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Poços de Caldas Report No. 10

Microbiological analysis at the Osamu Utsumi mine and Morro do Ferro analogue study sites, Poços de Caldas, Brazil

An international project with the participation of Brazil, Sweden (SKB), Switzerland (NAGRA), United Kingdom (UK DOE) and USA (US DOE). The project is managed by SKB, Swedish Nuclear Fuel and Waste Management Co.

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Microbiological analysis at the Osamu Utsumi mine and Morro do Ferro analogue study sites, Poços de Caldas, Brazil.

J.M. WEST[1], A. VIALTA[2] * and I.G. McKINLEY[3].

[1]British Geological Survey, Keyworth, Nottingham, NG12 5GG, (United Kingdom).

[2]Urânio do Brasil, Caixa Postal 913, Poços de Caldas, MG, 37700 (Brazil).

[3]Nationale Genossenschaft für die Lagerung radioaktiver Abfälle (NAGRA), Parkstrasse 23, CH-5401 Baden (Switzerland).

Abstract

The Poços de Caldas project is a wide-ranging natural analogue study focussed on a number of areas of concern in the performance assessment of the disposal of radioactive waste. Part of the work has involved characterising microbial populations and their influence in various processes. Core material and groundwaters have been sampled for microbiological content at various depths from boreholes at the Osamu Utsumi open pit uranium mine and Morro do Ferro Th/REE ore body. Microbes were found in all samples but numbers do not appear to be related to depth. Analyses of groundwaters gave higher numbers than with solid material and demonstrated the presence of sulphur cycle bacteria.

These observations have been compared with predictions of a model used in performance assessment to calculate the maximum biomass/microbial activity based on constraints set by available nutrients and energy. The main conclusions of this analysis are:

i) Low microbial activities can be supported by the energy and nutrients supplied by alteration processes at or around the redox front. The maximum annual production of ≈0.01 – 0.1 g biomass (dry)/m² of redox front is in reasonable agreement with observed standing populations.

ii) *The presence of high concentrations of sulphate reducing bacteria around the redox front indicate a complex sulphur geochemistry which may be predominantly microbially catalysed and could explain the nodular form of pitchblende concretions and the presence of secondary pyrite.*

iii) *There is little trace element mobilisation by organic byproducts and the main role of microbes in this system is to catalyse specific redox reactions.*

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*Now at Companhia de Desenvolvimento Technologico, Caixa Postal 6041, Campinas, SP. 13081 (Brazil).

Zusammenfassung

Das Poços de Caldas Projekt ist eine umfangreiche natürliche Analogstudie, die sich mit der Sicherheitsanalyse der Endlagerung radioaktiven Abfalls befasst. Ein Teil der Arbeit beschäftigt sich mit der Charakterisierung von mikrobiologischen Populationen und deren Einfluss auf verschiedene Vorgänge. Von unterschiedlichen Tiefen der Bohrlöcher beider Gelände wurden Grundgestein und Grundwasser auf deren mikrobiologischen Gehalt hin untersucht. In allen Fällen wurden Mikroben gefunden, aber ihr Vorkommen scheint nicht in Relation zur Tiefe zu stehen. Analysen des Grundwassers ergaben grössere Vorkommen als im Gestein und wiesen auf das Vorhandensein von Bakterien des Schwefelzyklus hin.

Diese Beobachtungen wurden mit Vorhersagen von Sicherheitsanalysemodellen verglichen, um die maximale Biomasse/Mikroben-Aktivität in Abhängigkeit von den vorhandenen Nährstoffen und Energiequellen zu berechnen. Die wichtigsten Schlüsse aus dieser Untersuchung sind:

i) Geringe mikrobiologische Aktivitäten können durch Energie und Nährstoffe unterstützt werden, die durch Prozesse in oder in der Umgebung der Redoxfront entstehen. Die maximale jährliche Produktion von ~0.01 - 0.1 g Biomasse (trocken)/m^2 der Redoxfront stimmt mit den Beobachtungen an bestehenden Populationen überein.

ii) Das Vorhandensein von hohen Konzentrationen an sulphat-reduzierenden Bakterien um die Redoxfront, deutet auf eine komplexe Schwefelgeochemie hin, die vorwiegend mikrobiologisch katalysiert zu werden scheint, und die teilweise die nodulare Form der Pechblenden Konkretionen und die Anwesenheit von sekundärem Pyrit erklären könnte.

iii) Es erfolgt nur eine geringe Mobilisation von Spurenelementen durch organische Nebenprodukte. Die Hauptaufgabe der Mikroben in diesem System ist es, die spezifischen Redoxreaktionen zu katalysieren.

Résumé

Le projet Poços de Caldas représente une vaste étude d'analogies naturelles visant divers domaines d'intérêt pour l'évaluation des capacités d'un dépôt final pour déchets radioactifs. Une partie des travaux a consisté dans la caractérisation des populations microbiennes et l'étude de leur influence sur divers processus. Des carottes et des eaux souterraines ont été prélevées à diverses profondeurs dans des forages réalisés et dans la mine d'uranium à ciel ouvert d'Osamu Utsumi et dans le gisement de Th/terres rares de Morro do Ferro pour l'étude de la teneur en éléments microbiologiques. Des microbes ont été observés dans tous les échantillons mais leur nombre ne semble pas être lié à la profondeur. Les analyses d'eaux souterraines ont livré des populations plus grandes que les échantillons solides et ont mis en évidence la présence de bactéries du cycle du soufre.

Ces observations ont été comparées avec les pronostics livrés par un modèle utilisé pour l'évaluation des performances le modèle permet de calculer l'activité maximale de la biomasse/microbienne maximale compte tenu des limites imposées par la disponibilité d'éléments nutritifs et d'énergie. Les principales conclusions de cette analyses sont:

i) Une faible activité microbienne peut être entretenue par l'apport d'énergie et d'éléments nutritifs fournis par les processus d'altération sur et aux alentours du front rédox. La production annuelle maximale de ~0.01-0.1 g de biomasse (sèche)/m^2 de front rédox se corrèle de manière satisfaisante avec les populations observées.

ii) La présence d'une forte concentration de bactéries réductrices de sulfates aux alentours du front rédox indique une géochimie complexe du soufre qui est peut-être essentiellement catalysée par des microbes et pourrait expliquer la forme nodulaire de concrétions de pechblende et la présence de pyrite secondaire.

iii) Il y a peu de mobilisation d'éléments traces par des sous-produits organiques et le rôle principal des microbes dans ce système est de catalyser certaines réactions rédox spécifiques.

