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RAPPORT

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Tittel:
Upconcentration of heavy metals in Norwegian biofilms and iron incrustations.
Oppkonsentrering av tungmetaller i norske biofilmer og jernutfellinger.

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Sammendrag:

Samples of metal-immobilising bacterial (MIB) biofilm and other apparently inorganic iron precipitates have been collected and have shown considerable up-concentration of heavy metals and some other elements (notably Fe, Ti, Al, P, Cu, V, Zr, Be, and Zn) in comparison with groundwater. These metals remain bound in the biofilm during "rinsing" with deionised water. This observation is important in the context of (i) toxicity of residues after treatment of clogged water wells or distribution systems (ii) accumulation of heavy metals at sewage treatment plants (iii) potential methods of bacterial treatment of heavy-metal contaminated water.

Prøver av metallimmobiliserende bakteriale (MIB) biofilmer, samt andre tilsynelatende uorganiske jernutfellinger er analysert. Biofilmene viser betydelig oppkonsentrering av tungmetaller og enkelte andre elementer (spesielt Fe, Ti, Al, P, Cu, V, Zr, Be og Zn) i forhold til grunnvannet. Disse metallene forblir bundet i biomassen under rensing med deionisert vann. Dette kan være viktig sett i lys av (i) mulig giftvirkning av residuer fra rehabilitering av tilgrodde grunnvannsbrønner og ledningsnett (ii) akkumulering av tungmetaller ved kloakkrenseanlegg (iii) mulige metoder for mikrobiologisk behandling av tungmetallforurenset vann.

Emneord: Hydrogeologi	Mikrobiologi	Grunnvann
Berggrunn	Grunnvannskvalitet	Jern
Løsmasser	Tungmetaller	Fagrapport

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1. INTRODUCTION[1]

This paper will focus on one particular aspect of groundwater microbiology in hard-rock aquifers, namely the group of bacteria known as the metal-immobilising bacteria (MIB), or more commonly as the "iron-bacteria". These bacteria have recently become the subject of much attention amongst hydrogeologists, due to their ability to form biofilms which can clog groundwater abstraction and drainage systems (Cullimore & McCann 1977, Howsam 1988, Banks 1992a), and their ability to immobilise a range of heavy metals (Banks 1992b).

MIBs appear to be ubiquitous in many groundwater environments, although particular species have different requirements for, for example, pH, oxygen and iron concentrations and organic carbon. The characteristics of the commonest genera are summarised in Table 1.

Genus	**Chemoautotrophic (C) or heterotrophic (H)**	**Preferred pH range**	**Other comments**
Gallionella	C	5.5 - 7.6	Oxidises Fe^{II}; microaerophilic; common in biofilms in water supply systems, iron removal plants, hydro-electric power plants.
Leptothrix	H	5.4 - 8.0	Requires low concentrations of soluble organic C; oxidises Mn^{II} and Fe^{II} extracellularly; microaerop-hilic; often in biofilms together with Gallionella.
Sphaerotilus	H	5.4 - 8.0	Similar to Leptothrix, but requires high concentrat-ion of soluble organic C; common in sewage sludge.
Thiobacillus ferroxidans	C	1.5 - 3.0	Oxidises Fe^{II} and inorganic S compounds simultan-eously; aerophilic; frequent in acid mine drainage waters.
Metallogenium	H	3.5 - 5.0	Oxidises Fe^{II} and Mn^{II}.
Siderocapsa	H	-	Utilises organic part of Fe / Mn humates; common in ground water.
Pseudomonas Arthobacter	H H	-	Utilise organic parts of Fe / organic complexes; some species produce siderophores. Pseudomonas com-mon in groundwater.
Pedomicrobium Hyphomicrobium	H H	-	Most deposit Fe oxides, some deposit Mn oxides.

TABLE 1. Main features of different genera of ferromanganese depositing bacteria (after Banks 1992b).

[1] These first sections are based on a paper given to the 1993 Congress of the International Association of Hydrogeologists at Ås, Norway. The full text of the paper can be found in Banks & Banks (1993).

Some (but not all) of the most problematic bacteria, in a groundwater context, actively metabolise iron and are microaerophilic, thus typically thriving where relatively reducing iron-containing groundwater encounters a more oxygen rich environment (e.g. in a borehole or tunnel). Although some species can fix CO_2, many species require a source of metabolisable organic carbon, and may particularly thrive in the vicinity of pollution sources or river water infiltration. It is as yet uncertain if these bacteria are introduced to deeper aquifers from soil during borehole drilling, but the weight of opinion seems to suggest that they can survive in pristine aquifers in limited numbers. The occurrence of such bacteria is worldwide, cases being reported from tropical (Cullimore & McCann 1977) and arctic (Asplan 1985) climates as well as from temperate ones.

2. MIB OCCURRENCE IN FRACTURED AQUIFERS

MIBs are widely recorded in the literature from a variety of porous and dual porosity aquifers throughout the world. Although never specifically identified in Norway it is overwhelmingly probable that iron-related bacteria exist in Norwegian sedimentary aquifers and have been responsible for clogging of a number of water supply wells

In contrast to many European lands, Norway has, in addition to Quaternary aquifers, a considerable number of small-scale abstraction boreholes in hard-rock aquifers. Despite the comprehensive literature on the subject of MIBs, there is little documentation of MIB clogging problems in fractured aquifers. Harker (1990) reports clogging problems in Chalk boreholes in England but these appear to be due to clogging of slotted casing rather than fractures themselves. Pedersen & Hallbeck (1985) and Pedersen (1987) report the occurrence of thriving populations of *Gallionella ferruginea* in groundwater from two 46 m deep boreholes in sideritic shales and sandstone at Helsingborg in Sweden, and a 60 m deep granitic well at Hindås, near Gøteborg, together with the existence of populations of MIBs in boreholes several hundred metres deep at Ävrö, near Simpevarp, and at Stripa mine.

