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Trace metal chemistry and silicification of microorganisms in geothermal sinter, Taupo Volcanic Zone, New Zealand

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Abstract

As part of a pilot study investigating the role of microorganisms in the immobilisation of As, Sb, B, Tl and Hg, the inorganic geochemistry of seven different active sinter deposits and their contact fluids were characterised. A comprehensive series of sequential extractions for a suite of trace elements was carried out on siliceous sinter and a mixed silica-carbonate sinter. The extractions showed whether metals were loosely exchangeable or bound to carbonate, oxide, organic or crystalline fractions. Hyperthermophilic microbial communities associated with sinters deposited from high temperature (92–94°C) fluids at a variety of geothermal sources were investigated using SEM. The rapidity and style of silicification of the hyperthermophiles can be correlated with the dissolved silica content of the fluid. Although high concentrations of Hg and Tl were found associated with the organic fraction of the sinters, there was no evidence to suggest that any of the heavy metals were associated preferentially with the hyperthermophiles at the high temperature (92–94°C) ends of the terrestrial thermal spring ecosystems studied. © 2001 CNR. Published by Elsevier Science Ltd. All rights reserved.

Keywords: Silica sinter; Arsenic; Mercury; Thallium; Boron; Antimony; Geothermal biogeochemistry; Thermophilic; Bacteria; Tokaanu; Waikite; Ohaaki; Wairakei; New Zealand

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1. Introduction

Arsenic, antimony, boron, thallium and mercury (As, Sb, B, Tl, Hg) are commonly found concentrated in silica sinter deposited around natural New Zealand hot springs (Weissberg, 1969), and in the sulphide scales of geothermal pipelines of some power stations of the Taupo Volcanic Zone (Brown and McDowell, 1983).

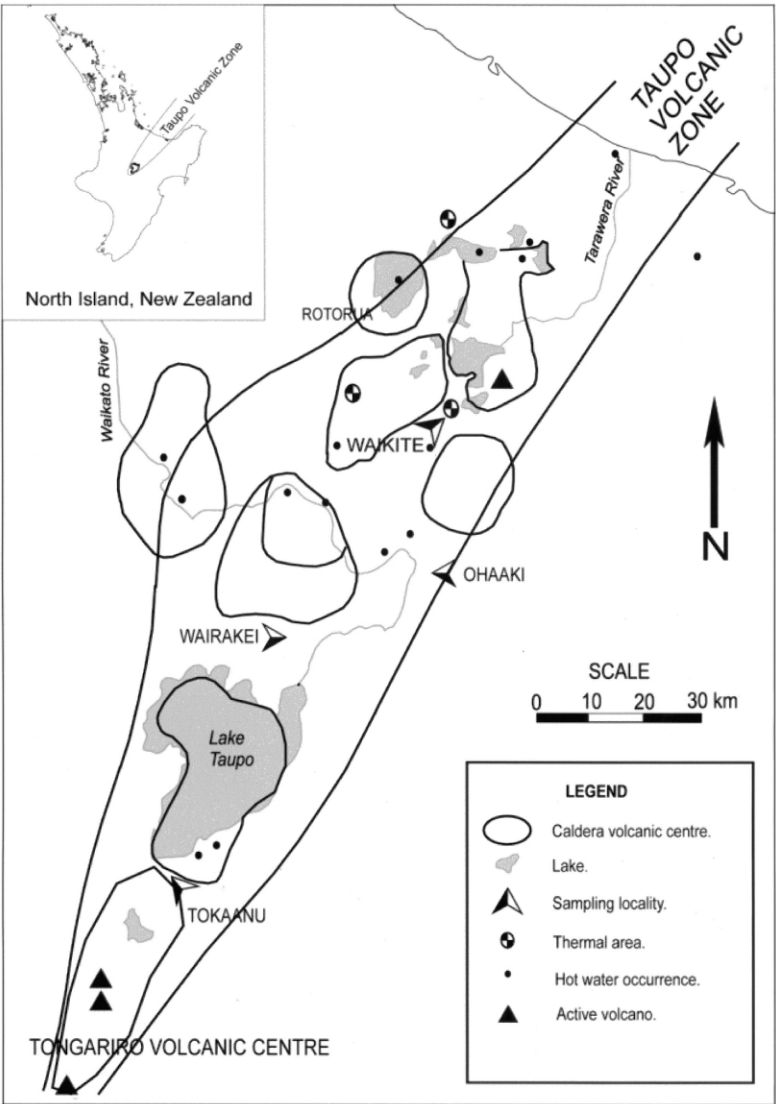


Fig. 1. Map of the Taupo Volcanic Zone showing sampling points (arrows) from north to south, including Waikite springs, Ohaaki pool, Wairakei geothermal power station and Tokaanu springs.

Release of these elements from geothermal waters, steam condensate and leachate into waterways can pose a health risk when the metals become concentrated in the aquatic food-chain. Investigation of the manner in which these elements are immobilised in silica sinter may reveal an avenue of environmental remediation where elements such as these cause contamination.

In water chemistry studies, microorganisms have traditionally been treated as inert colloids (e.g. Webster, 1989). However, since the surfaces and extracellular components of bacteria are highly reactive, *in vivo* and *post mortem*, they can provide ideal sorption surfaces for the nucleation of inorganic phases. For example, extracellular structures such as the paracrystalline protein ‘S-layer’ of bacteria (especially cyanobacteria) (Beveridge, 1995), can accumulate heavy metals and prevent them from entering the cell (Gadd, 1990). The cell wall structures of archaea, which are more diverse than those of bacteria, can also have S-layers (Schultze-Lam et al., 1995b) and sheaths, both of which can bind metals (McLean and Beveridge, 1990). At the present time, the types of interactions that occur between metals and archaea have not been extensively studied.

