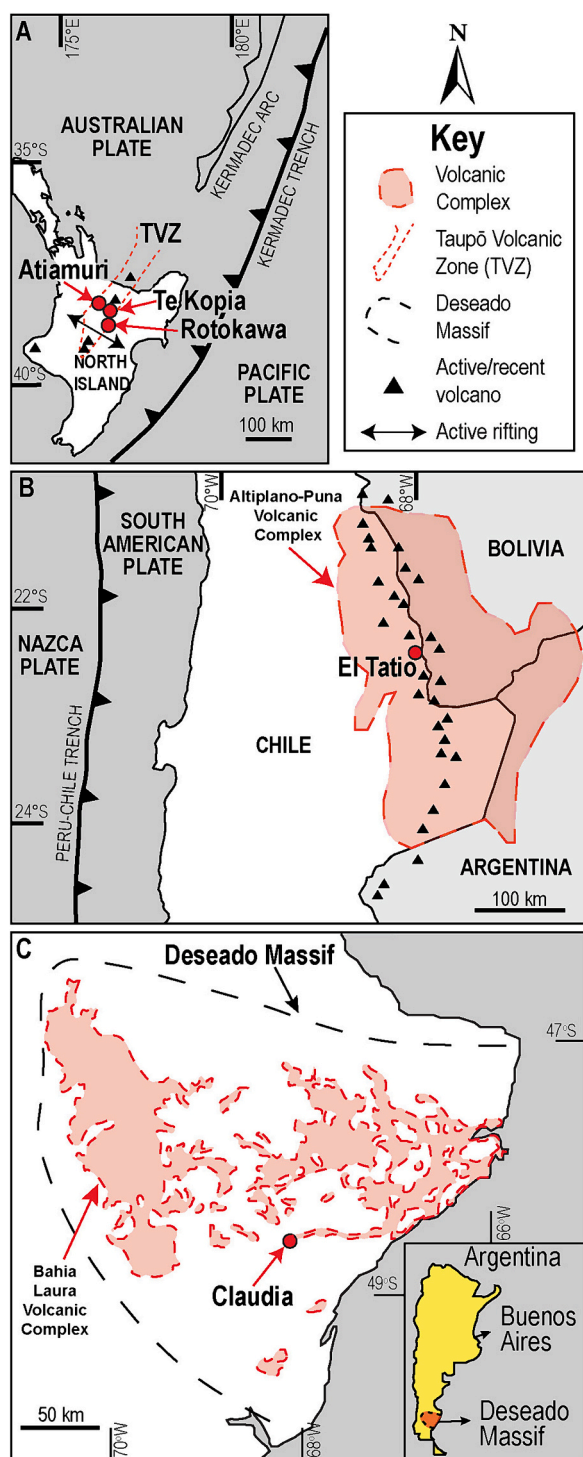


Fig. 1. Geo-tectonic setting of hot spring analog sites. (a) Location of the Atiamuri, Te Kopia, and Rotokawa geothermal systems within the Taupō Volcanic Zone [TVZ] relative to the North Island of New Zealand, and the Hikurangi subduction zone (direction of subduction indicated by “sawtooth” pattern). TVZ boundary adapted from Wilson et al. (1995). (b) El Tatio geothermal field location relative to the central Chilean subduction zone, and the Altiplano-Puna Volcanic Complex (de Silva, 1989), a major province of silicic volcanism. (c) The Deseado Massif, Argentina, a diffuse back-arc setting hosting Jurassic volcanics (red) and numerous hot spring deposits, including the Claudia locality (modified from Guido and Campbell, 2011). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

recrystallization from opal-A during diagenesis (Guido et al., 2019), and petrographic evidence of acid overprinting is evident (Teece et al., 2022).

2.2. Sample preparation

Individual digitate structures from each sinter hand sample were vacuum mounted in epoxy (EpoFix resin) during the work of Nersezova et al. (2023). For the electron microprobe analysis of this study, all mounts were repolished with silicon carbide paper (to 800 grit), followed by 3 and 1 μm diamond paste. Samples were then ultra-sonicated sequentially in soapy water, water, and ethanol.

In preparation for synchrotron x-ray fluorescence mapping, thin slices (averaging between 200 and 300 μm) were then cut from each polished epoxy mount using a diamond embedded wafering blade on a Buehler slow saw. These sections were carefully hand polished on the cut face using 600 then 800 grit silicon carbide polishing paper (Buehler) to even the thickness of the section of each sample to $\sim 150\text{--}250\text{ }\mu\text{m}$, while keeping the variation in the thicknesses across the section to $<30\text{ }\mu\text{m}$, creating polished thick sections. This sample preparation technique was employed to avoid the use of glass slides, which are often doped with trace elements and can produce unwanted background fluorescence (Howard et al., 2020), or the use of quartz slides which, although heavy-metal-free, are more fragile and difficult to prepare.

To obtain good quality Raman spectra and to accurately determine the distribution of primary carbonaceous matter, the samples needed to be polished avoiding the use of diamond-based polishing compounds, as well as all carbon-based oils, pastes, and liquids, due to the porous nature of the sawn silica. This issue has also been encountered in other Raman studies of porous sinters (e.g., Rodgers et al., 2004). Therefore, after the thick sections for the Synchrotron x-ray fluorescence mapping had been cut, the freshly exposed surfaces of the epoxy mounts (which had never been contaminated with diamond-based polishing compound) were polished using a series of silicon carbide polishing papers (Buehler) increasing from 400 through to 800 grit, and finished with 3 μm and 1 μm aluminium oxide flexible polishing papers (3M) until no scratches were observed under reflected light. This approach eliminated the issues of micro-diamond implantation on the sample surface as well as oil residue in the micro/nano-pores.

2.3. Petrography

Petrographic observations were carried out on the polished epoxy mounts and thick sections using reflected light microscopy, which also provided the foundation for the micro-scale geochemical analyses. Textures of interest (stromatolitic laminae, microbial microfossils, and mineralogical features) were identified for electron microprobe, micro-X-ray fluorescence, and Raman analyses. Images were taken on an Olympus DSX1000 Digital Microscope, using both bright field (BF) and polarized (PO) reflected light observational modes. This analysis was used to identify and understand the nature of the distribution of microbial features and bulk digitate textures for correlation with further trace elemental and carbon mapping.

2.4. Electron probe micro-analysis (EPMA) and Scanning electron microscopy (SEM)

Qualitative elemental distribution maps were collected using a field-emission electron probe micro-analyser (JEOL JXA-8530F Plus Hyper Probe). The analytical parameters for elemental mapping included an acceleration voltage of 15 kV, a probe current of 100 nA, and a probe diameter of 0.5–5 μm , which varied based on the pixel resolution of the maps. Dwell times were set to between 10 and 20 ms, to optimise acquisition time vs. size of each map. Mapping was undertaken in both the wavelength-dispersive spectroscopy (WDS) mode and the energy-dispersive spectroscopy (EDS) mode, in order to analyze the