Preface

The Poços de Caldas Project was designed to study processes occurring in a natural environment which contains many features of relevance for the safety assessment of radioactive waste disposal. The study area, in the State of Minas Gerais, Brazil, is a region of high natural radioactivity associated with volcanic rocks, geothermal springs and uranium ore deposits. It contains two sites of particular interest on which the project work was focussed: the Osamu Utsumi uranium mine and the Morro do Ferro thorium/rare-earth ore body. The first site is notable in particular for the prominent redox fronts contained in the rock, while Morro do Ferro was already well-known as one of the most naturally radioactive locations on the surface of the Earth, owing to the high thorium ore grade and the shallow, localised nature of the deposit.

The features displayed by these two sites presented the opportunity to study a number of issues of concern in repository performance assessment. The four objectives set after the first-year feasibility study were:

1. Testing of equilibrium thermodynamic codes and their associated databases used to evaluate rock/water interactions and solubility/speciation of elements.
2. Determining interactions of natural groundwater colloids with radionuclides and mineral surfaces, with emphasis on their role in radionuclide transport processes.
3. Producing a model of the evolution and movement of redox fronts, with the additional aim of understanding long-term, large-scale movements of trace elements and rare-earths over the front (including, if possible, natural Pu and Tc).
4. Modelling migration of rare-earths (REE) and U-Th series radionuclides during hydrothermal activity similar to that anticipated in the very near-field of some spent-fuel repositories.

The project ran for three and a half years from June 1986 until December 1989 under the joint sponsorship of SKB (Sweden), NAGRA (Switzerland), the Department of the Environment (UK) and the Department of Energy (USA), with considerable support from a number of organisations in Brazil, notably Nuclebrás (now Urânio do Brasil). The first-year feasibility study was followed by two and a half years of data collection and interpretation, focussed on the four objectives above.

This report is one of a series of 15, summarising the technical aspects of the work and presenting the background data. A complete list of reports is given below. Those in series A present data and interpretations of the sites, while those in series B present the results of modelling the data with performance assessment objectives in mind. The main findings of the project are presented in a separate summary (no. 15).

This report describes both the findings of the microbiological analytical programme and modelling work performed to define the importance and significance of the field observations.

Poços de Caldas Project Report Series

Series A: Data, Descriptive, Interpretation

Report No.	Topic	Authors (Lead in Capitals)
1.	The regional geology, mineralogy and geochemistry of the Poços de Caldas alkaline caldera complex, Minas Gerais, Brazil.	SCHORSCHER, Shea.
2.	Mineralogy, petrology and geochemistry of the Poços de Caldas analogue study sites, Minas Gerais, Brazil. I: Osamu Utsumi uranium mine.	WABER, Schorscher, Peters.
3.	Mineralogy, petrology and geochemistry of the Poços de Caldas analogue study sites, Minas Gerais, Brazil. II: Morro do Ferro.	WABER.
4.	Isotopic geochemical characterization of selected nepheline syenites and phonolites from the Poços de Caldas alkaline complex, Minas Gerais, Brazil.	SHEA.
5.	Geomorphological and hydrogeological features of the Poços de Caldas caldera and the Osamu Utsumi mine and Morro do Ferro analogue study sites, Brazil.	HOLMES, Pitty, Noy.
6.	Chemical and isotopic composition of groundwaters and their seasonal variability at the Osamu Utsumi and Morro do Ferro analogue study sites, Poços de Caldas, Brazil.	NORDSTROM, Smellie, Wolf.
7.	Natural radionuclide and stable element studies of rock samples from the Osamu Utsumi mine and Morro do Ferro analogue study sites, Poços de Caldas, Brazil.	MacKENZIE, Scott, Linsalata, Miekeley, Osmond, Curtis.
8.	Natural series radionuclide and rare-earth element geochemistry of waters from the Osamu Utsumi mine and Morro do Ferro analogue study sites, Poços de Caldas, Brazil.	MIEKELEY, Coutinho de Jesus, Porto da Silveira, Linsalata, Morse, Osmond.

Contents

1. Introduction

1.1. Microbiological studies in the Poços de Caldas project

The Poços de Caldas project is a wide-ranging natural analogue study of a number of areas of concern in the performance assessment of the disposal of radioactive waste. The background of the project is described by Smellie *et al.* (1987; 1989) and has consisted of three years of data gathering and analysis prior to detailed interpretation. Work has concentrated on two main sites: the Osamu Utsumi uranium mine (open pit) and the Morro do Ferro Th/REE ore body, both situated in the Poços de Caldas caldera in Minas Gerais, Brazil.

The microbiology component of the work forms part of objectives 1 and 2 as listed in the Preface and has been concerned with:

1. Determining the presence and activity of microbes known to be important in the uranium biogeochemical cycle (Zajic, 1969);
2. Defining their importance in the movement of uranium across the redox front;
3. Comparing field observations with the predictions of a microbiology model used to predict maximum biomass/microbial activity in repository safety assessment.

1.2. Microbiology in a nuclear waste disposal context

Various studies have shown that microbial contamination of nuclear waste repositories is inevitable and cannot be precluded from even a very deep repository for high-level waste (West *et al.*, 1982; West and McKinley, 1985).

The environment of such a repository is likely to be characterised by limited sources of available energy and nutrients (oligotrophic) which would constrain the maximum possible activity. A modelling methodology has been developed to predict such maximum activity levels based on nutrient and energy mass balances (McKinley *et al.*, 1985; Grogan and McKinley, 1990). Based on the activity present, the consequences of microbial growth for repository performance can be estimated.

The aims of this study are to test some aspects of the microbiology model:

1) The assumption that microbes can utilise chemical energy produced at a deep-lying redox front (assumed energy source in some repository conditions).

ii) To compare the microbial activity levels predicted by the model against field observations.

iii) To evaluate the direct consequences of such microbial activity on the geochemistry of the redox front, particularly with regard to the mobilisation, transport and retention of trace elements.

2. Sampling and measurement of microbial populations

2.1. Methods

These are described in detail in Appendix 1. Briefly, core material and groundwater from various sites and depths (Waber *et al.*; Waber; Nordstrom *et al.*, this report series; Reps. 2, 3 and 6) were sampled and analysed for:

1. Total heterotrophs (aerobic and anaerobic) using CPS medium (Collins, 1963);
2. Sulphate reducing bacteria (SRB) using Medium B (Postgate, 1984);
3. Sulphur oxidisers using the media of Silverman and Lundgren (1959) and enrichment media (Gerhardt *et al.*, 1981);
4. Total microorganisms using epifluorescence microscopy (West and Arme, 1985);
5. Iron oxidisers using the methods of Gerhardt *et al.* (1981) (for *Gallionella*) and Grainge and Lund (1969) (total iron bacteria). These techniques were used only in 1988.