To date, paired geothermal fluid-sinter studies are few, though Ellis and Sewell (1963), Ichikuni (1968), Ballantyne and Moore (1988), and Parker and Nicholson (1990) have examined the partitioning of As and B. Also, sequential extraction studies encompassing a suite of elements has been rare, with most studies focussing on a single element (e.g. Takahashi et al., 1987), except for Peralta et al. (1996).

2. Sample sites and analytical methods

The sampling sites for this study are known to contain high concentrations of some of the selected trace metals chosen for investigation (e.g. Healy, 1942; Ellis and Sewell, 1963; Mahon and Klyen, 1968; Weissberg, 1969; Ewers and Keays, 1977; Weissberg and Rohde, 1978; Liddle, 1982; Hedenquist, 1983; Finlayson and Webster, 1989; Parker and Nicholson, 1990; Glover et al., 1992, 1996; Christenson et al., 1994; Giggenbach, 1995; Severne, 2000). Elements such as Tl were included on the basis of their common occurrence with As, Sb and Hg in geothermal and hydrothermal environments. The sites (arrows, Fig. 1) include the natural hot springs at Waikite and Ohaaki, man-made environments such as drains and weirboxes at the Wairakei Geothermal Power Station, and an abandoned geothermal bore at Tokaanu. Brief descriptions of each site are given in Table 1.

2.1. Fluid analysis

Analyses of trace metals in the contact fluids were measured to ascertain whether the fluid chemistry alone could account for the concentrations of As, Sb, B, Tl, and Hg in the sinters. Water samples for As, Sb, B, Tl were collected in polyethylene bottles that had been soaked in acid to dissolve metals and other residues (Struempfer, 1973). Once collected, water samples were preserved with ~0.15 ml 100% HNO₃ to 500 ml sample (Subramanian et al., 1978) and analysed by ICP–MS (Perkin Elmer Elan 5100).

Table 1
Summary of principal features of study sites

Locality	pH	°C	Fluids	Distinguishing features
Healy's Bore 2, Tokaanu — southern end of Lake Taupo. (TK1 through TK3)	7.7	94	Alkali-chloride	Tokaanu hot spring waters are historically noted for their high mineral content. Run-off from the original bore has produced a geyser mound and an extensive terraced discharge apron
Ohaaki pool — northeastern margin of the Broadlands geothermal field (OHK)	8.3	69	Alkali-chloride, high sulphate. Pool appears milky from both high colloidal silica and sulphur content	Pool is currently filled with discharge water from the Ohaaki geothermal station. It has a large sinter apron from earlier periods of increased flow
Flash Plant 11 weirbox- Wairakei geothermal field, 8 km north of Taupo. (WKFP11)	8.2	94	Alkali-chloride	Flash plant 11 derives from several wells. White, vitreous silica scale encrusts the inner walls of the weirbox
Bore 116 weirbox- Wairakei geothermal power station (WKB116)	8.1	92	Alkali-chloride	White, vitreous silica scale encrusts the inner walls of the weirbox
Main borefield drain- Wairakei geothermal power station (WKMD)	8.1	74	Alkali chloride, plus acid-sulphate runoff	Sinter collected November 1998 was orange, with a sugary porous texture. In April 1999, a white, translucent silica deposit was observed, varying from smooth on the vertical faces of the drain to reticulated on horizontal surfaces
Eastern borefield drain- Wairakei geothermal power station (WKEB)	8.2	61	Alkali-chloride	When sampled in mid-November 1998, sinter had salmon-pink fibrous streamers that disappeared in early May 1999 but reappeared later in August 1999. The sinter has a sugary texture and is extremely friable
Waikite springs — base of Paeroa fault scarp. (WKT)	7.8	94	Bicarbonate	Waikite sinter is spongy and friable, and forms semi-circular ledges and lily pads alongside flow channels. White upper surface is noncrystalline silica, submerged underside of sinter covered with dark brown layer of calcite. The sinter contains approximately equal amounts of silica and calcite

Samples for Hg analysis were collected in glass bottles decontaminated prior to fluid collection by refluxing with fuming nitric acid. Upon reaching the sample site, a 6% potassium chromate, 98% sulphuric acid cleaning solution in the sample bottles was discarded, and the bottles were filled with geothermal fluid from the sampling site. Then 10 ml of 98% sulphuric acid was added to solubilise the mercury, followed by 10 ml of 6% potassium chromate to act as a preservative (Feldman, 1974). Samples were then analysed with ICP–MS.

2.2. *Sinter analysis*

Samples (detailed below) were collected from both naturally deposited sinters and geothermal power station sinters. In order to assess qualitatively sinter and microbial biofilm accumulation rates, pre-sterilised glass microscope slides were deployed as artificial substrates. Microscope slides provide a convenient mount for scanning electron microscopy (SEM) analysis. Bulk concentrations of trace elements were analysed semi-quantitatively using X-ray fluorescence (XRF) spectrometry (Siemens SRS 5000). Samples were dried at 40°C, finely ground, and mixed with a methylcellulose–ethanol–acetate binder (Potts, 1987). The mixture was placed in a mould and compressed into a briquette, then dried at 50°C for ~12 h.

2.2.1. *Sequential extractions*

Bulk samples were analysed to determine whether the trace metals in the sinters were exchangeable, or bound to oxides, carbonates, organics, or crystalline fractions by using a series of sequential extractions originally developed for soil analysis by Tessier et al. (1979), with modifications by Kim and Fergusson (1991). This method was chosen to reveal whether the trace metals were incorporated into inorganic phases, or whether they were associated with the organic fraction of the sinter. This technique cannot demonstrate whether the organics are biologically produced or abiotically produced organometallics.