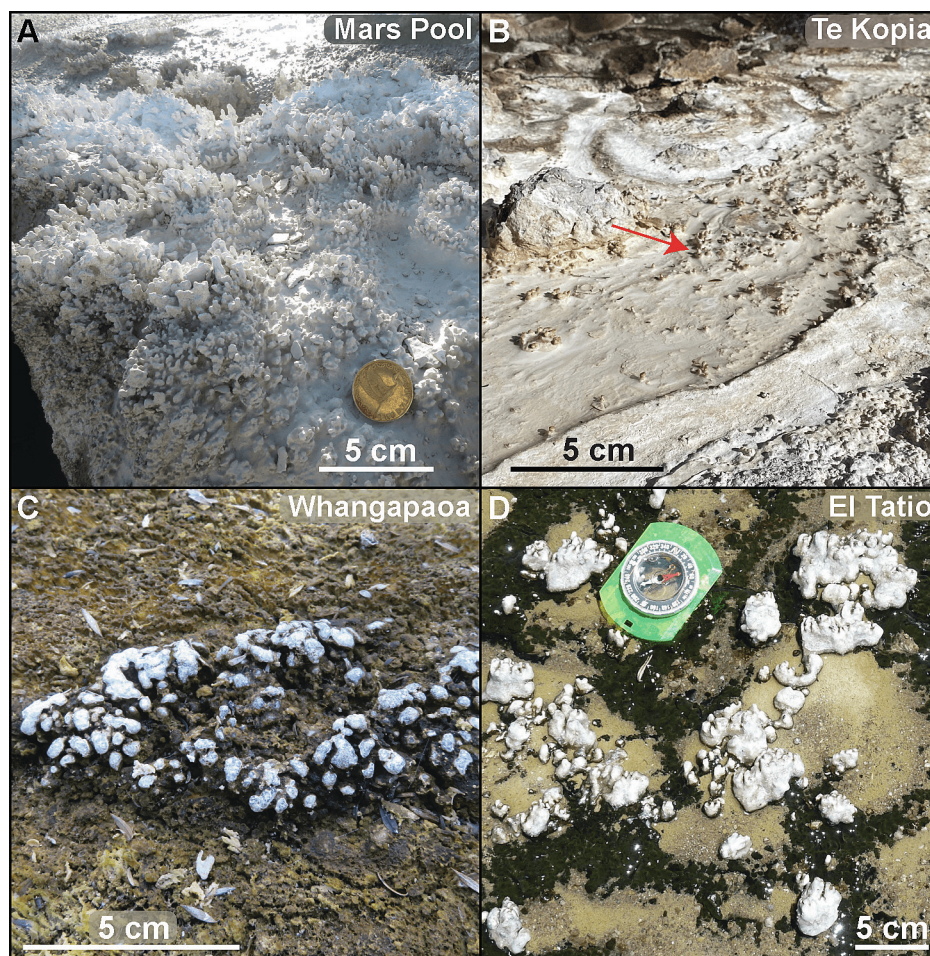


Fig. 2. Photographs of in-situ digitate sinter within representative hot springs of variable fluid chemistry. (a) Spicular digitate sinter deposited on a muddy substrate around the rim of currently inactive Mars Pool, likely of acid-sulfate-chloride fluid chemistry. (b) Fine, spicular, digitate sinter actively forming on hydrothermally altered muds in the shallow acid sulfate Te Kopia stream. (c) Whangapaoa spring bulgy digitate sinter, dried white during the summer, overlying green microbial mats. (d) Knobby digitate sinter actively forming in a shallow alkali chloride discharge channel at El Tatio, associated with dark green microbial mats. Photograph provided by Steve Ruff. Red arrows indicate in-situ digitate structures. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

qualitative distribution of Al, Na, Fe, S, Si (WDS) and C, O, Mg, Cl, K, Ca, Ti, Mn (EDS).

WDS was utilized for element mapping of light elements (lower atomic mass than Si) due to limitations of the Synchrotron micro-x-ray fluorescence (Howard et al., 2020). Secondary electron (SE) and backscatter electron (BSE) imaging (15 kV, 100 nA) on the instrument were used to micro-characterize textures on the polished surface of the digitate structures, at a higher resolution than what could be achieved by light microscopy. Additional SE images, focused on microbial textures in the digitate sinter samples, were collected on freshly broken surfaces at variable magnification on a FEI Quanta 200F ESEM at the University of Auckland Research Centre for Surface and Materials Science.

2.5. Synchrotron micro-X-ray fluorescence mapping (sXRF)

Synchrotron micro-X-ray fluorescence mapping (sXRF) was conducted at the XFM Beamline at the Australian Synchrotron to provide a high-resolution quantitative analysis of trace element concentration and distribution in digitate sinters. Two XRF detectors were utilized: the Maia 384-element ('Maia') silicon detector, and the Vortex ME3 3-element silicon drift detector. The Maia detector (incident beam energy of 18.5 keV) had a detection cut off at Si (cf. Howard et al., 2020), with good resolution achieved at or higher than Ca (resolution of ~ 300 eV versus 160 eV for the Vortex detector at the full width half max

(FWHM) of Mn K α). The Vortex ME3 detector (incident beam energy of 7.05 keV) was tested on two samples (ET1, WS1A) with the aim of trying to map and quantify aluminium fluorescence emission. Unfortunately, conditions were not favorable and the Al maps did not have good resolution. The Vortex ME3 detector did produce better resolution Si and S maps than the Maia detector. When using the Maia detector, the samples were oriented 90° to the X-ray beam, which passed through the detector onto the sample (Howard et al., 2020). With the Vortex ME3 detector, samples were oriented 45° to the X-ray beam to position the detector in the local scatter minimum.

The pixel resolution of sXRF maps was set to vary between 5 and 10 μm for overview maps of the entire large digitate structures, and 1–2 μm for overview maps of smaller digitate structures, as well as high-resolution small maps of microbial features and areas of interest. Dwell times of the beam on each pixel were set to vary between 1 and 10 milliseconds. Calculated attenuation lengths vary from ~ 13 μm (Ca) to ~ 700 μm (Sr) in SiO₂ (2.2 g/cm³, at a 90° beam incidence angle; Henke et al., 1993). The XRF spectra were analyzed using the GeoPIXE v 7.7 software (Ryan et al., 1990) on the Australian Synchrotron Computing Infrastructure (ASCI).

Results are primarily presented as intensity maps to demonstrate the spatial importance of chemical variability, with all sXRF maps presented as image files and image text files (.csv), available online. While each image pixel represents a full sXRF analysis, concentration information

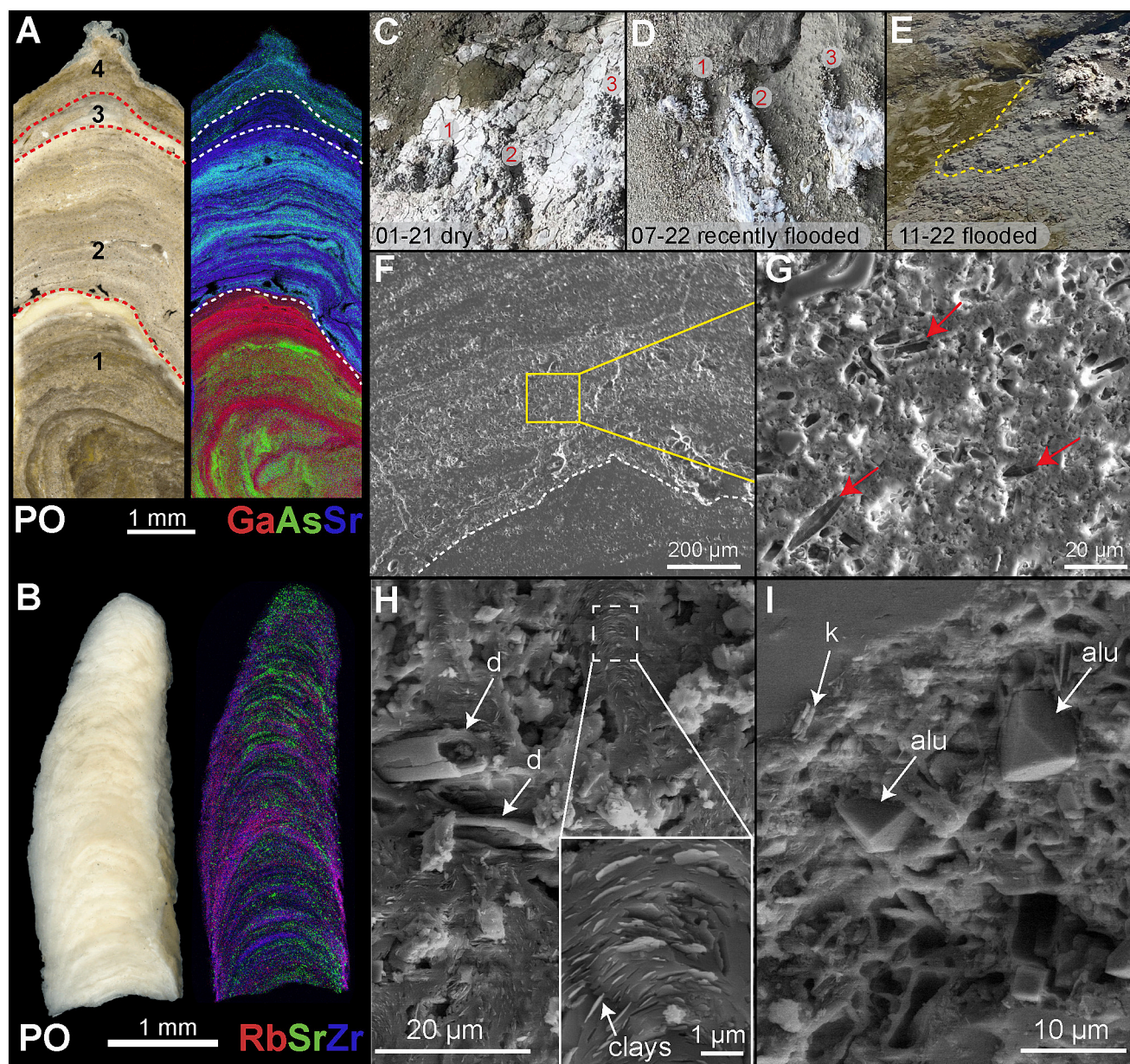


Fig. 3. Environmental and mineralogical influences on trace element variability in digitate sinter. (a) Polarized reflected light [PO] image and RGB elemental map of Ga (red), As (green), and Sr (blue) of a Mars Pool digitate structure, showing a clear compositional difference between Ga-enriched Horizon 1, and Sr-enriched Horizon 2. (b) Polarized reflected light [PO] image and RGB elemental map of Rb (red), Sr (green), and Zr (blue) of a Te Kopia digitate structure, showing laminae are concurrently enriched in Rb and Zr, alternating with particulate Sr in porous layers. (c) Water levels at Mars Pool in January 2021, showing dry and exposed digitate structures. (d) Water levels at Mars Pool in July 2022, showing still-wet mud coating digitate structures, indicating recent flooding from the greater shallow hydrothermal lagoon area. Numbers in (c) and (d) indicate matching features. (e) Water levels at Mars Pool in November 2022, showing flooded and mud-draped digitate structures. Yellow dashed line outlines an area of protruding digitate structures which are progressively coated in mud. (f) Secondary electron [SE] image of the polished surface of a Mars Pool digitate structure, showing alternating parabolic layers of porous and less porous silica. White dashed line indicates the boundary between Horizon 1 and Horizon 2. (g) Enlarged yellow box from (f), showing detail of Horizon 2 which indicates that the higher porosity is controlled primarily by diatoms (red arrows). (h) Diatoms [d] and micron-scale clay minerals interlaminated with silica [inset] within stromatolitic laminae in Te Kopia digitate sinter. (i) SE image of hexagonal kaolinite [k] and euhedral alunite [alu] crystals in a diatomaceous layer within Pretty Pool digitate sinter. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

has only been extracted from representative regions (Table 2).