Due to the importance of groundwater from hard-rock as a water resource for rural areas in Norway, the Geological Survey of Norway (NGU) has commenced a project to ascertain whether the growth of MIB biofilms in fractured aquifers can lead to clogging of fractures. The study consists of four parts:

(a) a questionaire survey of major Norwegian groundwater abstractors to ascertain the degree to which clogging, corrosion and general lack of maintainence pose a problem in Norwegian boreholes. Results are published by Banks (1992c).

(b) publication of a guide to borehole monitoring, maintainence and rehabilitation with emphasis on problems of corrosion and encrustation (Banks 1992a).

(c) monitoring of bedrock boreholes in Trondheim (Banks 1991) and Hvaler (Banks et al. 1993) with repeated test-pumping at two-yearly intervals, and monitoring of bacterial growth using simple "slides" (Banks 1993).

(d) collection of samples of biofilm and inorganic iron incrustations for chemical and bacteriological analysis (Banks 1992b, Banks & Banks 1993).

3. MIB OCCURRENCE IN SCANDINAVIAN BEDROCK AQUIFERS

MIB biofilm growth in Norwegian hydroelectric power tunnels in bedrock was reported by Cullimore and McCann in 1977. Carlsson and Olsson (1977) report ferruginous "sediments" deposited by water leaking from fractures in the Forsmark subsea tunnel in Sweden, which contained up to 73 % iron, 4.5 % manganese and 24 % [organic material + bound water]. These may be biofilms, but, due to the high iron content of the leaking water (ca. 3 mg/l), they may be purely inorganic deposits.

During the course of NGU's studies, biofilms have also been discovered in Norwegian road tunnels. In the Hvaler subsea tunnel, in Precambrian granite, (see Banks et al. 1992, 1993) several growths, predominantly of *Gallionella* bacteria, can be seen growing on leaking fractures in the walls of the tunnel. In the arctic Vardø subsea road tunnel, in Precambrian metasediments, (Asplan 1985) growths of predominantly *Leptothrix* and *Gallionella*, with associated precipitation of iron, aluminium and manganese, have led to severe clogging of drainage gravels and pipes, necessitating regular maintainence by jetting every two years (Tor-Erik Lynneberg, Veglab., Oslo, pers.comm. 1991). In the Ålesund subsea tunnel (Olsen & Blindheim 1987), reddish brown slimy deposits, presumably, but not confirmed to be, bacterial, cause similar problems (Oddmund Gussiås, Vegkontoret, Molde, pers.comm. 1991), and have necessitated replacement of clogged drainage.

Apparently inorganic iron oxyhydroxide deposits have also caused tunnel drainage problems. At a tunnel complex in pyritic keratophyre at Gråkallen, near Trondheim (Banks & Ottesen 1992), iron-rich drainage water caused incrustations in drainage systems which, after microscopic examination, appeared to be purely inorganic in character.

In boreholes, definite biofilm growth has been observed in only one borehole. A horizontal test borehole drilled in greenstone by SINTEF at Lade, Trondheim, yielding water with ca. 2.4 mg/l iron, exhibits biofilm growths apparently consisting of *Sphaerotilus* and/or *Leptothrix*. The free-draining nature of this borehole in pyrite-rich rock may have been responsible for aerobic, iron-rich conditions favorable for bacterial growth.

NGU's questionnaire investigation also revealed three boreholes in bedrock aquifers where a decline in capacity was diagnosed as being due to bacterial clogging. In the municipalities of Nes-in-Akershus and Ski, two bedrock boreholes in Precambrian gneiss were successfully rehabilitated by treatment with hydrochloric acid and shock chlorination. The solutions derived during treatment were shown to be iron and manganese-rich, indicating either inorganic or bacterial clogging of fractures. At Gaupen in Ringsaker municipality, a decline in capacity was observed in bedrock boreholes. Slimy red-brown deposits were observed in pipework, leading to a diagnosis of bacterial clogging. Treatment by hydrochloric and citric acids, with chlorination, yielded short-term improvements in yield, but after around six months the improvements had been reversed. In none of these three examples was bacterial involvement definitely proven.

4. METAL IMMOBILISATION BY IRON BACTERIA

Bacteria play a central role in the cycling of major elements including the metals, Fe, Mn, Cu, Zn, and Co (Zajic 1969, Olson 1983, McLean & Beveridge 1990) and are thought to be important in controlling fluxes of metal ions in aqueous environments.