2.2.2. *Sample preparation for SEM*

Samples for SEM, collected with implements rinsed with 70% ethanol before use, were transferred into sterile polypropylene tubes that contained a solution of 2.5% glutaraldehyde prepared by diluting a 25% stock solution with geothermal water that had been cooled to ambient temperatures and then filtered through 0.45 µm cellulose acetate filters at the sampling site. To ensure fixation of microbial cells, sinter samples were stored for a minimum of 8 h at 4°C in 2.5% glutaraldehyde. Samples for SEM were rinsed twice (5 min each) in distilled water, subjected to an ethanol dehydration series, and critical point dried to prevent volume loss (Ruffolo, 1974). They were then mounted on SEM stubs with epoxy resin or carbon adhesive and coated with iridium or platinum. The SEM specimens were either analysed with an Hitachi S-4000 FE–SEM with a Link Pentafet EDS (operating voltage of 30 kV), or a Philips XL30S FE–SEM with a Phoenix EDAX with an SUTW window (operating voltage 20 kV).

2.2.3. DNA preparation

To investigate whether DNA could be extracted from vitreous sinter, geyserite from Tokaanu was subjected to molecular analysis using a FastDNA SPIN Kit for Soil (Bio101, CA, USA). Samples for DNA analysis were collected with ethanol-cleaned implements and placed into sterile polyethylene tubes that were then over-filled with unfiltered geothermal fluid collected from the sampling site. Samples were stored at 4°C for 2 days, transferred into sterilised glass tubes, centrifuged, decanted and frozen at –22°C. The presence of bacterial DNA in the sample was investigated by polymerase chain reaction (PCR) amplification of a 200 base pair fragment of the small sub-unit 16S ribosomal RNA gene, using bacterial-specific primers. Samples with bacterial primer were placed in wells 1–4, as shown in Fig. 3F. Controls included a positive control to ensure that the amplification was working properly (wells 5 and 6), a blank of distilled water as a contamination check (well 7), and a ‘ladder’ to ensure complete sample migration (well 8). PCR products were separated by gel electrophoresis and identified under UV illumination.

3. Results

3.1. Fluids

Results for trace metal concentrations in geothermal fluids analysed by ICP–MS are shown in Table 2. All of the waters had very low dissolved oxygen levels, which is common for geothermal fluids. The silica concentrations in the fluid collected from the geothermal power developments were considerably greater than those of the natural features. Except for the Waikite site, the water samples display reasonably uniform concentrations of B, As, Hg, Sb, and Tl.

Table 2
Chemistry of geothermal waters^a

Component	TK	OHK	WKFP11	WKB116	WKMD	WKEB	WKT
Temp °C	94	69	94	92	74	61	94
pH	7.7	8.3	8.2	8.1	8.1	8.2	7.8
dO ₂	2.25	2.1	1.3	0.6	2.1	2.15	1.15
SO ₄	45	82	32	41	63	60	39
H ₂ S	b.d. ^b	0.376	0.086	0.1	b.d.	b.d.	b.d.
SiO ₂	287	407	547	560	561	400	183
Cl	1963	1341	2239	2115	1759	1437	170
HCO ₃	38	502	5	18	10	18	422
Tl	0.0037	0.0067	0.0106	0.0089	0.0076	0.0025	0.0001
Hg	0.00005	0.00014	0.00003	b.d.	0.00005	0.00012	0.00003
Sb	0.466	0.384	0.182	0.166	0.151	0.125	0.022
B	76.1	59.6	36.8	34.8	32.1	26.7	1.95
As	6.31	3.09	5.11	5.03	4.55	3.74	0.44

^a All values are in ppm.

^b b.d. = below detection limits.

3.2. Sinter

Results for trace metal concentrations in the bulk sinter, as analysed by XRF, are shown in Table 3. Except for the Waikite and the Tokaanu vent geyserite samples, the silica concentrations are all greater than 80%. The Waikite sample is a 1:1 mixture of silica and calcite as revealed by XRF (Table 3) and revealed indirectly through sequential extractions (the sample volume decreased by 50% when the carbonate fraction was dissolved). Among the trace metals, only As and Sb were found to be above the detection limits for XRF. However, total concentrations of all of the trace metals were calculated during the sequential extraction analysis. High iron concentrations were found in the Tokaanu vent geyserite, which also contained the highest arsenic concentration. The antimony concentrations are reasonably uniform for all samples.

After this study began, water draining from an acid iron-rich natural geothermal feature was diverted away from the Wairakei main borefield drain (WKMD). Consequently, two sinter samples from this site were analysed, one taken from the drain before the diversion, and one taken after the diversion. As shown by the bulk sample XRF analysis, the two WKMD samples were chemically quite different. Note that while arsenic and iron concentrations decreased, antimony concentrations increased (Table 3).

Table 3
X-ray fluorescence analyses of selected elements from sinter^a

Element	TK1	TK2	TK3	OHK	WKFP11	WKB116	WKMD before ^c	WKMD after ^c	WKEB	WKT
C%	b.d. ^b	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.	48.893
O%	0.135	b.d.	0.0282	b.d.	b.d.	0.0221	0.0606	0.0154	b.d.	13
SiO ₂ %	70.6	88.1	85.7	88.1	82.6	81.2	86.4	85.4	89	39.3
SO ₃ ppm	349	56	2070	1100	194	151	617	243	284	751
CaO%	2.35	0.407	0.524	0.262	0.477	0.625	0.297	0.427	0.509	36.4
MnO ₂ %	2.63	0.0516	0.0544	0.0039	0.0094	0.0143	0.0284	0.0141	0.0155	0.301
Fe ₂ O ₃ %	8.85	0.822	0.398	0.0759	0.0961	0.212	0.741	0.108	0.253	0.282
As ppm	1646	80	143	18	b.d.	b.d.	938	11	13	99
Sb ppm	349	160	494	245	157	211	b.d.	132	160	b.d.

^a Boron could not be detected and thallium and mercury values were below detection limits.

^b b.d. = below detection limits.

^c Separate WKMD samples were taken both before and after diversion of the Waiora stream.