2.6. Raman spectroscopy

Raman spectra were obtained at room temperature from 50 to 1750 cm^{-1} using a Horiba Labram HR Evolution confocal Raman spectroscope (University of Auckland) equipped with a 600 g/mm^{-1} diffraction grating and an air-cooled CCD detector (-60°C), and calibrated with a

silicon chip (520.7 cm^{-1}). The spectra were collected using a 785 nm laser passed through a neutral density filter to prevent sample damage, and focused through a $50\times$ objective lens (Olympus, N.A 0.5). The 785 nm excitation was selected to avoid autofluorescence in the samples, which was observed in the 532 nm excitation.

Spot measurements were taken in areas with well-preserved microbial material to refine measurement parameters (neutral density filter (10–25%), acquisition time (30–180 s), accumulations (2–3)). Variably

Table 2
Representative sXRF trace element concentrations for digitate sinter structures.

Sample	Location	K (ppm)	Ca	Ti	Mn	Fe	Cu	Zn	Ga	Ge	As	Rb	Sr	Zr	CsL	BaL	WL	PbL	HgL
MP1	Full	3059	—	1035	—	1319	17.4	2.38	38.8	25.8	1492	—	115	52	—	—	71.7	—	—
MP1	Base	—	—	327	—	1611	—	—	57.4	28.8	2206	—	67.9	35.5	—	—	83.3	—	—
MP1	Rim	3223	306	705	—	3070	6.9	2.4	9.7	22.3	1250	—	95.2	17.6	—	—	46	—	—
TK1A	Full	2609	—	454	51.6	772	—	—	—	—	—	43.6	59.3	214	—	586	—	19.3	—
TK1A	Base	2203	—	417	42	815	—	0.1	—	—	—	35.3	53.2	233	—	529	—	15.7	—
TK1A	Rim	2083	—	305	33	585	—	—	—	—	—	38.1	76.5	177	—	269	—	18.9	—
ET1	Full	—	18,848	—	—	1626	—	—	—	56.1	3691	51.3	82.2	3.27	—	—	—	—	—
ET1	Rim	—	26,191	—	—	1669	—	—	—	96.2	5666	57.8	91.8	—	—	—	—	—	—
ET1	Base	—	1941	—	1770	2050	143	3	5.5	—	588	36.3	51.1	7.9	—	—	—	—	—
ET1	Microbe1	—	3100	—	910	2642	—	—	387	—	385	49.1	51.8	10.5	—	—	—	—	—
ET1	Microbe2	—	5136	—	—	2116	—	—	255	—	249	43.3	49.8	2.9	—	688	—	—	—
ET1	Microbe2 (dense)	—	3386	—	—	3002	—	—	449	—	267	52.7	57	7.11	—	72	—	—	—
WS1A	Full	76	119	507	29.4	317	66.3	6.38	110	1.96	—	24.8	16.6	—	—	—	—	24.5	—
WS1A	Rim	—	—	129	429	183	107	11.5	593	12.9	—	57.5	32	—	—	—	—	8.6	—
WS1A	Microbe1 (rim)	1045	—	464	158	77	95	21.7	782	10.5	—	64.3	20.8	4.4	—	—	—	—	—
WS1A	Microbe1 (base)	151	—	388	365	66	162	33.3	1452	30.9	—	88.8	35.5	10.4	—	—	—	—	—
WS1A	Microbe2	679	4349	289	654	80	222	33	736	17	—	39.9	35.3	—	269	377	—	—	—
WS1A	Interior	328	181	1100	0.8	381	76	7.65	86.7	—	—	21.7	10.8	—	—	—	—	—	24.7

Locations correspond to identified locations in Fig. 4. “Microbe” locations in WS1A are within the rim area defined in Fig. 4d. Microbe locations 1 and 2 in ET1 are numbered in Fig. 4c. Full = the entire sinter surface. All concentrations in ppm. CsL, BaL, WL, PbL, and HgL analyzed with La X-ray lines. Italicized elements analyzed with the Vortex detector.

sized and spaced grids were set up to collect spectra at each area of interest, with each total map scan times between ~50–64 h total. Spectra collected from El Tatio digitate sinter did not exhibit fluorescence under these scanning parameters. Spectra collected from Mars Pool sinter exhibited fluorescence backgrounds. However, applying a multipoint, 5th order polynomial baseline correction on each spectrum in LabSpec6 sufficiently subtracted the fluorescent backgrounds to enable the identification of major spectral features.

3. Results and discussion

3.1. Trace elements recording digitate growth

The intermittent, wet-dry cyclicity of silica precipitation in digitate sinter growth is visualized through trace element variability preserved within the laminated digitate features (Fig. 3A,B). Fluid mobile elements, such as As, clearly delineate the draped, micro-stromatolitic growth horizons of these structures, consistent with micro-structural interpretations of their growth history (Figs. 3, 4; e.g., Gong et al., 2021; Nersezova et al., 2023). Alternating trace element enrichments and compositional shifts also record variations in fluid chemistry during silica precipitation (Fig. 4). In Mars Pool digitate sinter, As averages 1492 ppm (Table 2), but repeatedly cycles between 150 and 4780 ppm (Fig. 4B). Gallium, in the same Mars Pool sample, ranges between 35 and 98 ppm (57.4 ppm average) at its base, but following a step function decrease, subsequent precipitated silica has a maximum concentration of 41 ppm (9.7 ppm average) (Fig. 3A; Table 2). In the Te Kopia digitate features, where the compositional layering is less distinctive, Fe (410–1235 ppm) and Rb (18–75 ppm) demonstrate similar cyclicity in trace element distribution correlated to growth features (Fig. 3B; 4 A).

Detrital grains from aqueous or aeolian transport may introduce distinct trace elements into digitate structures (e.g. Wang et al., 2017). In Cretaceous sinter, alternating light and dark laminae record rare earth element and yttrium input, potentially resulting from terrestrial detrital contamination during wet seasons (You et al., 2024). In the present study, multiple visits to the Mars Pool site highlighted this wet-dry cycling with various states of flooding providing a mechanism for detrital transport (Fig. 3C-E). The detrital contamination is evident mineralogically as clays incorporated into the Mars Pool and Te Kopia digitate features (Fig. 3H, I), resulting in localized enrichments in Sr (Figs. 3A, B, 4; Grecco et al., 2012). The heterogeneous distribution of detrital grains is also evident in the high variability of trace elements recorded in compositional transects that apparently reflects the patchy nature of detrital contamination (such as Sr in Te Kopia, and Fe in El Tatio digitate structures; Fig. 4). These highly variable and cyclic trace element fluctuations (e.g. As, Sr, Fe, Rb, Zr) tend to be largely independent of silicified microbial content, with the exception noted below of the Whangapaoa Spring digitate sample (Figs. 3A, B, 4). Lynne et al. (2008) also found no correlation between variously colored sinter horizons, likely correlated to trace element differences, and filamentous microbial horizons.