This work required development of techniques for subsampling core taken from depth and for extracting 'uncontaminated' groundwater from depth. These techniques are detailed in West *et al.* (1988) and are summarised in Appendix 1.

2.2. Results

Six of the nine reference borehole cores and fourteen water sources (including all reference boreholes) were sampled between September 1986 and December 1988. The results are summarised in Tables I to VI and Appendix 2.

TABLE I

Borehole F1 at the Osamu Utsumi uranium mine sampled late 1986. CFU counts are maximum values (2 replicates) rounded to the nearest 10. Epifluorescence microscopy counts are the mean of 10 fields of view.

Code	Depth (m)	Heterotrophs (max CFU/g)		Epifluorescence microscopy (Nos/g)
		Aerobic	Anaerobic	
1–1A	0.34 – 0.45	100	50	390
1–1B	0.55 – 0.65	30	-	0
6–1A	5.91 – 6.01	30	-	100
10–1A	9.78 – 9.9	-	30	530
15–1A	14.97 – 15.17	30	-	20
20–1B	19.6 – 19.74	-	25	630
26–1A	25.2 – 25.37	-	-	Lost
31–1A	30.12 – 30.28	-	-	Lost
34–1C	33.88 – 34.04	-	-	10
41–1A	39.97 – 40.12	-	-	0
51–1A	50.51 – 50.63	-	10	Lost
60–1A	59.73 – 59.91	-	-	120
75–1A	74.17 – 74.3	-	-	Lost
91–1A	90.74 – 90.84	-	-	350

No sulphate reducing bacteria or sulphur oxidisers

- = No growth observed. CFU = Colony forming units.

Tables I and II give solid material data from two boreholes at the Osamu Utsumi uranium mine sampled in summer 86/87. Table I (Borehole F1) demonstrates microbial presence (heterotrophs) to a depth of 20 m, although there are indications that there may be some heterotrophs present to a depth of 90 m. However, epifluorescence microscopy shows much higher counts and to a depth of 90.8 m. No sulphur bacteria were found in any of the core samples. Groundwater taken from 95.5–126 m depth (Appendix 2) from 1986 to June 1988 suggests that more microbes are present in the groundwater than in the solid material, which is unusual as microbes generally sorb onto solid surfaces (Savage and Fletcher, 1985). It is likely that the preparation of the rock material for analysis failed to release the microbes from the rock, thus preventing their accurate assay.

TABLE II

Borehole F2 at the Osamu Utsumi uranium mine sampled early 1987. CFU counts are maximum values (2 replicates) rounded to the nearest 10. Epifluorescence microscopy counts are the mean of 10 fields of view.

Code	Depth (m)	Heterotrophs (max CFU/g) Aerobic	Anaerobic	Epifluorescence microscopy (Nos/g)
1A	0.8 – 0.93	-	-	880
5A	5.43 – 5.56	-	-	2390
10A	10.89 – 11.03	-	-	2960
16A	16.57 – 16.74	-	-	1740
20A	20.63 – 20.76	-	-	160
25A	25.74 – 25.87	-	-	280
31A	21.2 – 31.34	-	-	1370
40A	40.42 – 40.56	-	-	510

No sulphate reducing bacteria or sulphur oxidisers.

– = No growth observed. CFU = Colony forming units.

Table II (Borehole F2) shows that all culturing techniques gave negative results but microscopy (Table I) reveals quite large numbers of bacteria (up to ~2000 CFU (colony forming units)/g). It is usual to obtain higher counts from epifluorescence microscopy than from plate counts. However, these counts fluctuate with depth and it is difficult to define whether the numbers are a result of contamination (which should be mirrored to some extent in the culturing techniques) or whether they are an accurate representation of microbial presence. Groundwater samples were taken intermittently from May 1987 at a depth of 45–60 m (Appendix 2). High numbers of aerobic heterotrophs are revealed, but anaerobes are also present, including sulphate reducing bacteria. Heterotrophic numbers increase with time, indicating surface contamination. Large numbers of microbes were also counted using epifluorescence microscopy.

TABLE III

Borehole F3 at the Osamu Utsumi mine sampled in September 1987. CFU counts are maximum values (2 replicates) rounded to the nearest 10. Epifluorescence microscopy counts are the mean of 10 fields of view.

Code	Depth (m)	Heterotrophs (max CFU/g) Aerobic	Anaerobic	Epifluorescence microscopy (Nos/g)
01	4.93 – 5.02	-	-	200
02	9.9 – 10.03	-	140	200
03	14.54 – 14.68	-	70	100
04	19.70 – 19.86	4560	70	52700
05	25.18 – 25.31	2130	50	30000
06	29.67 – 29.77	140	2160	23200
07	39.97 – 40.11	20	70	28500
08	50.19 – 50.29	50	20	100
09	60.34 – 60.46	70	-	2200
10	74.82 – 74.92	70	-	50

No sulphate reducing bacteria or sulphur oxidisers.

- = No growth observed. CFU = Colony forming units.

TABLE IV

Borehole F4 at the Osamu Utsumi mine sampled in September 1987. CFU counts are maximum values (2 replicates) rounded to the nearest 10. Epifluorescence microscopy counts are the mean of 10 fields of view.

Code	Depth (m)	Heterotrophs (max CFU/g) Aerobic	Anaerobic	Epifluorescence microscopy (Nos/g)
01	5.36 – 5.47	10150	-	2880
02	10.05 – 10.17	1870	-	30400
03	14.54 – 14.65	216	-	14500
04	20.02 – 20.14	20	140	74200
05	24.88 – 25.10	70	3600	2300
06	30.05 – 30.18	20	20	69500
07	40.00 – 40.10	4510	4610	3200
08	47.94 – 50.04	860	-	482000
09	60.00 – 60.09	140	-	11800
10	80.09 – 80.20	100	-	38700
11	100.22 – 100.33	20	-	62600

No sulphate reducing bacteria or sulphur oxidisers.

- = No growth observed. CFU = Colony forming units.

Boreholes F3 and F4 at the mine were sampled in September 1987 (Tables III and IV) and give similar results to those from F1 and F2. Again, there are no sulphur bacteria but heterotrophs are detected. In F3 the highest numbers (by epifluorescence microscopy) are between 19.7 m and 40.11 m. No peaking of numbers occurs in F4, although anaerobes predominate between 20.02 m and 40.1 m. Groundwater sampling (Appendix 2) of F3 (0–75 m) and F4 (0–100 m) started in June 1988 with high numbers of heterotrophs present particularly in F4, probably as a result of surface contamination of the borehole. Again sulphur bacteria were found in both borehole waters.