Table 4
Trace metal concentrations as a ratio of silica concentrations

Ratio	TK	OHK	WKFP11	WKB116	WKMD	WKEB	WKT
B	0.00	0.01	0.00	0.00	0.01	0.00	0.11
As	0.08	0.00	0.00	0.00	0.17	0.00	0.10
Sb	0.23	0.17	0.42	0.39	0.29	0.38	0.75
Tl	0.77	0.14	0.19	0.23	0.09	0.36	4.66
Hg	3.86	0.00	1.61	0.00	0.63	0.03	0.24

3.3. Fluid-sinter ratios

To determine whether As, Sb, B, Tl and Hg were selectively concentrated in sinter, the trace metal concentrations in the fluid and sinter phases were normalised to the silica concentration of each sample. Tl in the Waikite sinter and Hg in the Tokaanu geyserite and Flash plant 11 sinter (with ratios greater than 1) were the only trace metals that were selectively concentrated in the sinter (Table 4).

3.4. Selective leaching

Results for sequential extraction analyses are shown in Tables 5–9. Arsenic (Table 5) does not show preferential association with any of the extractable fractions. Antimony is bound preferentially with the crystalline fraction of each of the sinters (Table 6). Although B is predominantly associated with the crystalline fraction, there are significant quantities associated with some of the other extracted

Table 5
Sequential extractions for arsenic in ppm^a

Location	Exchangeable	Carbonate	Oxide	Organic	Crystalline
TK1	9	33	420	39	709
TK2	2	2	17	14	4
TK3	6	15	108	53	33
OHK	7	8	5	2	4
WKFP11	3	1	2	b.d.	4
WKB116	1	1	1	1	4
WKMD	32	7	331	34	833
WKEB	4	2	7	2	8
WKT	1	34	38	b.d. ^b	11

^a Detection limit = 0.004 ppm.

^b b.d. = below detection limits.

Table 6
Sequential extractions for antimony in ppm^a

Location	Exchangeable	Carbonate	Oxide	Organic	Crystalline
TK1	1	5	7	6	250
TK2	b.d. ^b	1	2	3	144
TK3	2	8	8	5	174
OHK	b.d.	2	2	1	133
WKFP11	b.d.	b.d.	2	1	113
WKB116	b.d.	b.d.	1	1	91
WKMD	b.d.	b.d.	7	3	58
WKEB	b.d.	1	1	2	102
WKT	b.d.	b.d.	1	b.d.	34

^a Detection limit = 0.002 ppm.

^b b.d. = below detection limits.

Table 7
Sequential extractions for boron in ppm^a

Location	Exchangeable	Carbonate	Oxide	Organic	Crystalline
TK1	34	24	139	12	105
TK2	28	13	35	14	256
TK3	35	9	26	3	550
OHK	199	284	443	41	736
WKFP11	6	2	14	3	66
WKB116	3	2	15	2	51
WKMD	103	6	14	3	132
WKEB	12	10	33	4	137
WKT	13	4	14	2	445

^a Detection limit = 0.05ppm.

Table 8
Sequential extractions for thallium in ppb^a

Location	Exchangeable	Carbonate	Oxide	Organic	Crystalline
TK1	b.d. ^b	181	1007	3676	1776
TK2	b.d.	139	364	460	367
TK3	279	768	1467	902	612
OHK	71	420	551	224	842
WKFP11	b.d.	254	861	612	1275
WKB116	b.d.	287	304	908	1490
WKMD	b.d.	115	307	253	778
WKEB	b.d.	208	373	500	502
WKT	b.d.	b.d.	194	b.d.	271

^a Detection limit = 2 ppb.

^b b.d. = below detection limits.

Table 9
Sequential extractions for mercury in ppb^a

Location	Exchangeable	Carbonate	Oxide	Organic	Crystalline
TK1	10	b.d. ^b	b.d.	464	b.d.
TK2	9	b.d.	b.d.	b.d.	b.d.
TK3	b.d.	b.d.	b.d.	3529	351
OHK	b.d.	b.d.	b.d.	b.d.	b.d.
WKFP11	b.d.	b.d.	b.d.	73	b.d.
WKB116	b.d.	b.d.	b.d.	b.d.	b.d.
WKMD	b.d.	b.d.	b.d.	49	b.d.
WKEB	b.d.	9	b.d.	b.d.	b.d.
WKT	16	b.d.	b.d.	b.d.	b.d.

^a Detection limit = 1ppb.

^b b.d. = below detection limits.

fractions, especially at Ohaaki (Table 7) where it is found in anomalous concentrations in all of the fractions relative to the other sinter samples. Tl occurs variably in all fractions except the exchangeable fraction (Table 8). The highest concentrations of Tl are associated with the organic fraction in the high temperature (94°C) geyserite from Tokaanu. The only significant concentrations of Hg (Table 9) occur primarily with the organic fraction from the moderate temperature (53°C) silica oncoids at Tokaanu.

3.5. *Microbiota*

In this study we have grouped the microbial communities investigated according to their environmental niche, growth habitats described by Golubic et al. (1981) as epilithic (on upper surface), hypolithic (underneath), and endolithic (within). In the case of siliceous sinter structures that accrete away from the deposit in a direction subperpendicular to the horizon, we identified the microbial communities associated with the outermost accretionary surfaces as epilithic, and the communities associated with the undersides of sinter structures as hypolithic.

Epilithic microorganisms that grow attached to the upper- or outermost surfaces of sinters may be located either in subaerial, subaqueous, or mixed subaerial/subaqueous hydrodynamic regimes. Fig. 2A and B shows examples of the epilithic communities that colonised the permanently subaqueous geyserite from Tokaanu. Prior to collection, the Tokaanu geyserite sample was continually submerged in 94°C water with a pH of 7.7. Hyperthermophilic microorganisms that adhere to the geyserite surface, or to each other, display a similar morphotype that consists of long (> 100 µm), thin (~0.3 µm) filaments. Shown in Fig. 2C is an example of the filamentous hyperthermophiles (~0.3 µm diameter) that attached to a sterilised glass slide deployed at the effluent of Flash Plant 11 located at the Wairakei geothermal power station. The glass slide was continuously submerged in 92°C water (pH = 8.1) for 2 weeks. The epilithic community that colonises the subaqueous sinter surfaces at Flash Plant 11 and within the effluent at the Tokaanu geyser live at approximately the same temperature. However, in contrast to the Tokaanu epilithic hyperthermophilic community on the subaqueous geyserite, the hyperthermophiles that colonise Flash Plant 11 subaqueous sinter were often extensively silicified, and display a range of apparent diameters and lengths as shown in Fig. 2D.

