TRACE METAL CHEMISTRY AND SILICIFICATION OF MICRO-ORGANISMS IN GEOTHERMAL SINTER, TAUPO VOLCANIC ZONE, NEW ZEALAND

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SUMMARY—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. 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. There was little evidence to suggest that any of the heavy metals were associated preferentially with the hyperthermophiles at the high temperature ends of the terrestrial thermal spring ecosystems studied.

1.0 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). Release of these elements from geothermal waters, steam condensate and leachate into waterways can pose an environmental risk. 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. The surfaces and extracellular components of bacteria are highly reactive and they could provide ideal sorption surfaces for the nucleation of inorganic phases, however, so far, the interactions that might occur between metals and microbiota surfaces have not been extensively studied.

2.0 SAMPLE SITES AND METHODS

The sampling sites comprised the natural hot springs at Waikite (WKT), man-made environments such as drains (WKMD, WKEB) and weirboxes (WKEP11, WKB116) at the Wairakei Geothermal Power Station and the Ohaaki Pool (OHK), and an abandoned geothermal bore at Tokaanu (TK).

2.1 Analyses

The contact fluids were analysed for As, Sb, B, Tl, and Hg by ICP-MS. In order to qualitatively assess sinter and microbial biofilm accumulation rates, pre-sterilised glass microscope slides were deployed as artificial substrates at some sites. Bulk concentrations of trace elements were analysed semi-quantitatively using X-ray fluorescence (XRF) spectrometry. Bulk samples also were analysed to determine whether the trace metals in the sinters were exchangeable, or bound to oxides, carbonates, organics, or incorporated into the crystalline fractions, by using a series of sequential extractions originally developed for soil analysis by Tessier et al. (1979), with modifications by Kim and Ferguson (1991). Biological samples for SEM were preserved in gluteraldehyde, then rinsed twice in distilled water and subjected to an ethanol dehydration series, and critical point dried to prevent volume loss (Ruffolo, 1974).

2.2 DNA preparation

To investigate whether DNA could be extracted from vitreous sinter, geyserite from Tokaanu was subjected to molecular analysis. 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, in Fig. 2F. 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.
Table 1
Chemistry of geothermal waters
(all values in ppm) (b.d. = below detection limits)

<table>
<thead>
<tr>
<th>Component</th>
<th>TK</th>
<th>OHK</th>
<th>WKFP11</th>
<th>WKB116</th>
<th>WKMD</th>
<th>WKEB</th>
<th>WKT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp °C</td>
<td>94</td>
<td>69</td>
<td>94</td>
<td>92</td>
<td>74</td>
<td>61</td>
<td>94</td>
</tr>
<tr>
<td>pH</td>
<td>7.7</td>
<td>8.3</td>
<td>8.2</td>
<td>8.1</td>
<td>8.1</td>
<td>8.2</td>
<td>7.8</td>
</tr>
<tr>
<td>dO₂</td>
<td>2.25</td>
<td>2.1</td>
<td>1.3</td>
<td>0.6</td>
<td>2.1</td>
<td>2.15</td>
<td>1.15</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>45</td>
<td>82</td>
<td>32</td>
<td>41</td>
<td>63</td>
<td>60</td>
<td>39</td>
</tr>
<tr>
<td>H₂S</td>
<td>b.d.</td>
<td>0.376</td>
<td>0.086</td>
<td>0.1</td>
<td>b.d.</td>
<td>b.d.</td>
<td>b.d.</td>
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<tr>
<td>SiO₂</td>
<td>287</td>
<td>407</td>
<td>547</td>
<td>560</td>
<td>561</td>
<td>400</td>
<td>183</td>
</tr>
<tr>
<td>Cl</td>
<td>1963</td>
<td>1341</td>
<td>2239</td>
<td>2115</td>
<td>1759</td>
<td>1437</td>
<td>170</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>38</td>
<td>502</td>
<td>5</td>
<td>18</td>
<td>10</td>
<td>18</td>
<td>422</td>
</tr>
<tr>
<td>Ti</td>
<td>0.0037</td>
<td>0.0067</td>
<td>0.0106</td>
<td>0.0089</td>
<td>0.0076</td>
<td>0.0026</td>
<td>0.0001</td>
</tr>
<tr>
<td>Hg</td>
<td>0.00005</td>
<td>0.00014</td>
<td>0.00003</td>
<td>b.d.</td>
<td>0.00005</td>
<td>0.00012</td>
<td>0.00003</td>
</tr>
<tr>
<td>Sb</td>
<td>0.466</td>
<td>0.384</td>
<td>0.182</td>
<td>0.166</td>
<td>0.151</td>
<td>0.125</td>
<td>0.022</td>
</tr>
<tr>
<td>B</td>
<td>76.1</td>
<td>59.6</td>
<td>36.8</td>
<td>34.8</td>
<td>32.1</td>
<td>26.7</td>
<td>1.95</td>
</tr>
<tr>
<td>As</td>
<td>6.31</td>
<td>3.09</td>
<td>5.11</td>
<td>5.03</td>
<td>4.55</td>
<td>3.74</td>
<td>0.44</td>
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</tbody>
</table>