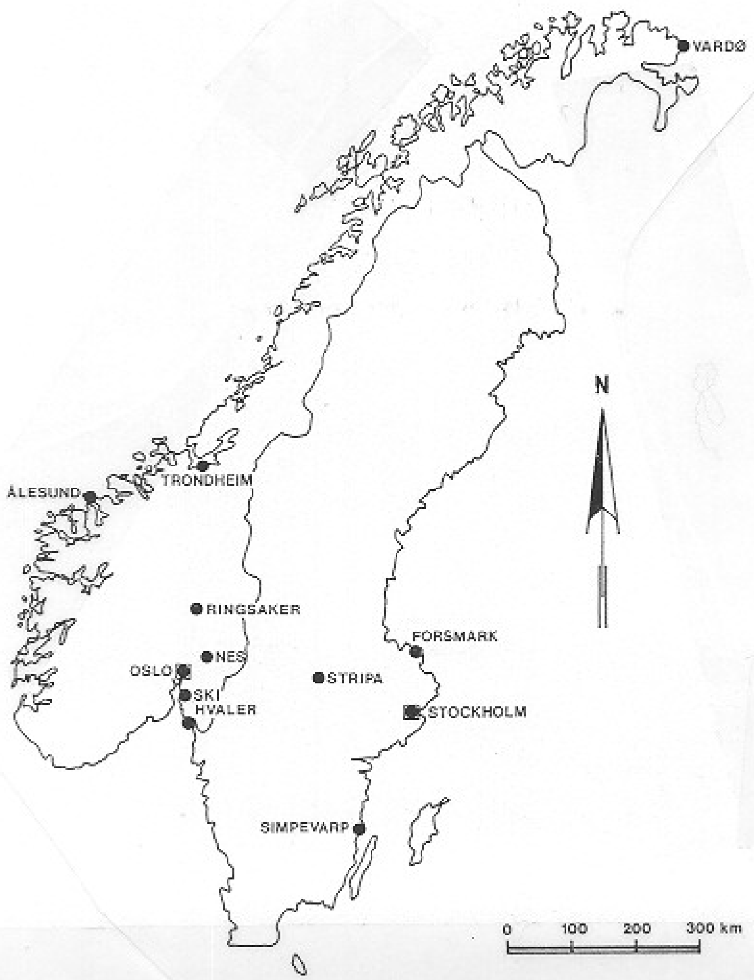


Fig. 1 Location of bacterial/ incrustation occurrences named in text.

Sample	Hvaler 1	Hvaler 2	Hvaler3	Trondheim 1	Trondheim 2	Gråkallen	Arekilen	Fisosen
Location Date of sampling	Hvaler tunnel, chainage 4120, 23/3/90.	Hvaler tunnel, chainage 3615, 29/5/91.	Hvaler tunnel, chainage 4120, 29/5/91.	SINTEF test-hole, Lade, Trondheim	SINTEF test-hole, Lade, Trondheim	Gråkallen tunnel, Trond-heim.	Arekilen municipal well, Hvaler, 9/4/92	Hot sulphur spring, Svalbard
Geology	Granite	Granite	Granite	Greenstone	Greenstone	Pyritic keratop-hyre	Moraine/ Glaciofluvial	
Bacteria	*Gallionella*	*Gallionella* (presumed)	*Gallionella* + others	*Leptothrix/ Sphaerotilus*?	*Leptothrix/ Sphaerotilus*?	Inorganic	Inorganic	Unknown
Chloride in groundwater	11600 ppm	17000 ppm	17300 ppm	110 ppm	110 ppm	Unknown	47 ppm	Unknown
Iron in groundw-ater	298 ppb	< 10 ppb	< 10 ppb	2.38 ppm	2.38 ppm	Unknown	127 ppb	15.2 ppb
Iron in biofilm	Not determ-ined for dry weight	258200 ppm	289200 ppm	124500 ppm	144300 ppm	343800 ppm	504200 ppm	2800 ppm
Weight loss on heating at 500-550°C	Not determ-ined	18.65 %	19.71 %	9.94 %	10.02 %	11.38 %	19.12 %	Unknown

TABLE 2. Summary of properties of the various biofilm / incrustation samples.

All bacteria require low levels of metals such as Fe, Co, Cu, Mo, Mn, Ni and Zn, as essential inorganic nutrients (McLean & Beveridge 1990, Gadd 1990), and some species use oxidation of, for example, iron or manganese, as an energy source. Most of these elements can, however, rapidly become toxic over a relatively narrow concentration range. Nevertheless, bacterial populations do exist in waters where levels of heavy metals (including Cd, Hg), may greatly exceed normal toxicity thresholds. One mechanism which enables bacteria to survive under such conditions is their ability to immobilise metals, by a variety of methods, on the outside of the cell. Entry of excessive amounts of unwanted metals into the cell is thereby prevented, enabling the cell to function normally, even in potentially toxic environments (McLean & Beveridge 1990; Ghiorse 1986). External immobilisation may be achieved by several methods including; (i) direct binding of metal ions to the cell wall, (ii) precipitation and (iii) extracellular complexation (Banks 1992b).

The immobilisation of iron by bacterial populations is actively exploited in several water treatment techniques, for example, the VYREDOX *in situ* treatment method for iron-rich groundwater (Hallberg & Martinell 1976). Metal immobilisation by bacteria also forms the basis for techniques for extraction of valuable metals from industrial waste water (Gadd 1990), for the proposed treatment of heavy-metal contaminated water (Banks 1992b), and is considered relevant to the mobilisation of heavy radioactive elements from waste repositories (Pedersen 1987). Even bacterial immobilisation of gold in Alaskan stream sediments has been observed (Hecht 1992).