Hypolithic microorganisms grow attached to the underside of various types of sinters and other objects that have fallen into the natural hot springs or in the drains located at the geothermal power plant. Fig. 3A–D shows an example of a hypolithic community that colonised the underside of a columnar geyserite knob from Tokaanu. Prior to collection the geyserite knob, located approximately 15 cm from the effluent, was exposed directly to periodic splashes of hydrothermal fluid ejected from the main vent (94°C). Although it was impossible to obtain direct temperature measurements of the fluid splashing on the geyserite column at the time the sample was collected, it is worth noting that we did not observe any visual evidence of a pigmented phototrophic community of microorganisms. SEM investigation revealed that the hypolithic community consists primarily of organisms characterised by two

different morphotypes. One morphotype consists of long rods $\sim 4\ \mu\text{m}$ in length and $\sim 0.75\ \mu\text{m}$ in width. The other morphotype consists of long filaments that reach up to $10\ \mu\text{m}$ or more in length and $\sim 0.25\ \mu\text{m}$ in width. The remnants of a thin, at least partially, silicified microbial biofilm matrix is shown in Fig. 3E. It is not known if the porous nature of the silicified biofilm remnant represents the distribution of the original extracellular matrix, or if it is an artifact that formed as a result of preparation of the sample for SEM analysis (e.g. it is possible that the non-silicified parts of the biofilm matrix were removed during SEM sample preparation, whereas the silicified parts remained intact). Molecular analysis of scrapings taken from the surface on the underside of the Tokaanu columnar geyserite shows the presence of bacterial DNA (Fig. 3F). Determination of the presence of bacterial DNA was achieved by adding bacterial primers to the sample and seeing whether the regions specified by the primer were amplified. In this case, amplification of the 16S rRNA gene revealed a band (Fig. 3F, wells 1–4, the brightest band) 200 base pairs long,

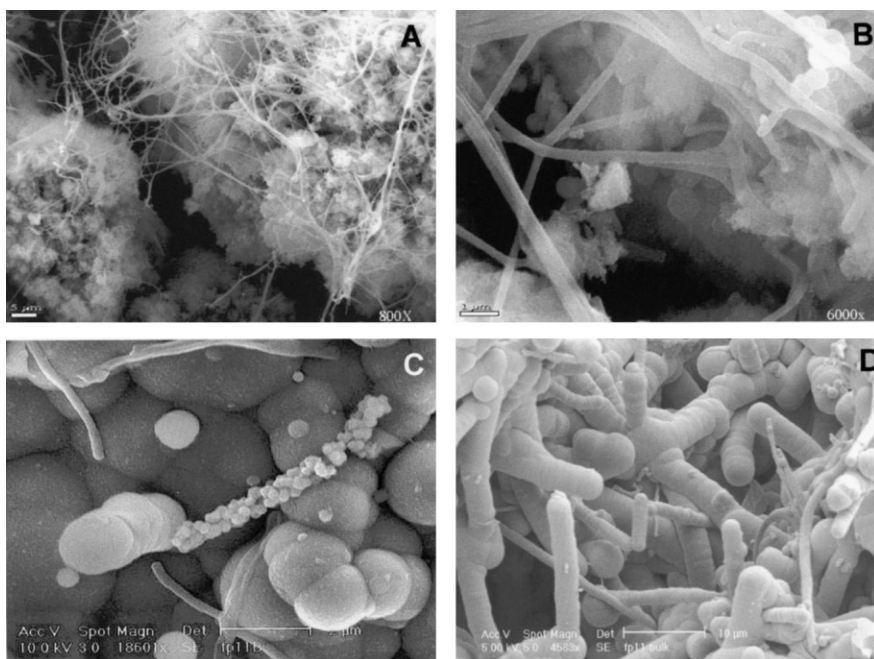


Fig. 2. A comparison of the subaqueous hyperthermophilic communities collected from the geyser at Tokaanu (A and B) and from the weirbox at Flash Plant 11 at the Wairakei geothermal power station (C and D). (A) and (B) Non-silicified filamentous hyperthermophiles that colonised permanently submerged geyserite ($T=94^{\circ}\text{C}$) collected from the geyser vent at Tokaanu. (C) Geothermal sinter that precipitated on a sterilised glass slide deployed for two weeks in the weirbox at Flash Plant 11 (94°C). Sinter colonised by viable filaments and outer remains of filaments that no longer retain any obvious intracellular contents. Another filament almost entirely entombed in silica colloids along the entire length of the filament. (D) Hyperthermophilic filaments and long rods on a permanently submerged geothermal sinter (94°C) collected from weirbox at Flash Plant 11 display various amounts of silicification, a process that alters the morphology of the silicified microbial fossils, especially their width.

typical of bacterial DNA. Endolithic phototrophic microorganisms were observed to occur within the outermost few millimetres of columnar geyserite at Tokaanu (Fig. 4A). The green and orange pigments of the phototrophic endoliths, visible in cross-sectional views of the sinter, are shown in Fig. 4A. Shown in Fig. 4B, within a few millimetres of the outermost geyserite surface, are endoliths exposed on a freshly fractured surface of the Tokaanu geyserite. Fig. 4C shows an image of endoliths in a