3.2. Microbe-metal interactions and biosignatures

Reflected light and secondary electron (SE) imagery of the studied digitate sinters reveal a variety of preserved microfossils which, from our samples, reflect the pH of the fluids from which the sinters were formed. Alkali chloride digitate sinters in this study primarily feature silicified sheaths of filamentous cyanobacteria, generally between ~6–10 µm diameter and up to 300 µm long, morphologically similar to *Calothrix* genus (Fig. 5A, B; cf. Ruff and Farmer, 2016). These filaments have variable pigmentation of their sheaths, ranging from yellow-brown in El Tatio sinter, to dark orange-brown and translucent sheaths in Whangapaoa Spring filaments (Figs. 5, 6A, B). Some filaments are aligned in tightly packed, vertical pillars characteristic of palisade fabric, oriented normal to the growth surface, whereas others are

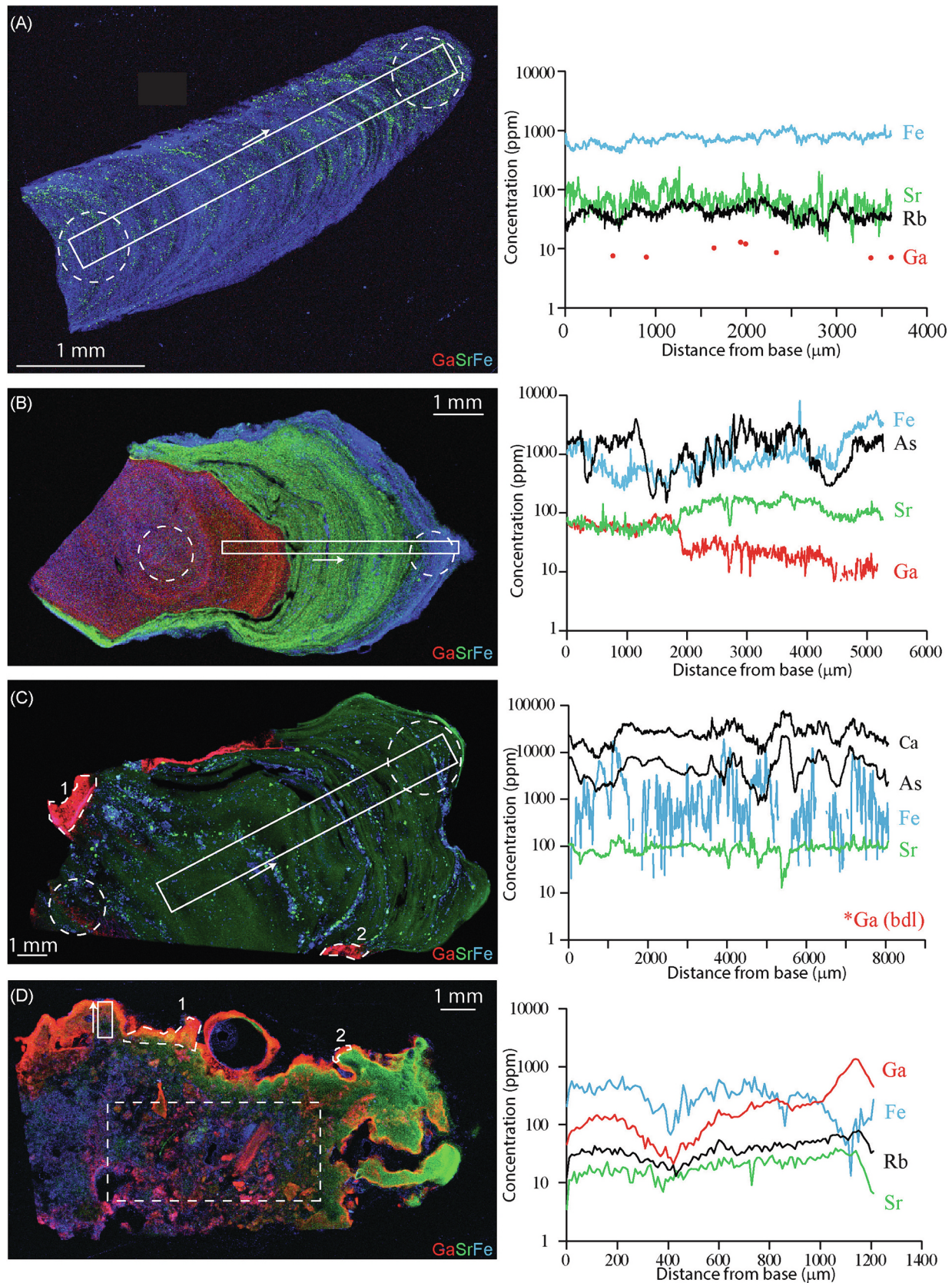


Fig. 4. Whole digitate sinter sXRF composite maps showing the relationship between Ga (red), Sr (green), and Fe (blue) in four digitate samples investigated in this study (A) Te Kopia, (B) Mars Pool, (C) El Tatio, and (D) Whangapaoa. Dashed regions are the integrated areas for representative chemical compositions reported in Table 2. Solid rectangles with white arrows show the direction and area of compositional traverses. Traverses are calculated as the average composition perpendicular to the transect direction within the rectangle (from base to top). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

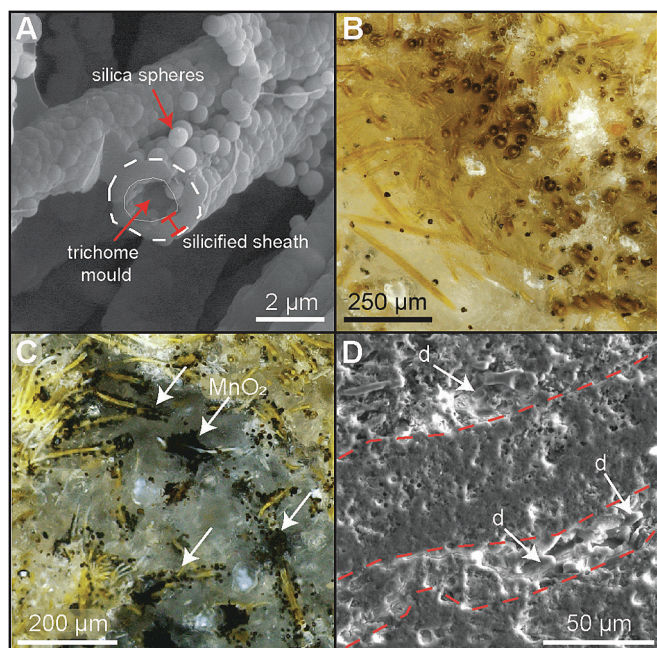


Fig. 5. Microbiota and mineralogical features of digitate sinter of this study. (a) Secondary electron [SE] image of a silica encrusted filament sheath in freshly broken Whangapaoa Spring digitate sinter. (b) Reflected light image of longitudinal and transverse filament cross sections in El Tatio digitate sinter, showing that pigmentation is concentrated in the microbial sheaths. (c) Manganese dioxide [MnO₂] particulates encrusting cyanobacteria [white arrows] in El Tatio digitate sinter. (d) Cut and polished surface of a Mars Pool digitate structure, showing diatomaceous laminae composed of diatoms [d] alternating with solid silica laminae. Layering is indicated by red dashed lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

apparently randomly distributed filaments in both transverse and longitudinal cross section. These sheaths are variably silicified, similar to the siliceous coatings around mucilaginous sheaths described by Gangidine et al. (2020). Solitary and colonial chains of pennate diatoms, morphologically similar to *Pinnularia* sp. (<30 µm long, <10 µm diameter), are well documented in acidic New Zealand hot springs (cf. Cassie and Cooper, 1989; Jones et al., 2000; Owen et al., 2008) and dominate the biota of acidic digitate sinters in this study. All observed diatoms are hollow, and some frustules have begun to merge with the surrounding opal-A matrix (Fig. 5D). Auxiliary microbiota morphotypes in acidic digitate sinters include coccoid (~0.5 µm diameter), rod-shaped (~1 µm long, 0.5 µm diameter), and filamentous (~4 µm diameter, <50 µm long) microbes, which likely represent bacteria as well as coccoidal algal cells (~2–3 µm diameter), the latter of which form the bright green microbial mats that are ubiquitous throughout the Parariki Stream floodplain in the Rotokawa geothermal system, and which have been attributed to *Cyanidium caldarium* (cf. Schinteie et al., 2007).

Qualitative WDS and quantitative sXRF maps show distinct enrichments of Al, Ca (~2.5 wt%), Fe (1000–5000 ppm), Ga (500–900 ppm), As (750–900 ppm), Rb (~45 ppm), and Sr (~100 ppm) in silica, outlining silicified cyanobacterial filaments in El Tatio digitate sinter (Fig. 6). Filamentous cyanobacteria are characterized by a trichome (a connected thread of cells) encased in a mucilaginous sheath composed of EPS (Weckesser et al., 1988), which is often coated with silica (Gangidine et al., 2020). In this study, comparison to SE imagery shows these enrichments correspond to the silica entombing the cyanobacterial sheath.