30 RESULTS

3.1 Fluids

Results for trace metal concentrations in geothermal fluids analysed by ICP-MS are shown in Table 1. 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.

3.2 Sinter

Results for trace metal concentrations in the bulk sinter, as analysed by XRF, show that 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. 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 (Table 2). 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 main (WKMD). Consequently, two sinter samples from this site were analysed, one taken from the drain before, and one taken after the diversion. The XRF analysis, showed that the sample with high Fe concentrations had low As concentrations. It was also noted that when the arsenic and iron concentrations decreased, antimony concentrations increased.

3.2 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 were the only trace metals that were selectively concentrated in the sinter.

3.3 Selective leaching

The sequential extraction analyses showed that As does not exhibit preferential association with any of the extractable fractions. Sb is bound preferentially with the crystalline fraction of each of the sinters. Although B is predominantly associated with the crystalline fraction, there are significant quantities associated with some of the other extracted fractions, especially at Chaeiki, 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. 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 occur primarily with the organic fraction from the moderate temperature (53°C) silica oncoids at Tokaanu.
Table 2
Trace element total concentrations in the sinters calculated from the sum of the sequential extractions. A single total (nonsequential) digest of TKI is also shown (ppm) (b.d. = below detection limits)

<table>
<thead>
<tr>
<th>Element</th>
<th>Sb</th>
<th>As</th>
<th>B</th>
<th>Hg</th>
<th>Ti</th>
</tr>
</thead>
<tbody>
<tr>
<td>TKI</td>
<td>269</td>
<td>1210</td>
<td>314</td>
<td>0.47</td>
<td>6.6</td>
</tr>
<tr>
<td>TKI single digest</td>
<td>504</td>
<td>2360</td>
<td>458</td>
<td>0.3</td>
<td>9.2</td>
</tr>
<tr>
<td>TK2</td>
<td>150</td>
<td>39</td>
<td>346</td>
<td>0.01</td>
<td>1.3</td>
</tr>
<tr>
<td>TK3</td>
<td>198</td>
<td>214</td>
<td>622</td>
<td>3.88</td>
<td>4.0</td>
</tr>
<tr>
<td>OHK</td>
<td>138</td>
<td>26</td>
<td>1703</td>
<td>b.d.</td>
<td>2.1</td>
</tr>
<tr>
<td>WKFPI I</td>
<td>116</td>
<td>10</td>
<td>91</td>
<td>0.07</td>
<td>3.0</td>
</tr>
<tr>
<td>WKB116</td>
<td>93</td>
<td>7</td>
<td>73</td>
<td>b.d.</td>
<td>3.0</td>
</tr>
<tr>
<td>WKMD</td>
<td>68</td>
<td>1237</td>
<td>258</td>
<td>0.05</td>
<td>1.5</td>
</tr>
<tr>
<td>WKEB</td>
<td>105</td>
<td>24</td>
<td>195</td>
<td>0.01</td>
<td>1.6</td>
</tr>
<tr>
<td>WKT</td>
<td>35</td>
<td>85</td>
<td>479</td>
<td>0.02</td>
<td>0.5</td>
</tr>
</tbody>
</table>

34 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). We therefore identified the microbial communities associated with the outermost accretionary surfaces as epilithic, and the communities associated with the undersides of sinters 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. Figs. 1A and 1B show 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 (>100μm, thin (~0.3μm) filaments. Shown in Fig. 1C is an example of the filamentous hyperthermophiles (~0.3μm 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 two 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. 1D.

Figs 2A-2D show an example of a hypolithic community that colonised the underside of a columnar geyserite knob from Tokaanu. Prior to collection, the geyserite knob was exposed directly to periodic splashes of hydrothermal fluid ejected from the main vent (94°C). SEM investigation revealed that the hypolithic community consists primarily of organisms characterised by two different morphotypes. One morphotype is comprised of long rods ~4μm in length and ~0.75μm in width. The other morphotype is recognised by long filaments that reach up to 10μm or more in length and ~0.25μm in width. The remnants of a thin, at least partially silicified microbial biofilm matrix is shown in Fig. 2E. Molecular analysis of scrapings taken from the surface on the underside of the Tokaanu columnar geyserite shows the presence of bacterial DNA (Fig. 2E). Amplification of the 16S rRNA gene revealed a 200 base pairs long, typical of bacterial DNA.