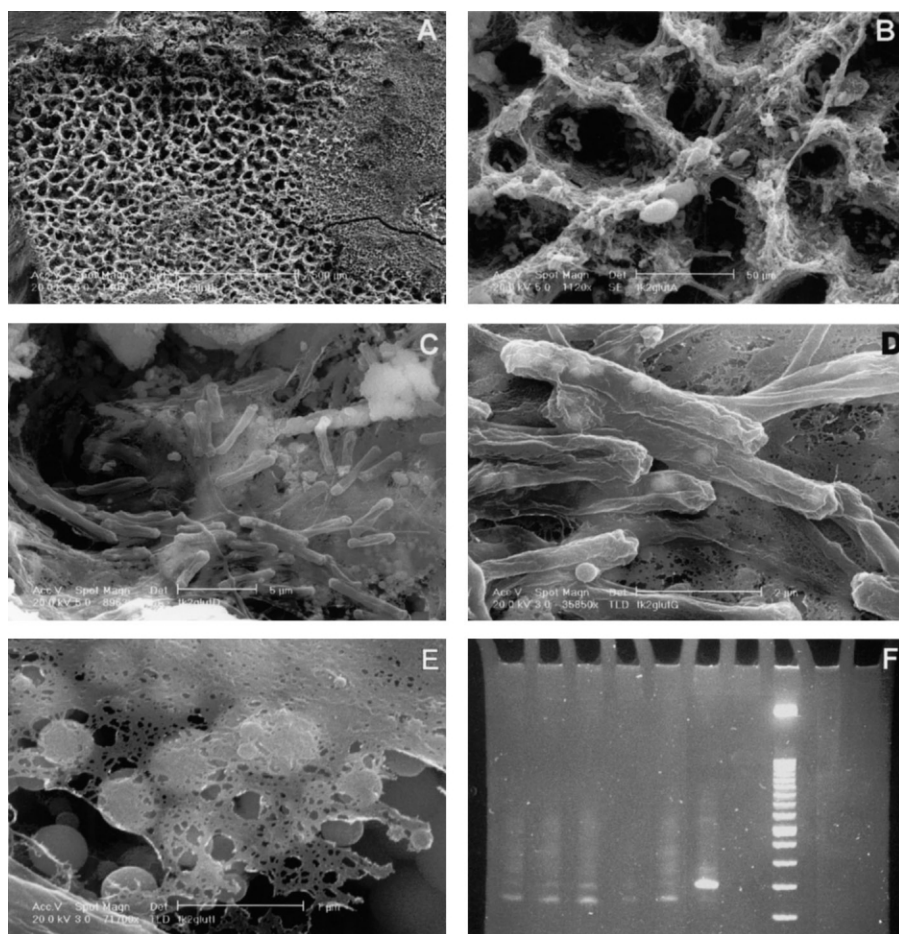


Fig. 3. Geyserite samples collected from Healy's Bore 2, Tokaanu (A–E). Underside of columnar geyserite collected within splash zone approximately 15 cm from geyser effluent at Tokaanu (94°C fluid in effluent). (A) Network dehydration texture observed in association with the underside of a geyserite knob. (B) Same area at higher magnification illustrating silicified filaments that comprise the silicified honeycomb-like network. (C) The interior of one of the cavities shown in B harbors microorganisms characterised by different morphotypes when compared to those comprising the outer edge of the network structure. (D) Early stages of silicification of short filaments presumably occur as a result of the deposition of silica colloids within the sheaths or interior of the cells. (E) Silica colloids associated with a silicified film that represents the remains of a microbial biofilm matrix. (F) Bacterial DNA extracted from the columnar geyserite collected at Tokaanu. Positions 1–4 (from left) contain sample. Positions 5–8 are controls. See text for further discussion.

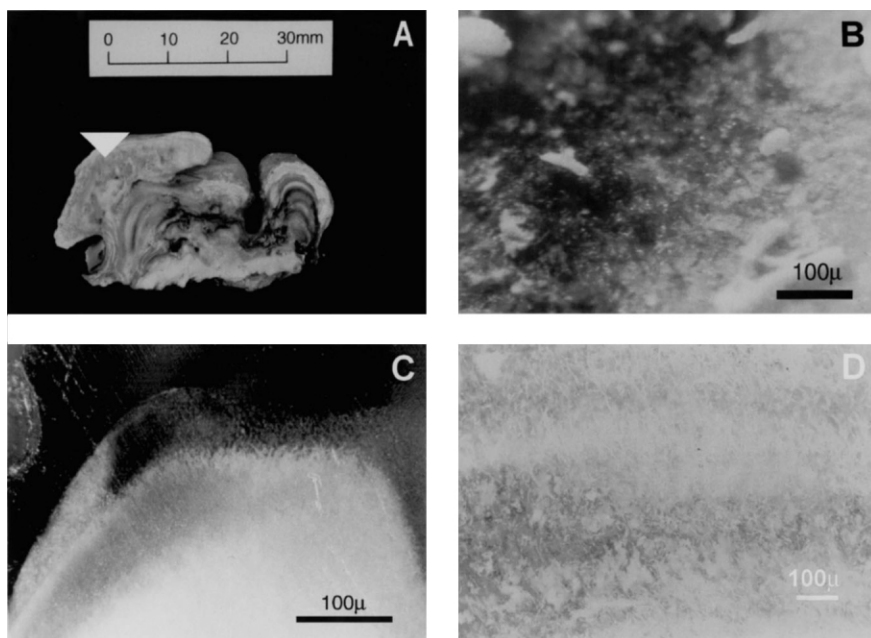


Fig. 4. Cryoendolithic zones of cyanobacteria in the marginal layers of silica that comprise the columnar geysers at Tokaanu. (A) Cross-section of geysers, showing green-pigmented zone located just below the accretionary surface. (B) Freshly broken piece of geysers that reveals green filaments in the zone below the sinter surface. (C) Discrete zones of green pigment interspersed with loosely aggregated silica. (D) Autofluorescent filaments visible in a thin section of the same sample as viewed using a fluorescence filter.

sawn cross-section of a sample as viewed through a binocular microscope. As shown in Fig. 4D, the phototrophic endolithic microbial community autofluoresces (Omega XF115 filter with excitation of $475\text{ nm} \pm 20$ and emission of $> 510\text{ nm}$).