TABLE V

Borehole MF10 at Morro do Ferro sampled in late 1986. CFU counts are maximum values (2 replicates) rounded to the nearest 10. Epifluorescence microscopy counts are the mean of 10 fields of view.

Code	Depth (m)	Heterotrophs (max CFU/g) Aerobic	Anaerobic	Epifluorescence microscopy (Nos/g)
1A	0.22 – 0.29	90	-	400
5A	5.59 – 5.68	30	-	-
9A	9.37 – 9.5	60	60	80
14A	14.35 – 14.51	-	-	410
20A	20.15 – 20.28	-	-	0
24A	24.77 – 24.86	30	30	Lost
29A	29.4 – 29.49	-	-	31
39A	39.25 – 39.98	-	20	1450
46A	46.87 – 46.96	-	-	0
61A	61.09 – 61.22	-	-	0

No sulphate reducing bacteria or sulphur oxidisers

- = No growth observed. CFU = Colony forming units.

Tables V and VI give the results for solid material from boreholes at Morro do Ferro. Borehole MF10 (Table V) sampled in late 1986 shows microbial presence to a depth of ~40 m although no sulphate reducing bacteria or sulphur oxidising bacteria were isolated. Again, microscopy gives higher counts. Aerobes were found to a depth of 9.5 m and anaerobes from 9.5 to ~40 m. Borehole MF12 (Table VI) sampled in May 1987 shows high aerobic heterotroph counts of up to ~4000 CFU (colony forming

TABLE VI

Borehole MF12 at Morro do Ferro sampled in May 1987. CFU counts are maximum values (2 replicates) rounded to the nearest 10. Epifluorescence microscopy counts are the mean of 10 fields of view.

Code	Depth (m)	Heterotrophs (max CFU/g) Aerobic	Anaerobic	Epifluorescence microscopy (Nos/g)
01	5.47 – 5.59	60	-	0
02	10.25 – 10.4	50	20	0
03	15.87 – 16.04	35	40	40
04	22.97 – 23.12	20	-	20
05	27.32 – 27.43	230	-	300
06	31.99 – 32.09	4300	-	280
07	40.76 – 40.94	140	-	30
08	51.32 – 51.5	4100	140	100
09	61.45 – 61.61	3300	-	760
10	70.02 – 70.16	1650	200	Lost

No sulphate reducing bacteria or sulphur oxidisers.

Mud used for drilling this borehole contained $1.5x10^4$ CFU/g aerobic and $1.2x10^3$ CFU/g anaerobic heterotrophs.

- = No growth observed. CFU = Colony forming units.

units)/g to a depth of 70 m. Much lower anaerobic heterotroph and microscopy counts were obtained. No sulphur bacteria were detected. These results probably indicate contamination during drilling. Microbial analysis of the mud used in the drilling gave aerobic heterotroph counts of $1.5x10^4$ CFU/g. Groundwaters from MF10 and MF12 were sampled at intervals from September 1987 together with water from MF11. All showed microbial content, but sulphur oxidisers were present only intermittently.

Other water sources were also sampled, including one further borehole at the Osamu Utsumi mine (Pilot hole), shallow holes (SW01; SW02; SW03), a shallow shaft and a piezometer also at the mine. All contained aerobic heterotrophs with few anaerobes. Sulphur cycle bacteria were usually present.

No iron oxidisers were detected in any solid or liquid sample using current methods.

2.3. Summary

Microbes were found in all solid and groundwater samples tested from the Osamu Utsumi mine and Morro do Ferro but their numbers do not appear to be related to depth. In general, epifluorescence microscopy numbers were higher than the plate counts and the former should be taken as a more accurate indication of numbers/ml present. Analyses of groundwaters gave higher numbers (particularly of aerobic heterotrophs) than solid material and demonstrated the presence of sulphur cycle bacteria. No iron oxidisers were detected in any samples. It is likely that the preparation of the rock material for analysis failed to release the microbes from the rock and thus they were not detected.

These quantitative results should not be viewed as definitive as they give no indication of microbial activity *in situ*, but merely demonstrate that certain microbes will grow when inoculated onto the given media. Results from epifluorescence microscopy are more reliable as they are based on direct counting of microbial cells. However, the results do show that microbes are present in every sample taken from the sites and that sulphur cycle bacteria can be detected.

3. Modelling and interpretation

3.1. The conceptual model

In a closed system, a microbial population will grow at a rate limited by either the availability of energy sources, the availability of the elements required to form the cells or the build-up of metabolic poisons. In a deep geological system with relatively low microbial populations, the last constraint (metabolic poisons) is unlikely to be important and the level of microbial activity will be limited by either the supply of chemical energy or essential elements (predominantly C, N, P and S).

The supply rates of energy and nutrient elements can be evaluated by mass balance calculations – in the former case considering all energy production reactions as redox couples and evaluating the supply of potential oxidants and reductants. In order to translate energies into microbial activity, a simple conversion factor of 65 kJ/g (dry weight) of biomass is used (McKinley *et al.*, 1985). This value is very uncertain as it includes global estimates for the efficiency of microbial utilisation of chemical energy (which would vary for different redox couples) and the energy requirement for

maintenance of the cell (which, to our knowledge, has never been studied for the very slowly growing organisms found in relevant oligotrophic environments).

The conversion of nutrient element supply to biomass utilised the average composition of a microbe from Stanier *et al.* (1977):

$C_{160}\,H_{280}\,O_{80}\,N_{30}\,P_2\,S$

Although various alternative formulations exist, their differences are relatively small. More significant, however, is the implicit assumption that such elements can be utilised with 100% efficiency regardless of their speciation in the groundwater. In cases where biomass is increasing, such a calculation would, certainly, give rise to maximum biomass estimates. In a fairly static system which reaches a steady state, nutrients may be recycled, with new microbes utilising the decay products of previous populations. Again there are no data on efficiencies of nutrient recycling in relevant ecosystems but, for scoping calculations, a value of $\approx$90% might be reasonable.

The conversion from biomass (in g (dry weight)) to numbers of organisms is made assuming an average of 1.5×10^{-15} g/organisms (dry) (McKinley *et al.*, 1985).

The model is described in more detail by Grogan and McKinley (1990).