5. HEAVY METAL IMMOBILISATION IN NORWEGIAN BIOFILMS

Seven samples of iron oxhydroxide incrustation have been analysed for around 30 elements using NGU's ICP apparatus. Three samples (Hvaler 1,2,3) were of *Gallionella* biofilm from the Hvaler tunnel (Banks 1992b), two samples (Trondheim 1,2) were of *Leptothrix/Sphaerotilus* biofilm from the horizontal SINTEF test borehole at Lade, Trondheim, the sixth sample was of inorganic iron oxyhydroxide incrustation from Gråkallen, near Trondheim (Banks & Ottesen 1992) and the seventh was also of an apparently inorganic iron oxyhydroxide incrustation from the dug well at Arekilen, Hvaler. Arekilen well is in Quaternary glaciofluvial deposits, and was clogged with iron oxyhydroxide precipitation, necessitating digging a new well.

The nature (bacterial genera, organic/inorganic) of the samples was determined by microscopic inspection by Prof. Kjell Eimhjellen, Inst. for Biotechnology, Norwegian Technical University, Trondheim (NTH).

An eighth sample is also discussed here, although it is not an iron bacterial or inorganic iron incrustation. This sample comes from Fisosen warm spring (15°C), emerging from limestone at Stormbukta near the South Cape on Svalbard. It was a whitish threadlike slime growing on the rock in the spring and drainage stream. The spring is highly reducing with a high content of H_2S. The sample was not subjected to microscopic analysis, but it is regarded as probable that the biomass sample collected at Fisosen is composed of sulphur-related (sulphur-oxidising ?) bacteria and / or algae. The sample was taken 15 / 7 / 93.

Samples were air dried at c.110°C. All results refer back to dried samples. One portion of each sample was dissolved in ultrapure nitric acid (in all cases with near-total dissolution) and analysed for 30 elements on NGUs ICP equipment. Another portion of each sample (except Hvaler 1) was "rinsed" repeatedly with several volumes of deionised water (allowing settling and decanting between each rinsing) to determine how tightly various elements were bound in the biofilm. The rinsed material was then dried and analysed as above. A third portion was heated to c.550°C for 1 hr, and weight loss was measured. This weight loss would be due to both loss of bound water (for example in hydroxides), to loss of CO_2 from carbonates (if present) and to ignition of organic material. Pure iron (III) hydroxide $Fe(OH)_3$ would undergo a weight loss of 25.3 % during dehydration to iron (III) oxide Fe_2O_3.

In the cases of the Hvaler (tunnel and Arekilen), Trondheim (SINTEF) and Fisosen samples it was possible to obtain a sample of the groundwater in which the biofilm was growing. For the Hvaler tunnel (samples 2 and 3) and SINTEF borehole, the groundwater sample for ICP analysis was field filtered using a 0.45 μm Millipore filter and acidified using a few drops conc. HNO_3. The Fisosen and Arekilen groundwater samples were not filtered or acidified. By assuming that the most soluble elements (e.g. sodium) were not significantly immobilised in the biofilm, it is possible to normalise the concentrations of elements in the biofilm by dividing by the ratio (conc. of sodium in biofilm / conc. of sodium in groundwater). The up-concentration factor for each element in the biofilm relative to groundwater (in turn relative to non-immobilised elements, e.g. sodium) can then be derived by simple division (see Appendix 1). This can be considered to be an estimate of how effectively the biofilm immobilises metals from groundwater. It must, however, be admitted that this is not a totally justified assumption - in the Hvaler tunnel, for example, metals such as lead could be derived from the exhausts of passing cars.

It must be mentioned that with some elements (e.g. Be) the extremely high levels of iron present render the ICP determinations somewhat innaccurate.

6. HVALER TUNNEL

Results are presented in Tables 2, 3 and 4, Figs. 2-5 and Appendix 1.

In the Hvaler biofilms, it will be noted that iron is by far the most effectively immobilised element, with upconcentration factors of several million. In the leaking saline groundwater, iron concentrations were, for biofilms 2 & 3, < 10 ppb (measured, however, after some contact with the biofilm), demonstrating that iron concentrations are seldom a limiting factor for iron-bacterial growth.

For biofilm sample 1, the iron content for the leakage water was higher, c. 300 ppb. The filtrate water from filtering of the biofilm was also analysed for sample 1, revealing iron concentrations of only 77 ppb, demonstrating effective iron removal in the biofilm "pore water".

Other elements which were effectively upconcentrated (around or over 1000 times) in at least one of the Hvaler samples include P, Ti, Cu, Zn, Zr and Be. Elements showing upconcentration factors of at least several hundreds include La, Si, Mn, Al and Ba. For many heavy metals it was only possible to calculate a minimum upconcentration factor as the concentration in the water was below the analytical detection limit. It is therefore likely that significant upconcentration occurs for more elements than those named above.

Mg, Ca, Li and K are not immobilised to a significant extent in the Hvaler biofilms.

7. SINTEF BIOFILMS

The absolute content of iron in these biofilms is a little lower than in the Hvaler biofilms. In the SINTEF Trondheim biofilms, however, the upconcentration factors for iron are considerably lower, reflecting the higher iron concentrations in the groundwater. Aluminium and titanium are, however, significantly immobilised in these biofilms (several 1000 to 10,000 times). Mg and Ca are immobilsed with factors of around 20 with respect to sodium. Potassium gives a factor less than one; the groundwater appears to be contaminated and contains high levels of this element.