Element enrichments associated with filaments are not consistent throughout sinters from different springs. For example, Whangapaoa Spring cyanobacterial filaments have a distinct S enrichment within the

silicified sheaths, while K (1045 ppm), Ga (782 ppm), Rb (64 ppm), Sr (21 ppm), and Cs (200–300 ppm) are present in the silica around the sheaths (Fig. 6; Table 2), along with qualitative enrichments in Al and Ca (EMPA; Fig. 6D–E). In addition to outlining of the microbial filaments by trace elements (Fig. 6A–C), both Ca and Ga have a secondary enrichment in laterally continuous zones perpendicular to the growth direction of filaments. Strong enrichment of Ga (~782 ppm), Rb (~64 ppm), and Sr (~20 ppm), are observed in the basal silica substrate beneath the filaments, often including a portion encompassing the lower parts of the filaments (Fig. 4D; Table 2). This result suggests trace metal variability is related to both microbial processes (Fig. 6A, B) and changes in fluid chemistry or environmental conditions through time (Fig. 3A, B). This observation contrasts with the El Tatio filaments, which are clearly outlined in metal-rich silica relative to the surrounding silica matrix (Fig. 6E). Moreover, no Fe-enrichment was associated with filaments in Whangapaoa Spring, potentially due to comparatively less availability of dissolved iron in its discharging fluids (Landrum et al., 2009; Power et al., 2018). Observations of elemental enrichment from the digitate sinter of this study are consistent with documented enrichments of Ga and Fe in recently silicified filaments (Gangidine et al., 2020) and in measurements of modern organic rich sinter (Churchill et al., 2021) from Yellowstone National Park, as well as enrichments of Al, Fe, and Sr in Devonian microfossils from the Drummond Basin (Gangidine et al., 2021) (Table 1). The enrichment of S in some samples, identified by electron microprobe analysis (Fig. 6E), could reflect primary sulfate groups in the bacterial sheath EPS, also found in archaea, which contributes to the overall negative charge of the sheath and its ability to sequester cations (Pereira et al., 2009; Zhang et al., 2019). However, the high porosity of the studied digitate sinters (Nersezova et al., 2023) means they are also susceptible to chemical alteration by later processes. For example, the spatial co-occurrence of silica and sulfur does not necessarily imply that their processes of formation occurred contemporaneously. It is widely recognized in terrestrial geothermal systems that late-stage, hydrothermal, acid-sulfate leaching often results in the overprinting of earlier alkali-chloride silica sinter by sulfur and sulfate minerals (e.g. Fig. 3I), typically occurring with a drop in the water table, waning flow, or episodic earthquake activity affecting fluid emission flux, such as in the Sub-Recent to Holocene Te Kopia geothermal area (Bignall and Browne, 1994), Ohakuri sinter (Henneberger and Browne, 1988), and Roosevelt Hot Springs sinter (Lynne et al., 2005; Ruff et al., 2020), as well as in the Miocene Atastra Creek sinter (Campbell et al., 2019) and the Late Jurassic El Macanudo paleo-geothermal field (Guido and Campbell, 2018). Therefore, S enrichments could alternatively reflect later acid-sulfate overprinting, with high S contents simply reflecting precipitation of sulfur-bearing minerals utilizing the increased porosity due to the loss of organic components from the filament trichome (Fig. 4A), or acid etching.

Cyanobacteria have long been recognized for their ability to act as metal sinks in aqueous environments by binding dissolved metals (e.g., Sigg, 1985; Urrutia and Beveridge, 1994; Phoenix et al., 2000; Yee et al., 2004). For example, *Calothrix* sp. cyanobacteria isolated from Icelandic hot springs bind metals via proton-reactive anionic carboxyl, amine, hydroxyl, and phosphoryl functional groups on both the cell walls and EPS sheaths (Phoenix et al., 2002; Yee et al., 2004). Some iron-oxidising bacteria are known to nucleate iron (III) oxyhydroxides (e.g., ferrihydrite) through sorption and oxidation of ferrous iron on the cell surface, producing sheaths of ferrihydrite (Inskeep et al., 2004). Bacteria can also behave as chemically reactive surfaces, adsorbing ferric iron and subsequently reacting with dissolved anions to act as sites for surface ferrihydrite precipitation (Fortin et al., 1997; Warren and Ferris, 1998). In this study, there is no petrographic evidence for any red-brown ferrihydrite precipitants around cyanobacterial sheaths in El Tatio digitate sinter (Fig. 4C), and no SEM evidence for calcite nor ferrihydrite nucleation on the sheaths (Fig. 5A). Furthermore, there is no spectral evidence for calcite nor ferrihydrite, which both have distinct Raman shifts (Fig. 7; Jia et al., 2006; Dufresne et al., 2018). Instead, it is more

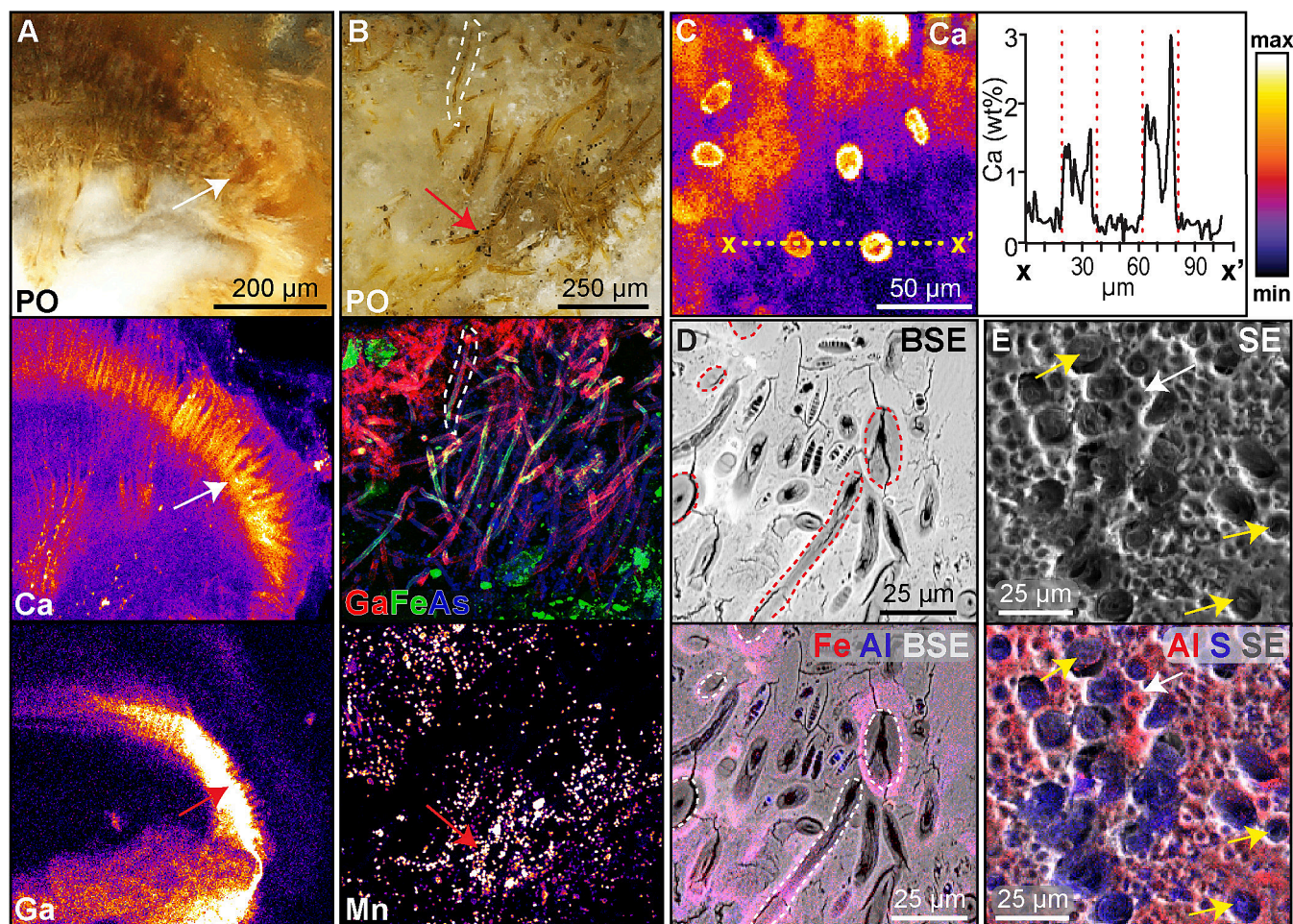


Fig. 6. Elemental enrichments and corresponding Raman spectra of cyanobacterial filaments in El Tatio and Whangapaoa Spring digitate sinter. (a) Elemental enrichments of Ca and Ga around cyanobacterial filaments from Whangapaoa Spring digitate sinter (sXRF). (b) Elemental enrichments of Mn, Ga, Fe, and As around cyanobacterial filaments from El Tatio digitate sinter (sXRF). PO = polarized reflected light. (c) Elemental enrichment of Ca around cyanobacterial filaments from El Tatio digitate sinter. Graph shows calculated Ca concentration across a traverse ($x - x'$). (d) Back scatter electron (BSE) image of cyanobacterial filaments in El Tatio digitate sinter (dashed red outline) with elemental enrichment of Fe (red) and Al (blue), superimposed on the same BSE image, around cyanobacterial filaments in El Tatio digitate sinter (EMPA-WDS). (e) Secondary electron (SE) of cyanobacterial filaments in Whangapaoa Spring digitate sinter. Yellow arrows indicate protruding sheaths; white arrow shows silica coating around sheath; elemental enrichment of Al (red) and S (blue) superimposed on the SE image (EMPA-WDS). S is enriched in the sheaths, whereas Al is dominantly in the silica coatings around the sheaths. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

likely these ions are being sorbed onto cyanobacterial EPS and that silicification is occurring through cationic bridging (Phoenix et al., 2002; Urrutia and Beveridge, 1994; Yee et al., 2004). This lack of observed mineralization thus suggests most elements (excluding Mn, as discussed below) appear to be preserved within siliceous coatings on the sheath exterior, rather than precipitating on the sheath as discrete metal (oxyhydro)oxides or carbonates. This is especially likely considering the large range of non-specific metal cationic species observed to be sequestered around filaments in the alkali chloride sinter samples of this study (Table 2).