3.2. Supply of energy and nutrients at the Osamu Utsumi study site

In terms of redox reactions, these are clearly focussed at the marked redox fronts in the mine. The main reaction at these fronts is the oxidation of pyrite to form Fe^{III} oxyhydroxides. The complete oxidation of pyrite can be written as:

$$Fe\,S_2 + 3.5\,H_2O + 7.5\,O_2 \rightarrow Fe(OH)_{3(am)} + 4\,H^+ + 2\,SO_4^{2-} \qquad (1)$$

with ΔG_r° = -1358 kJ/mole FeS_2 at pH 7. As an acid-producing reaction, the free energy change involved is strongly pH-dependent. Various studies of the redox front movement give rates $\approx$1–50 m/10^6 years and a value of 10 m/10^6 years will be taken for further calculations. The pyrite content in the reduced rock is $\approx$2%, thus giving a pyrite oxidation rate of 3.4×10^{-3} moles/year for each m^2 of redox front. For reaction (1) above, this rate would correspond to an energy supply of 4.6 kJ/year or an annual biomass production of 7×10^{-2} g (dry weight)/m^2. Expressed as a standing population assuming a 1 year turnover time, this would correspond to 4.7×10^{13} organisms.

The long-term nutrient balance is somewhat harder to estimate, as sources from both rock weathering and infiltrating groundwater have to be taken into account. The rock itself has rather significant quantities of P, C and S – ranges of 0.2 – 14‰, 0.6 – 4‰ and 13 – 23‰ respectively in the hydrothermally altered phonolite. Unfortunately, however, there are no nitrogen measurements from the rock. Assuming the ranges above result from low-temperature alteration around the redox front, the supply rates of these elements for a 10 m/10^6 y front movement rate could be ≈0.35, 0.09 and 0.58 g/m^2 redox front per year for P, C and S respectively (using the extreme values of the ranges given and assuming a rock density of 2.5 Mg m^{-3}).

The supply from groundwater cannot be directly estimated from current measurements as both the water flux and the water chemistry have been changed considerably by the presence of the mine and would, in any case, vary with time due to climate, topography and land use. Very simplistically, an average water flux can be calculated from assumed (guessed) permeability (5x10^{-7} m/sec) and hydraulic gradient (0.02) values to be ≈0.3 m^3/year through 1 m^2 of front. For scoping calculations, C and S in infiltrating (soil) water are both taken to be ≈2 mg/l and N and P to be ≈0.05 Mg/l. Combining these figures gives supply rates for C and S of 0.6 g/year and for N and P of 0.015 g/year. Comparison of these data with the rock supply rates above indicate that they are of comparable orders of magnitude.

In terms of the elemental composition of an average organism (cf. previous section), P and S are plentiful relative to C and N. Assuming equal efficiency of uptake, N would be the limiting element with a supply rate of ≈10^{-3} moles/year to the reference m^2 of front. Converted to biomass, this would correspond to ≈0.1 g (dry). This value is about 50% higher than the limit set by energetics but, given the uncertainties involved, this difference should not be over-interpreted.

Taking the numbers above at face value, the model would predict that the maximum biomass supported in the rock column around the redox front would be ≈10^{13} organisms/m^3. This value is directly dependent on the redox front migration rate assumed but, in any case, would have an associated uncertainty of at least an order of magnitude.

Due to the complex 3D structure of the redox fronts at the Osamu Utsumi mine, it is not easy to clearly define microbial populations associated with a particular redox front. For the sake of calculation, we assume an average of 10^5 organisms/g of rock and 10^3 organisms/ml of water (~5–10% porosity). A region extending 1 m on either side of the redox front would thus contain ≈5x10^{11} organisms. Given the great uncertainties in both model and measurements, this could be considered to be a

reasonable agreement and to justify using the model data to examine the consequences of such activity on redox front processes.

3.3. Possible significance of microbial activity at the redox front

In principle, we have to explain the following observations (McKinley, this report series; Rep. 12):

i) On the oxidising side of the redox front, pyrite is oxidised to iron oxyhydroxide and pitchblende nodules are dissolved.

ii) On the reducing side of the front, pitchblende nodules form which are associated with secondary pyrite. The sulphur isotope analysis indicates that the pyrite formation is biologically catalysed.

iii) In the transition from oxidising to reducing zones, the groundwater pH stays relatively constant (or increases slightly), sulphate increases by a factor of $\approx$ x3, the ionic strength increases by a factor of $\approx$ x4, the Eh drops by $\approx$ 100 mV and the Fe(II) concentration increases by a factor of $\approx$ x8.

iv) At discharge sites, pH in surface waters drops very rapidly to $\approx$ 1.5 – 2 associated with growth of dense algal mats.

These observations are incompatible with the assumption of complete oxidation of pyrite by dissolved oxygen as given in equation (1) above, which would result in a very significant drop in pH over the redox front. Even if the pH was buffered by rock weathering reactions, the formation of secondary pyrite cannot be explained.

Despite many years of study, the oxidation mechanisms of pyrite are poorly understood. The transfer of 7 electrons per sulphur atom required for the S_2^{2-} to SO_4^{2-} oxidation will certainly not proceed in a single step and a number of intermediate S species are to be expected.

Species which have been invoked as intermediates include polysulphides, elemental sulphur and a range of sulphoxy anions (e.g. Granger and Warren, 1969; Lowson, 1982; Moses *et al.*, 1987). As an illustration of the complexity of the chemistry involved, Figure 1 shows some of the sulphur species which have been reported to be stable in aqueous solutions (Rimstidt *et al.*, 1986). It should be emphasised that, under natural conditions, in particular those found at the Osamu Utsumi mine, many such species would be very unstable and thus play an insignificant role here.