Other elements which were effectively upconcentrated (around or over 1000 times) in at least one of the SINTEF samples include Zn and V. Elements showing upconcentration factors of at least several hundreds include P, Cu, Mo, Cr, Ba, Zr and Sc. For many heavy metals it was only possible to calculate a minimum upconcentration factor as the concentration in the water was below the analytical detection limit. It is therefore likely that significant upconcentration occurs for more elements than those named above.

8. INORGANIC INCRUSTATIONS

In terms of dry mass, the two apparently inorganic incrustations from Arekilen and Gråkallen contain more Fe than any of the definite biofilms, namely 504 g/kg and 344 g/kg respectively. Pure $Fe(OH)_3$ and Fe_2O_3 contain 523 g/kg and 699 g/kg Fe respectively. The incrustation from Arekilen thus approaches the composition of pure $Fe(OH)_3$. The dry Arekilen incrustation also contains very much Ca (13 g/Kg) probably representing $CaCO_3$ precipitation from the calcium rich (102 ppm) groundwater.

The accumulation factor for iron for Arekilen (383,000) is somewhat less than for Hvaler biofilms 2 & 3 (>4,000,000), but greater than for Hvaler 1 (47,000). Mn shows an extremely high upconcentration factor of > 40,800. Other elements which were effectively upconcentrated (around or over 1000 times) in the Arekilen sample include Zn, Mo and Ba. Elements showing upconcentration factors of at least several hundreds include P, Zr, Be, Ce and probably Ni.

9. FISOSEN BIOMASS

As mentioned before, the Fisosen sample is not strictly comparable with the other samples, and does not appear to consist to any great degree of iron oxyhydroxide incrustation. Its main inorganic elemental component was Ca (23 g/kg); most likely present as carbonate, given composition of the water. It appears likely that organic and to some extent inorganic (carbonate), carbon, hydrogen and oxygen are responsible for much of the remaining mass. Other elements present at over 1 g/Kg include P, Si, Al, Fe, Mg, K, Na and Ba.

Al appeared to be the most accumulated element (factor 174,000), followed by Fe (94,000) and Zn (> 15,000). Other elements which were effectively upconcentrated (around or over 1000 times) in the Fisosen sample include Ti, Mn, V, Cu and Cr. Elements showing upconcentration factors of at least several hundreds include Si, P, Pb, Ni, Co and Mo.

10. RINSING OF BIOMASS /INCRUSTATIONS

When considering the ratio of concentrations in rinsed biofilm to unrinsed biofilm, the majority of heavy elements remain unchanged (i.e. difficult to extract by water rinsing). Only Na and K were significantly removed in all biofilm samples. Mg, Li, Ce, and, surprisingly, in one sample (Trondheim 2) Pb, Ni, B and Co, were removed to a lesser degree.

	Hvaler 2	Hvaler 3	Trondheim 1	Trondheim 2	Fisosen	Arekilen	Gråkallen
Si	129	55,6	3100	2700	2000	< 20	<2
Al	1300	124,5	6700	7300	15600	< 20	2000
Fe	258200	289200	124500	144300	2800	504200	343800
Ti	116,6	9,6	1400	1400	100	5.2	751
Mg	10100	11100	8000	7700	6100	212	1400
Ca	17400	23900	29700	28400	23000	13900	520,9
Na	58300	63100	2200	1300	1700	243	302,2
K	1800	1800	<40	<40	4200	< 200	771,2
Mn	1600	532,5	1100	1200	100	846	107,7
P	1300	601,8	541,6	513,2	2300	324	1200
Cu	18,8	0,2	21,5	22,5	34	< 2	367,6
Zn	165,9	62,6	69,9	113,3	145	376	189,9
Pb	<5	<5	<10	77,7	42	< 50	98,7
Ni	<2	<2	36,9	40,2	31	37,3	5,1
Co	4,2	3	3,2	5	10	< 10	<1
V	<1	<1	87,2	85,3	37	< 10	20,5
Mo	<2	<2	43	54	6	158	98,2
Cd	<2	<2	<4	<4		< 20	<2
Cr	3,7	<1	29	31,2	21	< 10	8,6
Ba	30,7	30	707,4	503,8	2200	207	60,5
Sr	467	498,5	94	89,5	469	172	13,4
Zr	75,5	18,5	16,3	16,9		20,1	68,3
Ag	<1	<1	<2	<2		< 10	<1
B	255,5	242,1	7,2	69,5	70	25,5	<1
Be	17,3	18,7	<1	<1		13,2	8,7
Li	2,1	1,8	2,2	3,1		< 5	1,3
Sc	<0,5	<0,5	11,4	11,3		< 5	0,9
Ce	20,2	<3	15,8	34,6		78,4	69,6
La	35,5	27,6	4,9	<1		< 5	2,9
Y							

TABLE 3. Concentrations of various elements in biofilms / iron precipitates; concentrations in mg/Kg dry weight.