Black particulates are observed in close spatial association with microbial textures in El Tatio digitate sinter, varying in occurrence between individual particles scattered within groups of filaments, to clusters of particles clearly associated with, and/or coating, individual filaments (Fig. 5C). The black particulate material is less abundant in areas without petrographically visible cyanobacterial filaments. Raman analyses identify this mineral as manganese dioxide (MnO_2), with ~1–3 wt% Mn measured in sXRF maps (Fig. 6B). The close spatial association of these MnO_2 particles with filaments, especially in cases where the mineral completely coats the filaments, suggests this mineralization is mediated by microbial activity. In laboratory-cultured cyanobacteria

(Chaput et al., 2019), patterns of Mn (II) oxidation varied between MnO_2 precipitants coating some cells, to distributed as clumps throughout biofilms by overlying cell colonies (with or without clear association to particular cells). These laboratory experiments align with and strongly support the hypothesis of this study that patterns of MnO_2 spatial distributions observed in El Tatio digitate sinter are mediated by microbial activity. Moreover, bacterial EPS has been reported as locations for adsorption of Mn(II) (Tebo et al., 2004), where the adsorbed manganese is oxidised on the EPS surface and precipitated as MnO_2 (Chaput et al., 2019). Cyanobacterial EPS is both associated with the cell (e.g., extracellular sheaths) and also occurs as biofilms composed of polysaccharides released into the surrounding environment (Pereira et al., 2009). For the El Tatio digitate sinter of this study, the direct association of MnO_2 with both extracellular sheath EPS and scattered within biofilm-forming EPS suggests that both types of cyanobacterial EPS are likely able to adsorb and oxidise Mn (II) and precipitate MnO_2 (Fig. 5C). In addition to occurring broadly in phototrophs, biogenic Mn(II) oxidation has also been documented among a wide range of non-phototrophic bacteria (e.g., Tebo et al., 2004; Villalobos et al., 2006; Miyata et al., 2007), and has recently been shown to fuel the growth of chemolithoautotrophic Mn-oxidising bacteria (Yu and Leadbetter,

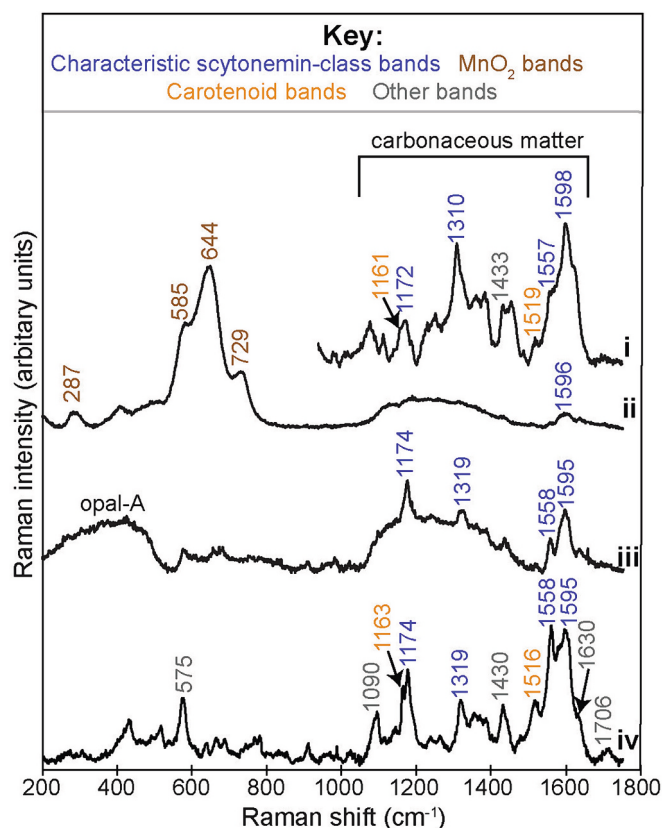


Fig. 7. Raman spectra showing the variability of interpreted scytonemin-class and carotenoid organic molecules (tentatively assigned to scytonemin and β -carotene) and inorganic minerals (opal-A silica and manganese dioxide) detected in cyanobacterial filaments from Whangapaoa Spring (i) and El Tatio (ii-iv).

2020).

The observed sequestration of Ca, Fe, and Mn around cyanobacteria supports the hypothesis that these bio-essential elements could be actively sequestered as a reservoir of essential nutrients (Phoenix and Konhauser, 2008). Interestingly, the consistent sequestration of Ga across microbial filaments (Figs. 6A, B; 8B) is problematic for cyanobacterial viability, as gallium is known to have clinical applications as a potent antimicrobial agent (e.g., Bonchi et al., 2014; Kelson et al., 2013; Li et al., 2022). Gallium, which is not redox-active, is known to replace Fe in bacterial redox enzymes and impair their function (Bonchi et al., 2014). Therefore, iron-mimicry could be a likely mechanism for the observed sequestrations of Ga (up to 900 ppm), as well as other toxic trace elements observed in alkali chloride digitate sinters.

WDS and sXRF mapping of diatomaceous laminae indicate trace element abundances in diatom frustules are indistinguishable from trace element concentrations in the surrounding silica matrix (Fig. 9). Diatoms are unicellular algae with characteristic opal-A cell walls (frustules), produced by intracellular, biologically controlled mineralization via uptake of dissolved silicic acid from the surrounding environment (Kröger et al., 1999; Reid et al., 2021). Diatoms have long been documented to absorb trace elements in the formation of their frustules, including both bio-essential elements (e.g., Martin and Knauer, 1973) and heavy metal contaminants from their surroundings (e.g., Gélalbert et al., 2007). Laboratory experiments to culture and dope biogenic diatom silica with trace elements have reported that trace element uptake is proportional to the concentration of the trace elements in the aqueous medium (Riley and Roth, 1971), consistent with our observations in digitate sinter.

3.3. UV protective pigments as biosignatures

Raman spectra of cyanobacteria filaments from the Whangapaoa digitate sinter have clearly defined bands at 1161, 1172, 1310, 1433, 1519, 1557 and 1596 cm^{-1} (Fig. 7). In El Tatio cyanobacteria, similar bands as observed at Whangapaoa are also observed at 1163, 1174, 1319, 1516, 1558, 1595 cm^{-1} , with additional bands evident at 575, 1090, 1430, 1630, and 1706 cm^{-1} (Fig. 7). A broad band in the ~ 200 –500 cm^{-1} range indicates the presence of opal-A (Hellowell et al., 2015). Furthermore, spectral features at 729, 644, 585, 287 cm^{-1} correspond to manganese dioxide (MnO_2 ; Figs. 5C, 7), consistent with published spectra for a romanechite/todorokite-type α - MnO_2 polymorph (Gao et al., 2008; Post et al., 2020). The major spectral bands in the studied cyanobacterial filaments in the wavenumber range between ~ 1000 and 1750 cm^{-1} are assignable to scytonemin class molecules (tentatively, assigned to scytonemin) (1595, 1558, 1319, 1174 cm^{-1}) and carotenoids, (tentatively assigned to β -carotene) (1516 and 1163 cm^{-1}) (Fig. 7) based on comparison to published Raman spectra (Edwards et al., 2000; Merlin, 1985). From Raman spectra alone, in the absence of standardization, it is impossible to unambiguously identify scytonemin and β -carotene (de Oliveira et al., 2010; Jehlička et al., 2014b). Scytonemin and carotenoids are microbial pigments commonly produced by cyanobacteria and are known to produce strong and characteristic Raman signals (Edwards et al., 2005; Vitek et al., 2010; Wynn-Williams et al., 1999). Raman signatures of scytonemin in cyanobacteria were first reported by Wynn-Williams et al. (1999) and subsequently described in greater detail by Edwards et al. (2000), who noted the characteristic corroborative scytonemin bands of ~ 1590 , ~ 1549 , ~ 1323 , and ~ 1172 cm^{-1} . Carotenoid pigments represent a large group of molecules with a conjugated polyene backbone chain of different lengths, and with different end-groups (Jehlička et al., 2014b). The ~ 1000 cm^{-1} band usually present in carotenoid Raman spectra is characteristically weak (Edwards et al., 2005; Jehlička et al., 2014a). The absence of this band in the spectra indicates that concentration of carotenoid is minor (or masked by fluorescence), and more intense Raman signals for the scytonemin class molecules indicate this is the primary microbial pigment in the studied samples. Similar Raman spectra have been identified in Fe-Ga-Mn sequestered silicified filaments from Yellowstone National Park sinter (Gangidine et al., 2020), with spectral signatures characteristic of β -carotene; however, scytonemin was not identified from the Raman results of the Yellowstone study. The use of the 785 nm laser in this study minimized the impact of fluorescence background produced using shorter laser wavelengths, which have previously proved problematic for molecule identification in opal-A silica (Rodgers et al., 2004), while still allowing for a reasonable timeframe for area-imaging measurements compared to the use of longer wavelength laser lines.