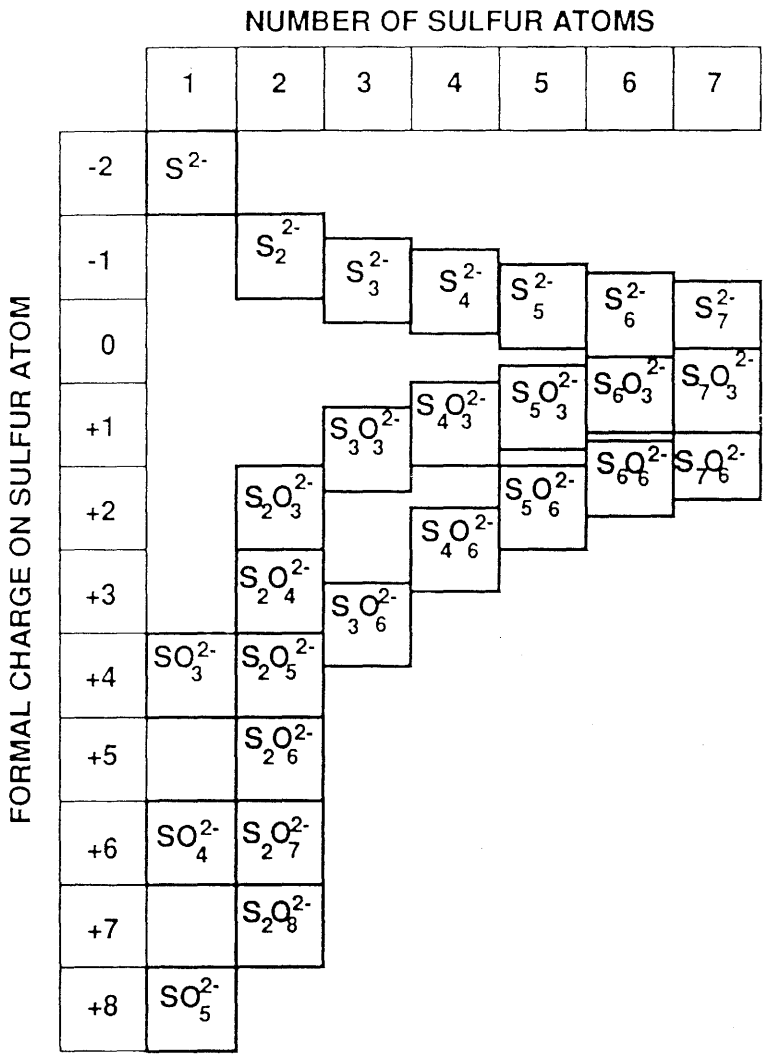

Figure 1. Diagram showing the sulphur species that are reported as stable in aqueous solutions. (From Rimstidt et al., 1986; figure kindly supplied by J.D. Rimstidt).

When bacteria catalyse pyrite oxidation, however, the chemistry becomes even more complex. A range of organo-sulphur species may then act as intermediates. Particularly important here may be adenosine phosphate complexes which are known to be subject to enzymatically catalysed redox reactions, which directly produce energy-rich compounds which are further used for microbial metabolism (Ehrlich, 1971). Such reactions give rise to the sulphur isotope fractionation commonly observed.

A key problem, however, is that both the pitchblende and secondary pyrite formed on the reducing side of the front require the presence of some active reductant in this part of the system. In many natural systems, microbes utilise organic carbon as a reductant to form pyrite, although other reductants such as hydrogen are also known to be utilised (e.g. Ehrlich, 1981). At Osamu Utsumi, however, the reduced rock does not contain measurable quantities of organic carbon, certainly less than the minimum of $\approx 0.1\%$ (dry weight) considered necessary for mineralisation on the scale observed (Ehrlich, 1981). For the very slowly moving fronts, dissolved organic carbon is also a possible reductant. Although the flux could be quite significant (assuming a DOC concentration of 1 ppm and a water flux of 0.3 m^3/m^2 as considered in section 3.2), it is difficult to explain why organic carbon, which is not labile during its transport through fully oxidising zones, should be oxidised by SO_4^{2-} under conditions of very low oxygen fugacity. From a purely thermodynamic point of view, however, this interpretation would be acceptable.

An alternative explanation is that the initial pyrite oxidation does not go to completion but produces Fe oxyhydroxide plus metastable sulphur species (e.g. polysulphides, elemental sulphur, thiosulphate). The sulphur species could then be transported to the reducing side of the front where they could be further used by microbes in fermentation-type reactions (Bak and Cypionka, 1987; Kelly, 1987) which provide energy for the microbes, but also may produce the required reduction. The discovery of the sulphur fermentation metabolism is relatively recent and little is known about the full range of mechanisms possible. Nevertheless, from the algebraic point of view, at least, it is possible to write a series of reactions in which pyrite is initially oxidised by O_2 or Fe^{III} to give $Fe(OH)_3$ plus polysulphide/elemental sulphur/thiosulphate etc. plus either protons or hydroxyl. The intermediate sulphur species on the reduced side of the front then disproportionate (the microbial fermentation reaction) in the presence of Fe^{III} to give pyrite plus more oxidised sulphur species (e.g. sulphite, thiosulphate, sulphate), plus again either protons or hydroxyls. The latter stage could also include UO_2^{2+} as an electron acceptor to give production of pitchblende. It should also be noted that the oxidised sulphur species produced in the

disproportionation could, in principle, still be capable of further oxidation and this could thus explain the acid waters formed at groundwater discharge sites.

Although the database is rather limited, the fundamental feasibility of the postulated mechanisms could be examined by simple thermodynamic calculations. A key aspect of all the reactions above is the production of either OH^- or H^+, making the thermodynamics of marginal reactions very pH-dependent. A common feature associated with microbial growth is the formation of microenvironments within "biofilms" which may have Eh/pH conditions very different from those of the bulk groundwater. Nevertheless, it should be possible to conduct some scoping calculations.

A common role attributed to microorganisms is the mobilisation of trace elements (and especially actinides) due to complexation by their organic degradation products. A biomass production rate of 10^{-2} g/year per m^2 of front could give rise to 10^{-3} g of potentially complexing products if a 10% efficiency is assumed. In the reference flux of 0.3 m^3/year, this would give a concentration of $\approx 3 \times 10^{-6}$ g/l, which is small relative to the organic carbon which may be expected in infiltrating surface water ($\approx 10^{-3}$ g/l). In general, therefore, mobilisation by microbial products would be considered negligible, unless very specific chelating agents are produced. The main effect of microbial activity is catalysis of specific redox reactions. Observations of trace element distributions around the redox front indicate that, in fact, trace elements tend to be concentrated in the zones in which microbial activity is expected (MacKenzie *et al.*, this report series; Rep. 7). As discussed both by MacKenzie *et al.* and Bruno *et al.* (this report series; Reps. 7 and 11 respectively), the trace element concentrations are most probably due to scavenging by secondary iron minerals formed by oxidation or reduction reactions. It is thus likely that the most significant aspect of microbial activity, from the viewpoint of trace element mobility, is the catalysis of redox reactions. It is, however, possible that trace element incorporation into such secondary phases may be directly influenced by the role of microbes in the formation of the minerals themselves (e.g. Ferris *et al.*, 1987).

4. Conclusions

In terms of the original aims (section 1.1) this study has indicated that:

i) Microbial populations were found in rock and groundwater samples to the maximum depth sampled.

ii) Microbial populations found at depth in the Osamu Utsumi mine could be supported by chemolithotrophic organisms (predominantly sulphur bacteria) which derive their energy from redox front reactions.

iii) Maximum populations predicted by a modelling approach used for performance assessment based on nutrient and energy constraints were roughly comparable with measured populations.

iv) Enhancement of uranium or trace element mobility by organic metabolites is unlikely to be significant but microbial catalysis of specific redox reactions may play a key role in defining redox front chemistry/mineralogy.