	Hvaler 1	Hvaler 2	Hvaler 3	Trondheim 1	Trondheim 2	Fisosen	Arekilen
Si	299,75	6,35	1,95	33,07	48,73	397	< 0,28
Al	>1,20	176,52	14,32	7796,86	14371,89	174272	ERR
Fe	46705,75	> 4207267	> 4124888	1607,09	3151,26	93633	382886,14
Ti	>1,61	1376,78	58,77	> 4301,08	> 7276,51	>5083	>50,27
Mg	1,03	1,50	1,44	19,04	31,00	79	2,60
Ca	1,54	3,05	2,43	17,63	28,52	77	13,20
Na	1,00	1,00	1,00	1,00	1,00	1	1,00
K	0,89	1,46	1,59	< 0,24	< 0,40	49	< 2,61
Mn	11,36	128,43	27,42	23,63	43,62	1177	>40882,07
P		> 2118,30	> 858,35	> 166,39	> 266,74	943	>313,60
Cu	>5,27	1021,13	6,63	> 330,26	> 584,72	7201	<69,05
Zn	>11,84	2017,37	686,82	> 429,49	> 1177,75	>14741	>7267,71
Pb	>1,11	ERR	ERR	ERR	> 80,77	>427	ERR
Ni	ERR	ERR	ERR	> 28,34	> 52,23	>394	>90,15
Co	>0,97	> 68,44	> 42,79	> 9,83	> 25,99	>508	ERR
V	>5,88	ERR	ERR	> 535,79	> 886,69	>3761	ERR
Mo	>2,90	ERR	ERR	> 132,10	> 280,67	>305	>1525,46
Cd	>0,98	ERR	ERR	ERR	ERR		ERR
Cr		> 60,29	ERR	> 89,09	> 162,16	>1067	ERR
Ba	>0,62	215,62	149,09	280,06	337,44	3057	1527,55
Sr	2,77	6,50	5,52	15,84	25,52	39	30,91
Zr		> 2460,49	462,93	> 100,15	> 175,68		>388,62
Ag		ERR	ERR	ERR	ERR		ERR
B		12,32	13,33	3,34	54,48	38	39,57
Be	>42,03	> 1409,48	> 1333,60	ERR	ERR		>638,03
Li	ERR	2,19	1,42	> 33,79	> 80,56		ERR
Sc		ERR	ERR	> 175,12	> 293,66		ERR
Ce		> 65,83	ERR	> 9,71	> 35,97		>151,58
La		> 578,46	> 393,66	> 15,05	ERR		ERR
Y		ERR	ERR	ERR	ERR		

TABLE 4. Up-concentration factor, for the various samples. ERR = under detection limit.

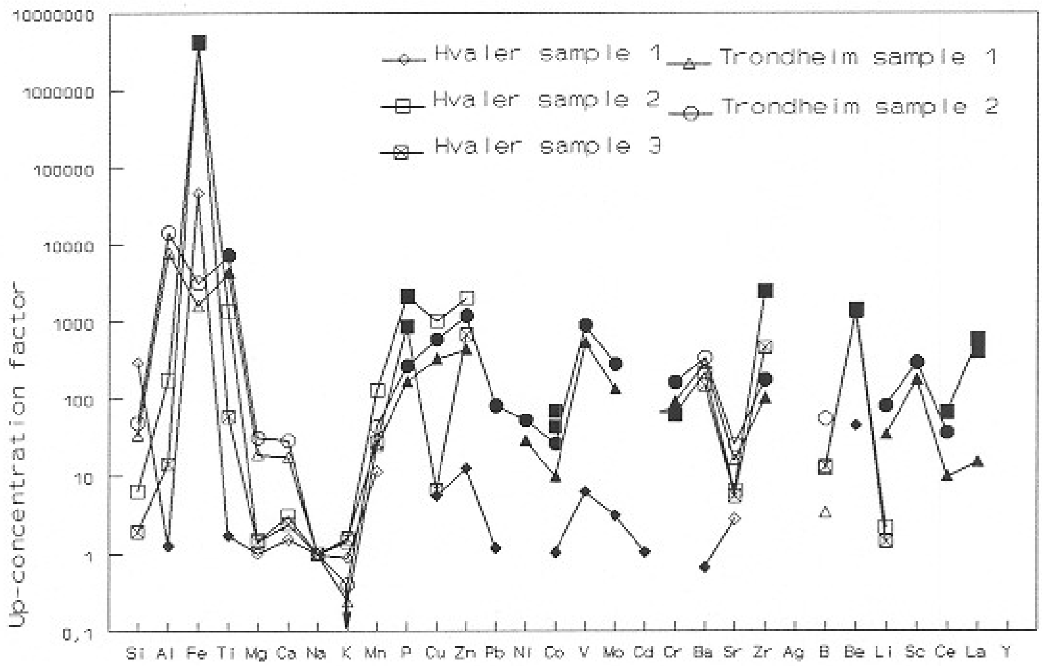
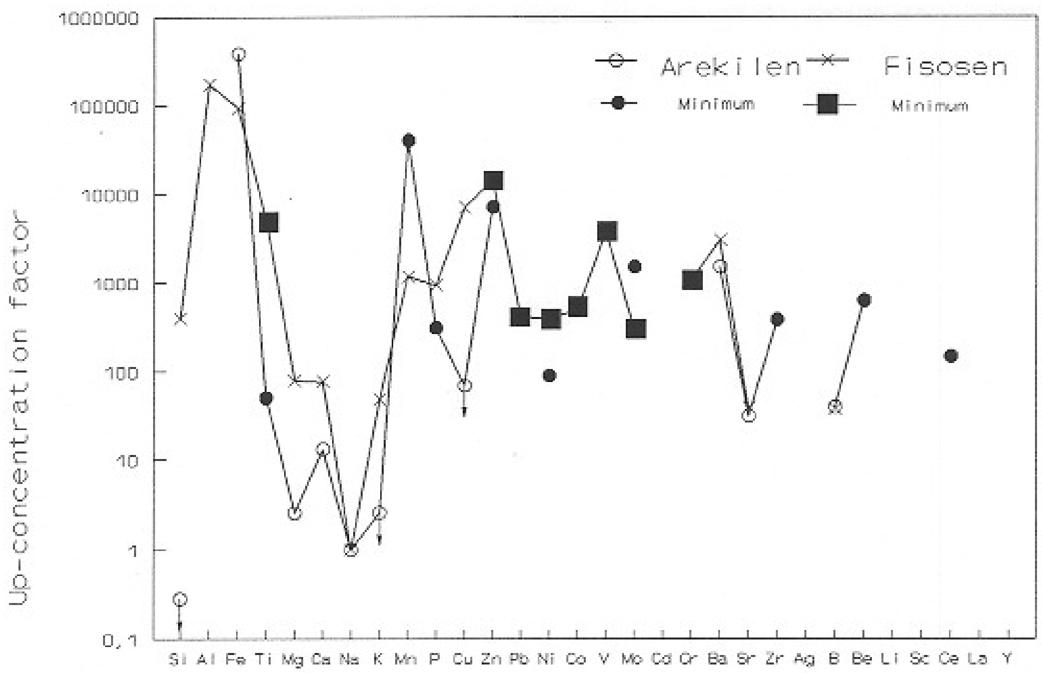