Scytonemin is a UV-absorbing pigment produced exclusively by certain cyanobacterial species to protect and screen the cells against harmful UV irradiation (Brenowitz and Castenholz, 1997; Wynn-Williams et al., 1999; Dillon and Castenholz, 1999). UV-screening mechanisms, such as microbial pigments, are employed by cyanobacteria to screen against detrimental UV radiation in an organism which still requires some photosynthetically active radiation to produce energy (Phoenix et al., 2006). A yellow-brown, lipid-soluble pigment, scytonemin, is produced as part of the extracellular sheath surrounding cyanobacterial filaments (Dillon and Castenholz, 1999; Garcia-Pichel and Castenholz, 1991), and is documented to be abundantly produced in *Calothrix* sp. cyanobacteria (Brenowitz and Castenholz, 1997). In this study, scytonemin class pigments are also likely responsible for the yellow-brown pigmentation observed in the polarized reflected light images of silicified filaments in El Tatio digitate sinter (Fig. 6B).

β -carotene is a common carotenoid and is an orange pigment produced by many prokaryotic and eukaryotic microorganisms, including cyanobacteria and a variety of extremophiles. Along with other carotenoids, β -carotene plays an important role as an antioxidant, scavenging

free radicals that result from exposure to UV radiation (Kelman et al., 2009). These photoprotective antioxidant properties explains why carotenoids are considered Mars-relevant potential molecules, as they represent important potential adaptations in Martian environmental conditions with high UV and oxidising species (e.g., Parnell et al., 2007; Baqué et al., 2022; Gill et al., 2023). This contrasts with the protective mechanism of scytonemin, which absorbs UV radiation due to its molecular structure and acts as a microbial ‘sunscreen’ rather than an antioxidant, and hence could account for the lower carotenoid signal intensity in the spectra of this study, as the molecule is destroyed by UV radiation. In the geological record on Earth, β -carotene typically undergoes diagenesis to form β -carotane (e.g., Marshall and Olcott Marshall, 2010), a process recorded in the Jurassic Claudia geothermal field (Teece et al., 2022). Some Mars-relevant degradation studies have examined the effect of UV irradiation on β -carotene, and reveal an alternative diagenetic pathway where there is signal loss but the carotenoids remain intact (Dartnell et al., 2012), supporting the above potential process. In this study, the interpreted UV-protective pigment groups like scytonemin and carotenoids in silicified cyanobacterial filaments (inferred as *Calothrix* morphotype; cf. Cady and Farmer, 1996) within El Tatio digitate sinter indicates that molecular UV-screening is one of the strategies these organisms employ to ensure their survival in a high-UV environment, such as at the El Tatio geothermal field in the Atacama Desert (Phoenix et al., 2006).

The spatial co-location of scytonemins with observed metal sequestration (Al, Ca, Fe, Ga, As, Rb, Sr) and MnO_2 mineralization in the studied El Tatio cyanobacterial sheaths suggests there could be a correlation between these two phenomena (Figs. 6B,D, 7). Therefore, a possible mechanism for metal sequestration could be metal co-ordination and complexation by sheath pigments. Varnali and Edwards (2013) calculated theoretical Raman shifts for a Fe(III)-scytonemin complex in order to inform future investigations searching for experimental evidence of this complex as a potential biosignature. The theoretically predicted Raman spectra show that the iron-scytonemin complex can be spectrally differentiated from experimentally observed scytonemin, primarily by the replacement of the characteristic weak scytonemin shifts at 1656 and 1710 cm^{-1} by new bands at 1767 and 1754 cm^{-1} , respectively (Varnali and Edwards, 2013). As all interpreted scytonemin spectra from El Tatio digitate sinter retain the 1706 cm^{-1} shift, as well as other characteristic scytonemin Raman bands (Fig. 7), there is no spectral evidence in this study for scytonemin chelation of Fe or other heavy metals.

3.4. Application of novel biosignatures

The utility and application of any morphological, organic molecular, or inorganic chemical biosignature is inherently dependent on its preservation potential into the ‘deep time’ geological record, both on Earth and on Mars (e.g., Farmer and Des Marais, 1999). Controls on microbial pigment and trace element preservation, spatially and temporally, include the impacts of UV radiation, mineralization, and diagenesis.

3.4.1. Pigment preservation

Phoenix et al. (2006) found that the amorphous silica surrounding cyanobacterial filaments was the most effective at absorbing UV-C radiation (200–280 nm), without silicification inhibiting photosynthesis (Phoenix et al., 2000), suggesting that silicification acts a significant UV shield for cyanobacteria, especially against biocidal UV-C. However, scytonemin is known to absorb UV most strongly in the UV-A region (320–400 nm), with less, but still significant, absorption in the shorter wavelength UV-B (280–315 nm) and UV-C (200–280 nm) regions (Dillon and Castenholz, 1999; Edwards et al., 2000). In environments like early Mars and Precambrian Earth, with higher levels of UV-C radiation compared to today (Cockell et al., 2000), silicification of microbes may have provided an additional UV screening method to work in concert with biosynthesis of UV-protective pigments to effectively screen against

all wavelengths of UV radiation. Indeed, Dillon and Castenholz (1999) postulated that scytonemin may have evolved in cyanobacteria during the Precambrian to allow colonization of more exposed shallow waters and/or terrestrial habitats.

If photosynthetic microbes were present in hydrothermal environments and contributed to the formation of suspect digitate sinter on the surface of early Mars, which had similar UV-B and UV-C fluxes to early Earth (Cockell et al., 2000), then scytonemin-like microbial UV-screening pigments may serve as excellent organic biosignature targets. Both *Perseverance* (NASA Mars 2020 mission) and *Rosalind Franklin* (ESA ExoMars 2028 mission) rovers have Raman spectrometers on board that are capable of searching for key spectral biosignatures characteristic of extremophile activity (Farley et al., 2020; Rull et al., 2017).

High UV-fluxes on the surface of present-day Mars and throughout the last ~3.6 billion years also have the potential to impact the preservation (and therefore detection) of microbial pigments. Due to their mechanism of neutralizing UV-generated free radicals, carotenoids are destroyed by UV irradiation (Dartnell et al., 2012), while scytonemin appears to be relatively stable when exposed to UV radiation (Fleming and Castenholz, 2007). The latter has been found preserved in the sedimentary record in >1000-year-old lake sediments (Leavitt et al., 1997; Verleyen et al., 2005), in ~8 ka Black Sea sediments (Fulton et al., 2012), and in ~300 ka carbonate ooids (Sun et al., 2019). However, metamorphic alteration degrades these molecules, with observations of scytonemin in sediments older than the Middle Pleistocene yet to be reported in the literature. In contrast, saturated carotenoid derivatives represent the oldest preserved biomarkers in ~1.7 Ga rocks in the McArthur Basin (Vinnichenko et al., 2020). Given such different planetary conditions, although carotenoids appear more geologically stable on Earth, scytonemin may be more stable under martian surface conditions.

In this study, Raman spectral measurements of cyanobacteria in El Tatio digitate sinter showed that the signal intensity of the scytonemin assigned Raman shifts were reduced in the spectra measured in areas with relatively more opal-A, and that the scytonemin assigned bands were nearly completely absent in areas of MnO_2 mineralization (Fig. 7). This finding suggests that the degree of microbial mineralization via silica or MnO_2 , which appears to be spatially variable, decreases the preservation of microbial pigments within individual filaments at the micrometer scale. This phenomenon may be further complicated in older geological deposits, as diagenesis in sinters is known to be spatially patchy (e.g., Campbell et al., 2001; Lynne and Campbell, 2003). Hence, recrystallization during silica mineral phase maturation, from opaline silica to microcrystalline quartz, would likely add an additional factor influencing the degree of pigment preservation. Further research should be undertaken to characterize the impact of diagenesis in the preservation potential of microbial pigments.