4. Discussion

4.1. *Microbes and silica*

While hot springs have been recognised for several decades as ideal ecosystems for the preservation of thermophilic microorganisms (Walter, 1976; Schultze-Lam et al., 1995a; Cady and Farmer, 1996; Renaut et al., 1996), Cady and co-workers (Cady et al., 1995; Cady and Farmer, 1996) only recently demonstrated that the hyperthermophilic biofilms associated with geysers can also become preserved in these environments.

In this study we have found that the rapidity with which epilithic and hypolithic hyperthermophilic communities become silicified can be correlated with the concentration of dissolved silica in the hydrothermal fluids. A comparison of the

permanently subaqueous hyperthermophilic communities observed in association with the natural sinters collected from the effluent at Flash Plant 11 and in the vent at the Tokaanu geyser, and from glass slides that were deployed for two weeks at both locations, illustrate our finding.

As shown in Fig. 2, various morphotypes of hyperthermophiles can be found in the geyser vent at Tokaanu (Fig. 2A and B) and around the effluent at Flash Plant 11, Wairakei geothermal power station (Fig. 2C and D). The hyperthermophiles associated with the epilithic community on the geyserite specimen shown in Fig. 2A and B do not appear to be mineralised when viewed with the SEM — differences in the secondary electron contrast along the length of the microbes reveal overlapping, nearly electron transparent, filaments (see especially filaments located on left hand side of Fig. 2B). Evidence that this hyperthermophilic community is not extensively silicified is surmised from the presence of the remains of the biofilm matrix, which appears as a fluffy amorphous substance associated with the geyserite and interspersed amongst the filaments. The extracellular matrix of microbes can be up to 99% water, and collapses during the dehydration required for SEM imaging. It is worth noting that silica did not precipitate onto sterilised glass slides deployed for 2 weeks in the geyser effluent at Tokaanu.

In contrast to the unsilicified microbes observed from the vent at Tokaanu, the hyperthermophiles collected from a glass slide deployed for 2 weeks at the effluent for Flash Plant 11 (Fig. 2C and D) display various degrees of silicification. Fig. 2C demonstrates that the diameter of the cells, the remains of cells, and a cell almost entirely entombed in silica colloids ($< 0.5 \mu\text{m}$), are all approximately the same size ($0.3 \mu\text{m}$). Fig. 2D reveals that the hyperthermophiles associated with the sinter deposit at Flash Plant 11 can be heavily silicified, and characterised by a range of apparent diameters. Although long and short filaments can be seen in this sample, it is impossible from such an image to determine the true morphological diversity of the hyperthermophilic community members. Merging and agglomeration of silica colloids with time and continued silica deposition produced filament-type objects of several different sizes. However, size analysis of several non-silicified microbes revealed only one morphotype attached to the sinter surfaces in viable populations. Decreased silicification of microbes and the lack of sinter deposition at Tokaanu is consistent with the lower silica concentrations (Table 2) in the waters at that location (287 ppm), which are less than half that of the fluid at Flash Plant 11 at Wairakei (547 ppm). These observations illustrate that in the hyperthermophilic ecosystems characterised by fluids with supersaturated dissolved silica concentrations, the formation of heavily silicified hyperthermophilic microfossils is likely to be a common occurrence.

We have also observed that some members of the hypolithic community found on the underside of a columnar geyserite collected in the splash zone at Healy's Bore 2 at Tokaanu display the very earliest stages of a type of silicification (Fig. 3D). Inside nearly all of the cavities of the silicified honeycomb structure shown in Fig. 3B are short filaments, most of which display secondary electron contrast that indicates they are not silicified. However, as shown in Fig. 3D, one particular cavity contains

short filaments inside which have formed electron dense silica colloids ($\sim 0.25 \mu\text{m}$ diameter).

Communities of cryptoendolithic phototrophs are also commonly observed around geyser effluents. As shown in Fig. 4, a stratified cryptoendolithic community occurs in the marginal layers of silica from a columnar geyserite collected in the splash zone at Tokaanu. The cryptoendolithic community in the geyserite is superficially similar to the cryptoendolithic community of cyanobacteria that occurs as a rind in surface sandstones from the dry valleys of Antarctica (e.g. Johnston, 1989). However, one has to keep in mind that the rate of mineral deposition in the two contrasting environments is very different; the siliceous geyserite has a relatively rapid accretion rate, whereas the accretion rate of the Antarctic sandstone is likely to be orders of magnitude slower. In fact, at times the sandstone may even be weathering. Silica associated with the cryptoendoliths is most porous at its outer margin, where the secondary porosity of the silica has not yet been occluded to form a dense, impenetrable deposit as it does inside the geyserite (Fig. 4C). The phototrophic endoliths are located at similar depths in the interior of the two different types of deposits: 0–2.5 mm for the geyserite and 0.5–3 mm for the Antarctic sandstone. The Tokaanu geyserite endoliths are also similar to the Antarctic endoliths in having blue-green and black pigments. Fig. 4D shows discrete layered zones with vertically oriented filaments and zones that contain very few autofluorescing filaments. While a stratified endolithic community clearly occupies the outermost millimetres of the Tokaanu columnar geyserite, the presence of numerous, strongly pigmented (especially dark green) layers in the interior of the geyserite suggests another possible explanation for the phototrophic filaments in some of the columnar geyserite laminations. If periods of time occur when fluid flow from the geyser effluent wanes, it is likely that a phototrophic biofilm consisting of motile cyanobacteria would develop on the upper surfaces of the geyserite. Communities of viable phototrophic cyanobacteria were observed in topographic lows around the geyserite columns where splashed water accumulates and cools rapidly. At a later time, if the effluent activity increased, it is likely that the phototrophic community would rapidly become encased in silica as geyserite accretion resumed. Prior to the time the cyanobacteria pigments faded, they could be visible in thin sections of the geyserite, and give the appearance of ‘endolithic’ layers.