Figure 2a,b: Up-concentration of elements in biofilm relative to groundwater (normalised with respect to sodium). Solid symbols indicate minimum up-concentration factors. Arrows indicate maximum factors.



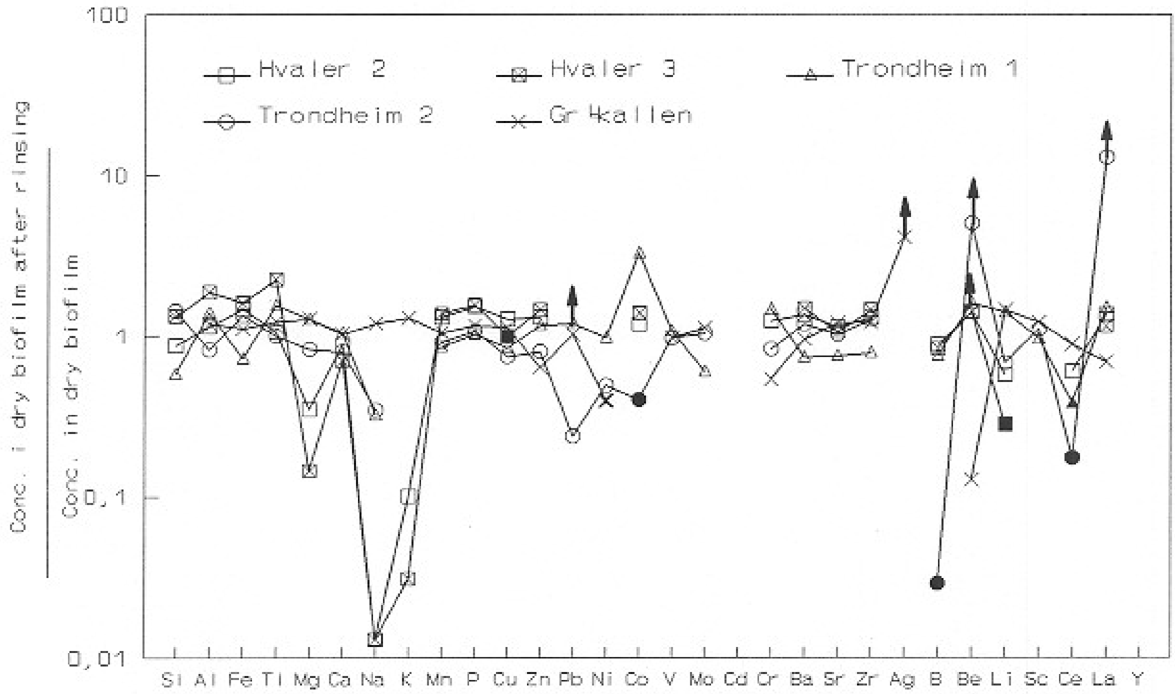
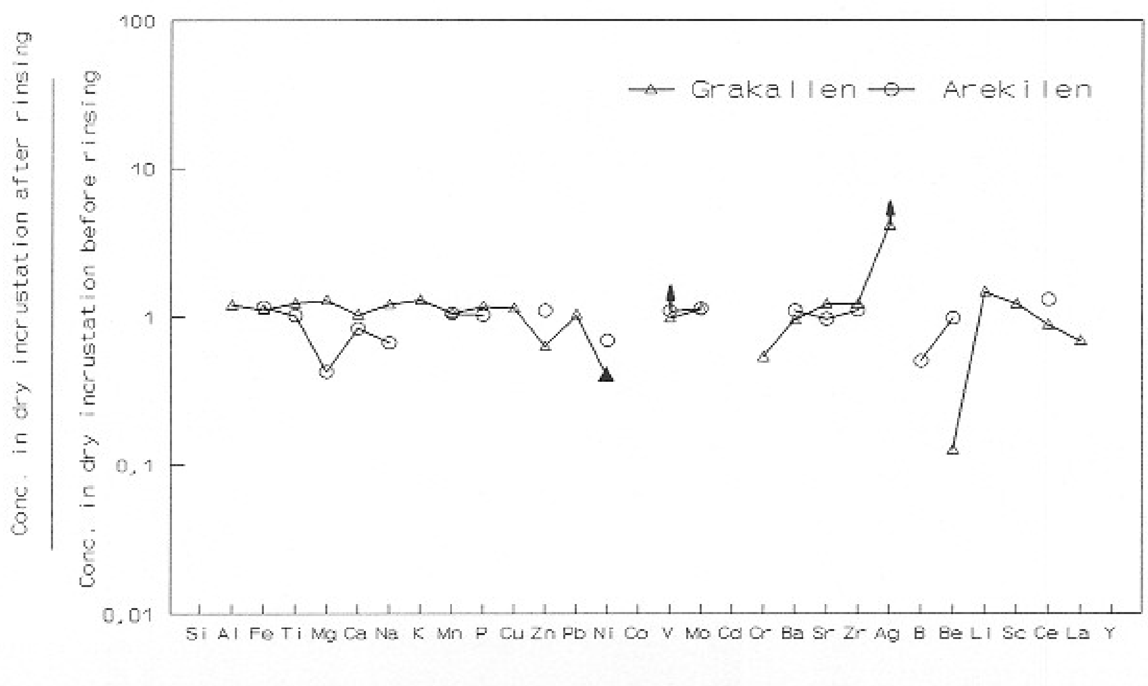