The role of microbes in catalysing pyrite oxidation and, probably, influencing aqueous sulphur speciation could be important in explaining mineralisation on the reduced side of the redox front (McKinley (Ed.), this report series; Rep. 12).

5. Acknowledgements

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Appendix 1

Sampling and laboratory procedural details.

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1. Sampling

Both groundwater and core samples should be taken for microbiological analysis. It is important that, once the samples are collected, analysis should take place as soon as possible. Only in extreme cases can the material be stored at low temperatures (~2–5°C) and this must be in anaerobic conditions and only overnight.

1.1. Solid material

Core will be abstracted to about 150 m depth. It is recommended that core for microbiological analysis should be taken at the following intervals: 0.5 m, 1 m, 10 m, 15 m, 20 m, 25 m, 30 m, 40 m, 50 m, 60 m, 75 m, 90 m, 110 m, 130 m, 150 m. Obviously it will be necessary to be flexible in this respect as the exact selection of core will be dependent on the other requirements of the project. Sampling responsibility should be coordinated between the geologist and microbiologist.

Samples of core should be ~20 cm in length if possible and upon removal from the main body of the core, should be placed in clean plastic bags. The air from the bags should be expelled and the bag sealed. Samples should be stored at 5°C in a cool-box until microbiological analyses are conducted. Analysis should take place rapidly.

1.2. Groundwater

Groundwater analysis will be a slower procedure as abstraction relies on the efficiency of pumps/packers. Samples should not be taken until the water is considered representative of the formation in hydrogeological and hydrochemical terms. Samples should be taken in autoclaved bottles of ~500 ml volume and every effort made to maintain an aseptic technique. In field conditions, plastic bottles are probably best. Bottles should be filled to overflowing and the lid replaced without touching the lip of the bottle. Again the samples should be stored at ~5°C and microbiological analysis should take place as soon as possible after collection.

Groundwater sampling intervals should be roughly the same as for core material. Unfortunately this will not be possible, particularly where the intervals are small, but theoretically this should be the goal. The aim is then to match the core microbiology with that of the groundwater.

1.3. Chemistry

All known chemistry (including Eh/pH) must be made available to the microbiologists so that results can be collated. This should give an indication of in-situ conditions so that, if these are totally different from the laboratory situation, modifications can then be made. For example, shallow water samples may be oxygenated so that storage under anaerobic conditions may not be appropriate.

2. Laboratory procedures

2.1. Subsampling of core

Intact core must be taken at the site. If the material has disintegrated then contamination will have occurred and it is not worthwhile proceeding with the analysis.

Subsampling of the core material in the laboratory using strict aseptic techniques is essential if good results are to be obtained. Ideally this should be done in a laminar flow cabinet which has been previously sterilised using a UV lamp. All implements which are to be in direct contact with the area of subsampling must be sterilised. Subsamples should be taken parallel to the core axis. Several techniques, depending on the hardness of the material, have been developed in Poços de Caldas for subsampling the cores. In all cases the core should have the ends removed aseptically and the first and last centimetre of the subsample discarded as they will have been exposed to surface contamination. These methods are (in order of use):

a. Cork borer

This is the first method to be attempted. The core should be put in a holder (developed at Poços de Caldas) and clamped at an angle. Material can then be collected through the hollow bore handle in a sterile plastic bag held over the end of the cork borer.

b. Slow percussion drill

This method with a hollow bit can be very effective providing it is done slowly to avoid heat generation. The core will have to be clamped upwards. The drill will have to be removed at intervals for material to be collected in a sterile plastic bag. Do not allow the material to become compacted as removal from the bit will be difficult.

c. Drill

This method produces a fine powder and generates a lot of heat. It is not recommended unless all else fails.

For all samples collect about 20 g of material.

Split this amount into 2 portions of 10 g each. Place 10 g in a sterile flask and add glutaraldehyde (0.5% final concentration diluted in 0.1% pyrophosphate) which will fix the material for epifluorescence microscopy. Store aerobically.

The remaining 10 g can be left in the plastic bag. Expel all air from it and store anaerobically at about 5°C if necessary. A slurry will have to be prepared from this subsample so that inoculations can be made. This should be done using filter-sterilised (0.2 μm filter) groundwater from the borehole or, if this is not possible, from a nearby hole. The subsample should be reduced to a powder using a hammer etc. whilst still in the bag or, alternatively, a sterile pestle and mortar can be used. The material should then be put in a sterile flask and 90 ml of the groundwater added aseptically. If the material is very fine then the water must be added slowly to give a fine suspension. The slurry must then be shaken or stirred for about 30 minutes in an anaerobic environment so that no excess air is introduced into the system.

Ideally, the subsampling/preparation of the slurry and inoculation should be completed in one day. However, there are stages where the routine can be satisfactorily stopped. These are:

a. Core storage

b. After subsampling

c. After slurry preparation

Do not store for more than 24 hours. Always store anaerobically at ~5°C.

Ideally, several subsamples should be taken from each core to give statistical accuracy. This may not be feasible.

2.2. Groundwater

Groundwater should not need treatment if collected properly. It should be stored at ~5°C in anaerobic conditions once collected. Shake the sample, wait 2 minutes for large particles to settle out and then remove 200 ml of the supernatant. Fix 100 ml with glutaraldehyde (50:50 mix). The remaining 100 ml will be used in inoculations.

Once again the water collection, preparation and inoculation should be completed in one day, but there can be breaks in the routine. These are:

a. Water storage

b. After subsampling

Do not store for more than 24 hours. Always store anaerobically at ~5°C unless the *in-situ* geochemistry gives indications that the water is oxygenated.

Filter-sterilised groundwater from the same borehole/area should be used in the preparation of slurries and for the serial dilutions and will thus contain little air if collected as described. If this is not possible, then other groundwater which has not been kept anaerobically should be used. This will have to suffice.

Ideally, several samples of water from each location should be analysed for microbiological content to provide statistical accuracy.

2.3. Total aerobic/anaerobic heterotrophs

In brief,

a. CPS medium at full strength and at 1% concentration.

b. 0.1 ml inoculations in triplicate and incubated aerobically and anaerobically.

c. Incubations at ~25°C over 10 days.

d. Results + or -. Count colonies and estimate total numbers per ml.