4.2. Sequential extractions

The comprehensive series of sequential extractions was obtained for a suite of trace elements in order to determine how the As, Sb, B, Tl, and Hg are bound in the sinter. For a given element, the sum of the trace metal concentrations released for each fraction can be used to estimate the total concentration of that element in the sinter (Table 10). This method propagates some error through each stage of the sequential extraction. To determine how the error propagation affects the accuracy of the calculated total trace metal concentrations, the results obtained by summing the concentrations obtained from each sequential extraction were compared with the concentrations determined from a single total digest of the Tokaanu vent geyserite

Table 10

Trace element total concentrations in the sinters calculated from the sum of the sequential extractions. A single total (nonsequential) digest of TK1 is also shown (ppm)

Element	Sb	As	B	Hg	Tl
TK1	269	1210	314	0.47	6.6
TK1 single digest	504	2360	458	0.3	9.2
TK2	150	39	346	0.01	1.3
TK3	198	214	622	3.88	4.0
OHK	138	26	1703	b.d. ^a	2.1
WKFP11	116	10	91	0.07	3.0
WKB116	93	7	73	b.d.	3.0
WKMD	68	1237	258	0.05	1.5
WKEB	105	24	195	0.01	1.6
WKT	35	85	479	0.02	0.5

^a b.d. = below detection limits.

sample (Table 10). The total digest yielded concentrations for As and Sb approximately double those of the sum of the sequential extractions. The concentrations for As and Sb measured by XRF (Table 3) fall between those calculated from the selective leaching totals and the single digest (Table 10), showing good agreement between the two methods for those two elements. The accuracy of the calculated total concentrations measured by sequential extraction for trace elements in concentrations too low to be measured by XRF could not be evaluated at this time. However, the trace element concentrations obtained by summing the concentrations measured at each sequential extraction provide the only determination of Hg, Tl and B, since all three elements were present in the sinter in concentrations below the detection limit of XRF analysis.

4.2.1. Arsenic and antimony

The results for As are in agreement with the data obtained by sequential extractions of geothermal sinter undertaken by Takahashi et al. (1987), which showed that As was variably dispersed among the different fractions in silica sinter. Finlayson and Webster (1989) and Swedlund (1996) have established the co-precipitation of As with silica and Fe oxides. This study confirms that As precipitated in the sinter declined from 938 to 11 ppm when precipitated Fe decreased from 7410 to 1080 ppm (see Table 3). This 'iron effect' could explain the high As concentrations in the Tokaanu vent geyserite sample. However, non-quantitative EMPA analyses (McKenzie, 2000) detected localised concentrations of sulphur/sulphides and Mn in the sinters. It is possible that an additional, as yet unidentified, mechanism may be responsible for As concentration at Tokaanu.

Sb increased from zero to 132 ppm when Fe and As decreased (Table 3). Gallup (1993) and Cappetti et al. (1995) have found that Sb preferentially precipitates with silver and sulfide. For the TK1-3 samples, there is a correlation between Sb and SO₃ (Table 3). A similar effect is also present for the Wairakei samples. We also observed an inverse relationship between As and Sb concentrations, for which we have no

ready explanation. XRD analysis of powdered sinter indicates that the primary silica phase is noncrystalline. However, submicroscopic-size silica particles would not be detected by XRD, and may account for the concentration of some trace metals. It is worth noting that microscopic Sb–As–Mg–S crystals were observed to occur in the geyserite sample from Tokaanu.

4.2.2. *Boron*

From the sequential extraction analysis it appears that the majority of the boron is bound in the crystalline fraction of the sinter. Given the tendency of boron to replace silicon and aluminium silicates (cf. Grew and Anovitz, 1996), it is possible that a considerable proportion of boron could exist within submicroscopic-size silica phases in the sinter.

4.2.3. *Mercury and thallium*

Selective extraction analyses of the seven different sinter deposits revealed that Hg and Tl are the only trace metals found to be associated primarily with the organic fraction from Tokaanu. The relative fluid-sinter concentration ratios of Tl and Hg in water samples from these localities were also confirmed to be high (Table 4). The sample from Waikite is unusual in that there is no detectable Tl associated with the carbonate or organic sites despite this sinter consisting of 50% calcite. In a separate artificial substrate experiment at Waikite, no microorganisms were found to colonise the substrate over a two week period. These observations were consistent with the absence of trace metals associated with the leachate for the organic fraction from Waikite. The organic fraction may originate from either sulphide fractions or the thermophilic biomass; however, total organic carbon comprises less than 0.5% of the sinter mass. This does not preclude the possibility of microbial involvement in the sequestering of these elements, as mass calculations do not take surface interactions into account, and microorganisms and their biofilm matrices have extremely large surface areas.

5. Conclusions

Silica concentration in geothermal fluids affects the style and rapidity with which hyperthermophilic microorganisms are silicified.

It is difficult to distinguish endolithic from epilithic microorganisms in dynamic near-vent environments because of the rapidity of silicification and likely changes in flow characteristics of the hot spring.

Although the sequential extraction method results in some loss of sample, calculated totals compare reasonably well with other methods such as XRF.

Sb will preferentially precipitate with silica when there is no Fe present, and As will not precipitate with silica unless the Fe is present. There is a positive correlation between Sb and sulfur.

Hg and Tl were preferentially associated with the organic fraction in some of the sinters studied. Some studies (e.g. Gadd, 1988; Poole and Gadd, 1989; Brierley,

1990; Ferris, 1990; Mann, 1990; Urrutia and Beveridge, 1994; Beveridge, 1995; Schultze-Lam et al., 1995b; Konhauser and Ferris, 1996) have shown that heavy metals are associated preferentially with microbes. For the hot springs investigated in this study, the preferential adsorption of heavy metals other than mercury or thallium by hyperthermophilic microorganisms was not commonly observed in high-temperature siliceous sinters.

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