3.4.2. Trace metals and diagenesis

Microbial sheaths are the primary morphological cellular feature preserved in sinter through time due to its durability and good preservation potential under rapid silicification conditions (Cady and Farmer, 1996; Farmer and Des Marais, 1999; Campbell et al., 2015; Guido et al., 2019). Consequently, this taphonomic characteristic suggests that elemental enrichments of the sheath, or of the silica coatings immediately adjacent to microbial sheaths, as have been identified in the present study, are more likely to be preserved through geologic time than any enrichments associated with the poorly preserved microbial trichome. Metalloids such as Sb and B, commonly bound by microbially induced processes (e.g., Phoenix et al., 2005) and/or sorbed onto co-precipitated impurities within the opal-A matrix (Alsina et al., 2014), tend to be removed through recrystallization during silica diagenesis (Sanchez-Yanez et al., 2017). Such results suggest that enrichments associated with microbial textures that are present as ‘impurities’ within the silica matrix are less likely to be preserved through silica diagenesis.

Existing research on trace element biosignature preservation through the geologic record is sparse, and distinguishing geologic and microbial signatures is a continuing challenge (Dong et al., 2022). Recent work by Lyon et al. (2023) on ~16 ka sinter samples from the fossil Ohakuri geothermal system (Taupō Volcanic Zone, New Zealand; Henneberger and Browne, 1988) indicates similar enrichments of Ga are correlated with microbial textures from both high and low-temperature sinter facies. Furthermore, sXRF mapping performed during this study on Jurassic sinter deposits (recrystallized to quartz) from the Deseado Massif in Argentine Patagonia indicate that Ga enrichments are still preserved around filaments in these deposits, although not with the same consistency as observed in modern sinters (Fig. 8). Nonetheless, gallium enrichment was not reported in Devonian microfossils from the Drummond Basin sinter (Gangidine et al., 2021). However, given the relatively poor preservation state of sinter textures of the Drummond Basin sinters (Walter et al., 1996), it may be that extensive diagenetic recrystallization removed signs of this element from the deposit. In putative Archean stromatolites from the Dresser Formation (3.48 Ga), observations have focused on coarse textural features, highlighting mineralogical variability (Hickman-Lewis et al., 2023), as well as variations in trace elements such as Ni, As, Sr, Zn and Ca (Baumgartner et al., 2022) broadly associated with sedimentary laminae. However, the mm- to cm-scale observations of Hickman-Lewis et al. (2023) and Baumgartner et al. (2022) are too coarse for direct comparison to the microbe-scale chemical mapping presented here. Coarse recrystallization observed in these Archean Dresser materials has likely eliminated many of the fine-scale microbial metal enrichments observable in younger materials (e.g. Fig. 8; Lyon et al., 2023), but additional study from less weathered core materials ought to be the focus of future investigations.

Overall, the preservation potential of microbial pigments and trace element enrichments supports their utility as chemical biosignatures (organic and inorganic, respectively) in deep geologic time (Fig. 8). However, as the >3.6 Ga silica deposits on Mars are still opal-A (Squyres et al., 2008), rather than being recrystallized to quartz as occurs during siliceous sinter diagenesis on Earth (Herdianita et al., 2000), it is possible that potential microbial textures, organic molecules, and trace element enrichments may still be preserved in siliceous hydrothermal deposits on the red planet.

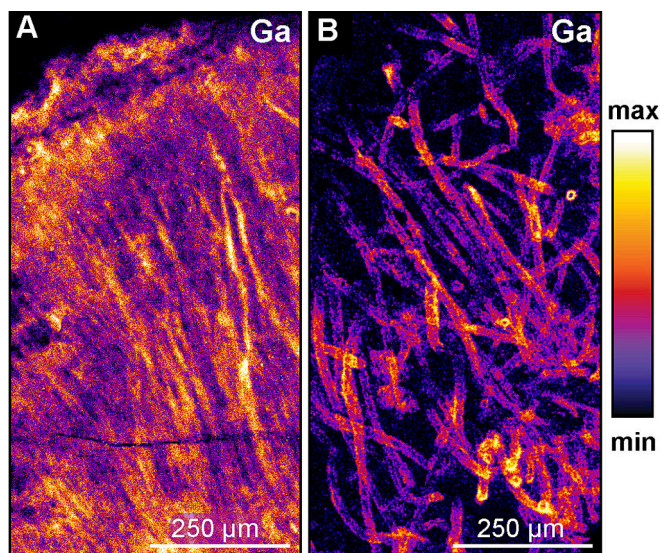


Fig. 8. Preservation of microbial gallium enrichment through space and time. (a) sXRF map of gallium in Jurassic fossil sinter from the Deseado Massif, showing preserved filaments enriched in Ga. (b) sXRF map of gallium in modern El Tatio digitate sinter, revealing preserved cyanobacterial filaments enriched in Ga. Maps have the same scale.

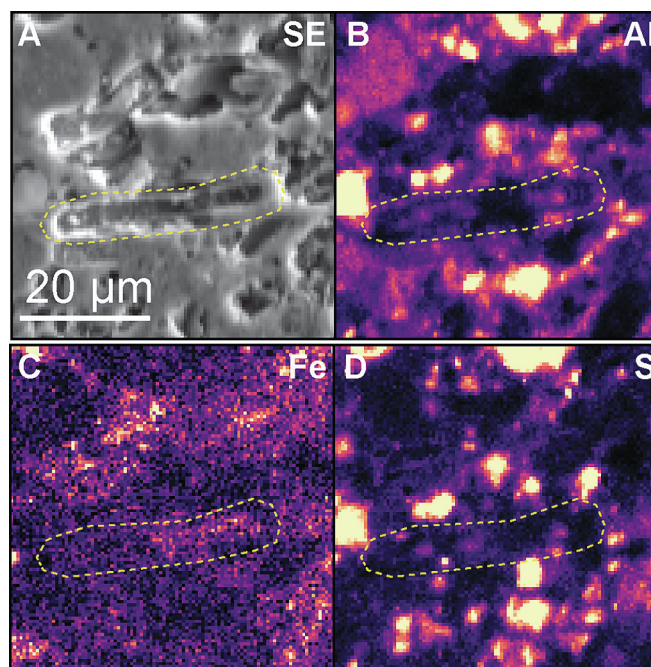


Fig. 9. (a) SE image and (b) Al, (c) Fe, and (d) S elemental distribution (EMPA-WDS) of diatoms in acidic digitate sinter from Mars Pool. Dashed lines outline a longitudinal transect of a diatom in each image. Porosity in the SE image is generated almost entirely from distributed diatom fragments.

4. Conclusions

Terrestrial analogs to digitate silica features on Mars provide an opportunity to investigate possible novel chemical biosignatures, namely inorganic trace metals and organic pigments, as potential tools for recognition of evidence of life in ancient and extreme environments. Modern digitate sinter in New Zealand and Chile encompasses a wide range of fluid compositions; however, potential trace metal and organic biosignatures are more likely to be preserved in alkali chloride hot spring fluid-derived digitate silica structures, where filamentous cyanobacteria flourish. Of trace metals enriched around microbial filaments (Ca, Na, Al, Fe, Mn, Ga, Sr, Rb, and Cs), Ga appears to be the most likely to persist through diagenesis, preserving concentrations up to 40× that normally observed in adjacent geologic materials. Gallium enrichment around microbial filaments is even observed in Jurassic-aged microbial sinter from the Deseado Massif, Patagonia, Argentina. Both environmental (abiotic) and biotic processes may significantly modify trace element concentrations in silica – particularly evident in more fluid mobile elements such as As or vapour-transported elements like S. This is especially significant given the porous nature of most sinter on Earth. Organic chemical biomarkers (pigments) identified by Raman analysis, as scytonemin class molecules and carotenoids, both of which provide UV protection to microorganisms, are co-located with trace element enrichments in this study. Whether trace metal enrichments provide a similar function as the pigments remains an open and important question, which may aid in understanding the mechanism of trace metal enrichment and subsequent preservation. With good resolution of trace metals and organic signatures in filamentous bacteria, but their poor or limited preservation in diatoms, care must be taken to assess how widely applicable the observations of this study are to other extremophiles – and therefore the applicability of this work to future planetary exploration. Therefore, in the future it is critical to investigate further the relationship between trace metal analysis and textural observations and, if possible, the analysis of organics, to robustly identify evidence of past life on early Earth and perhaps Mars.

CRediT authorship contribution statement

Ema E. Nersezova: Writing – original draft, Visualization, Methodology, Investigation. **Michael C. Rowe:** Writing – original draft, Visualization, Supervision, Investigation, Funding acquisition, Conceptualization. **Kathleen A. Campbell:** Writing – original draft, Investigation, Funding acquisition, Conceptualization. **Andrew Langendam:** Writing – review & editing, Methodology, Investigation. **Cherie Toll-emache:** Writing – review & editing, Methodology, Investigation. **Barbara Lyon:** Writing – review & editing, Investigation. **Amanda Galar:** Writing – review & editing, Investigation. **Diego M. Guido:** Writing – review & editing, Funding acquisition. **Bronwyn L. Teece:** Writing – review & editing, Validation. **Trinity L. Hamilton:** Writing – review & editing, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Synchrotron microXRF image files of digitate sinter supporting this research are available for download from Figshare (Doi:10.17608/k6.auckland.25591119).

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