2.4. Sulphur oxidising bacteria

In brief,

a. Two enrichment media: Medium S and Medium R.

b. Medium S is to be at pH 5 whilst Medium R is at pH 7.5. Adjustments to be made after autoclaving with sterile NaH and HCl.

c. Dispense the medium into sterile tubes in 10 ml aliquots.

d. Inoculate the medium with 0.1 ml inoculum in triplicate from the 10 g/90 ml slurry, or from the groundwater. Incubate aerobically at ~25°C for two to three weeks.

e. Monitor the pH of the controls immediately after inoculations of the other tubes.

f. Monitor pH daily.

g. Look for pH drop when compared with the controls. Compare results from all inoculations. Changes in colour can also indicate growth.

2.5. Sulphate reducing bacteria

In brief:

a. Two media: Postgate's B (qualitative) and Postgate's E (quantitative).

b. The media should be freshly prepared. Use screw-top tubes.

c. Two inoculation strengths should be used for the qualitative test – 2 ml and 0.2 ml in triplicate. If results are not satisfactory with the slurries, solid material can be tried if the material has been stored anaerobically.

d. Serial dilutions for the quantitative test are required (1/10, 1/100, 1/1000). These should be done using filter-sterilised groundwater. 1 ml inoculations for this test should be carried out in triplicate.

e. Incubate anaerobically if unsure of tube preparation. Otherwise the medium should remain anaerobic if the method has been adhered to. Incubate the tubes at ~25°C for 2 to 3 weeks. Controls are essential.

f. Qualitative test results – blackening of media when compared to control. Quantitative test results – count colonies and calculate number per ml.

Appendix 2

Microbial composition of groundwaters from the Osamu Utsumi mine and Morro do Ferro study sites.

Appendix 2

Microbial composition of groundwaters from the Osamu Utsumi mine and Morro do Ferro study sites.

CFU counts are maximum values (2 replicates) rounded to the nearest 10. Epifluorescence microscopy counts are the mean of 10 fields of view.

Site	Depth (m)	Heterotrophs (max CFU/ml) Aerobic	Anaerobic	SRB	SO	Epifluorescence microscopy (Nos/ml)
a. Sampled early 1987						
F1	95.5 – 126	150	10	+-	+-	NT
Pilot Hole	79.7 – 85	10	10	+	+-	NT
Shaft	0 – 40	3	0	+-	+-	NT
Piezo 22	>20	20	2	+-	-	NT
b. Sampled May 1987						
F1	95.5 – 126	145	20	+	+-	1270
F2	45 – 60	1410	530	+	-	5950
Pilot Hole	79.7 – 85	45	1	-	+-	2200
Shaft	Not tested					
Piezo 22	>20	30	4	-	-	733
c. Sampled September 1987						
F1	95.5 – 126	>4000	>4000	+	-	NT
F2	45 – 60	>4000	>4000	+	-	NT
Pilot Hole	79.7 – 85	2460	2440	+-	-	NT
MF10		>4000	150	+	-	NT
MF12		1660	50	+	-	NT
Shaft	0–40	430	380	+	-	NT
Piezo 22	>20	4000	60	-	-	NT

- = Negative + = Positive +- = Variable result CFU = Colony forming units.

NT = Not tested SRB = Sulphate reducing bacteria SO = Sulphur oxidisers.

Site	Depth (m)	Heterotrophs (max CFU/ml) Aerobic	Anaerobic	SRB	SO	Epifluorescence microscopy (Nos/ml)
d. Sampled June 1988						
F1	95.5 – 126	840	-	+	+-	460
F2	45 – 60	2500	-	+	+-	280
Pilot Hole	Not tested					
F4	0 – 100	4000	-	+	+-	470
F3	0 – 75	Contami-nated				
MF10	0 – 61	Contami-nated				
MF11	-	1780	-	+	+-	310
MF12	0 – 70	1600	200	+	+-	230
SW01	0 – 10	1100	10	-	+-	120
SW02	Not tested					
SW03	-	510	-	-	+-	47
Shaft	0 – 40	250	-	+	+-	870
Piezo 22	>20	40	-	-	-	730

No iron oxidisers found in any samples.

- = Negative + = Positive +- = Variable result CFU = Colony forming units.

NT = Not tested SRB = Sulphate reducing bacteria SO = Sulphur oxidisers.

Site	Depth (m)	Heterotrophs (max CFU/ml) Aerobic	Anaerobic	SRB	SO	Epifluorescence microscopy (Nos/ml)
e. Sampled September 1988						
F1	0 – 91	1570	850	+	-	NT
F2	45 – 60	> 4000	> 4000	+	+ -	NT
Pilot Hole	Not tested					
F4	Not tested					
F3	0 – 75	850	90	+	+ -	NT
MF10	0 – 61	> 4000	> 4000	+	-	NT
MF11	-	> 4000	920	-	-	NT
MF12	0 – 70	> 4000	> 4000	+	-	NT
SW01	0 – 10	310	10	+	+ -	NT
SW02	Not tested					
SW03	-	1030	570	-	+ -	NT
Shaft	Not tested					
Piezo 22	Not tested					

No iron oxidisers found in any samples.

- = Negative + = Positive +- = Variable result CFU = Colony forming units.

NT = Not tested SRB = Sulphate reducing bacteria SO = Sulphur oxidisers.

Sample	Depth (m)	Heterotrophs (max CFU/ml) Aerobic	Anaerobic	SRB	SO	Epifluorescence microscopy (Nos/ml)
f. Sampling from Borehole MF12 in November 1988						
1	5.9	140	-			
2	6.68	70	-			
3	8.39	-	-			
4	10.37	-	-			
5	13.59	2110	-			
6	15.54	-	-			
7	20.27	-	-			
8	25.13	50	-			
9	33.52	53710	-			
Not tested for sulphur oxidisers, sulphate reducing bacteria and iron oxidisers.						
g. Sampled December 1988						
F1	0 – 91	950	330	+	+-	NT
F2	45 – 60	>4000	-	+	+-	NT
Pilot Hole	Not performed					
F4	0 – 100	>4000	250	+	+-	NT
F3	0 – 75	500	-	+	+-	NT
MF10	0 – 61	>4000	800	-	-	NT
MF11	-	>4000	1580	+-	-	NT
MF12	0 – 70	>4000	1620	+	+-	NT
SW01	0 – 10	320	-	-	+-	NT
SW02		490	-	+-	+-	
SW03		570	-	+-	+-	NT
Shaft	0 – 40	>4000	20	+-	+-	NT
Piezo 22	>20	990	-	+-	+-	NT
F5		>4000	10	+	+-	NT
No iron oxidisers found in any samples.						

- = Negative + = Positive +- = Variable result CFU = Colony forming units.

NT = Not tested SRB = Sulphate reducing bacteria SO = Sulphur oxidisers.