Endolithic phototrophic microorganisms were also observed to occur within the outermost few millimetres of columnar geyserite at Tokaanu. Green and orange pigments of the phototrophic endoliths were visible in cross-sectional views of the sinter. Within a few millimetres of the outermost geyserite surface, we found endoliths exposed on a freshly fractured surface of the Tokaanu geyserite and this phototrophic endolithic microbial community autofluoresces.
Figure 1. SEM photomicrographs of hyperthermophilic communities collected from Tokaanu (1A and 1B), and Flash Plant 11 at Wairakei (1C and 1D).

Figure 2. Geyserite samples collected from Healy's Bore 2, Tokaanu at increasing magnification (A through E); Bacterial DNA (F) extracted from the columnar geyserite collected at Tokaanu.
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; Cady and Fanner, 1996; Renaut et al., 1995; Cady and co-workers (Cady et al., 1996), Cady and co-workers (Cady et al., 1996) only recently demonstrated that the hyperthermophilic biofilms associated with geyserite also can become preserved in these environments.

By comparing high and low silica concentration environments, 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.

As shown in Fig. 1, various morphotypes of hyperthermophiles can be found in the geyser vent at Tokaanu (Figs. 1A and 1B) and around the effluent at Flash Plant 11, Wairakei (Figs. 1C and 1D). The hyperthermophiles associated with the epilithic community on the geyserite specimen shown in Figs. 1A and 1B do not appear to be mineralised. Evidence that this hyperthermophilic community is not extensively silicified also 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. It is worth noting that silica did not precipitate onto sterilised glass slides deployed for two 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 two weeks at Flash Plant 11 (Figs. 1C-D) display various degrees of silicification. Fig. 1C demonstrates that cell diameters are approximately the same size (<0.3 µm) even though they occur as collapsed, unsilicified, or almost entirely entombed in silica colloids (<0.5 µm diameter). Fig. 1D 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 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 also have observed that some members of the hypolithic community found on the underside of a columnar geyserite collected in the splash zone at Healy's Boxes 2 at Tokaanu display the very earliest stages of a type of silicification (Fig. 2D). Inside nearly all of the cavities of the silicified honeycomb structure shown in Fig. 2B are short filaments, most of which display secondary electron contrast that indicates they are not silicified. However, as shown in Fig. 2D, one particular cavity contains short filaments inside which have formed electron dense silica colloids (~0.25 µm diameter).

Figure 3. Cryptoendolithic community in sinter from Tokaanu. A) The mow shows the position of a green pigmented zone. B) Stratified autofluorescent filaments visible in a thin section.

A stratified cryptoendolithic community occurs in the marginal layers of silica from a columnar geyserite collected in the splash zone at Tokaanu (Fig 3). This community is superficially similar to the cryptoendolithic community of cyanobacteria that occur as a rind in surface sandstones from the dry valleys of Antarctica (e.g., Johnston, 1989). Silica associated with the geothermal cryptoendolith 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. The phototrophic endoliths are located at similar depths in the interior of the two different types of deposits: 0.2-2.5 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. 3B 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 could develop on the upper surfaces of the geyserite. Communities of viable phototrophic cyanobacteria were observed in topographic lows around the geyserite columns where splash 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.

42 Sequential extractions

The series of sequential extractions was obtained for the suite of trace elements in order to determine how the As, Sb, B, Tl, and Hg are chemically bound in the sinter. 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 XRD analysis.

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 Wedlund (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 ppm to 11 ppm when precipitated Fe decreased from 7410 ppm to 1080 ppm. 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. It is not known why there is an inverse relationship between As and Sb concentrations. 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.

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 also were confirmed to be high. 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.

50 CONCLUSIONS

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

- Sb will preferentially precipitate with silica (although the mechanism is unknown) when there is no Fe present, and As will not significantly precipitate with silica unless the Fe is present.

- Hg and Tl were preferentially associated with the organic horizon in some of the sintered studied. Some studies (e.g., Gadd, 1988; Poole and Gadd, 1989; Brierley, 1990; Ferris, 1990; Mann, 1990; Urrutia and Beveridge, 1994; Beveridge, 1995; Schultz-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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7.0 REFERENCES