Fig. 3a,b. Ratio of element concentration in rinsed biofilm to unrinsed biofilm. Solid symbols denote maximum values of ratio. Arrows indicate minimum values.



In the apparently inorganic sample from Gråkallen, only Ni and Be appeared to be significantly removed by rinsing, and to a lesser degree Cr and Zn. In the Arekilen sample, only Mg was significantly removed, and to a lesser extent Na, Ni and B.

11. CONCLUSION

Biofilms of MIBs are well known in groundwater boreholes in porous aquifers, but have been relatively sparsely reported from fractured aquifers. NGU's study has, however, registered several occurrences of biofilm formation in tunnels in crystalline rocks, often causing problems with clogging of drainage. Iron-bacteria have also been observed in hard-rock boreholes.

Samples of MIB-bacterial biofilm have been collected and have shown considerable up-concentration of heavy metals, and some other elements, in comparison with groundwater, notably of Fe, Ti, Al, P, Cu, V, Zr, Be, and Zn, often with upconcentration factors of 1000 or more. These metals remain bound in the biofilm during "rinsing" with deionised water.

Mn and Ba often give more modest upconcentration factors of up to several hundred in biofilms. Ni, Pb, Co, Mo, Cr, Sc, Ce and La have factors of at least several tens or hundreds, but were present in groundwater at too low concentrations to allow detection.

The two apparently inorganic incrustations (Gråkallen and Arekilen) also show significant contents and upconcentration factors for many heavy metals. These metals differ sometimes from those which were most significant at Hvaler and SINTEF, Trondheim, (e.g. high upconcentration of Mn in the Arekilen deposit) but this is quite probably due to site-specific reasons. These two incrustations also show higher total iron contents than the known biofilms. Despite being categorised as "apparently inorganic" it is regarded as quite possible that there also is bacterial involvement in the incrustations from Arekilen and Gråkallen, although not distinguished under the microscope.

It may thus be that extracellular co-precipitation with iron is the main mechanism for heavy-metal immobilisation in both biofilm and apparently inorganic samples; i.e. accumulation of heavy metals in biofilms may not be significantly different from truly inorganic iron incrustations. Where biofilms may prove useful in e.g. treatment of heavy metal contaminated water is in acting as biological "catalysts", promoting iron and heavy-metal precipitation in waters with low iron content where inorganic precipitation is thermodynamically unfavorable.

The high degree of accumulation of heavy metals in iron bacterial biofilms is relevant to several practical problems;

(i) the question of toxicity of biofilm residues following rehabilitation of clogged water wells or distribution systems

(ii) the accumulation of heavy metals in iron bacterial biofilms in sewage treatment plants

(iii) the potential for treatment of heavy-metal contaminated waters using iron-bacterial biofilms

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Hvaler biofilm - sample 1			Water leakage ppm	Biofilm[2] "mg/l"	Normalised Biofilm "mg/l"	Accumulation factor
	Filtrate[3] ppm	Filtrate ppm				
	F	FS				
Si	4,08	4	4,52	27500	1354,88	299,75
Al	<10	<10	<10	244	12,02	> 1,20
Fe	0,066	0,077	0,298	282500	13918,31	46705,75
Ti	<0,4	<0,4	<0,4	13,1	0,65	> 1,61
Mg	1200	1200	1100	23000	1133,17	1,03
Ca	1100	1100	1100	34400	1694,83	1,54
Na	7500	7600	7400	150200	7400,11	1,00
K	158,6	139	143,7	2600	128,10	0,89
Mn	3,1	3,1	2,8	645,7	31,81	11,36
Cu	<0,1	<0,1	<0,1	10,7	0,53	> 5,27
Zn	<0,6	<0,6	<0,6	144,2	7,10	> 11,84
Pb	<9	<9	<9	202,3	9,97	> 1,11
Ni	<4	<4	<4	<40	<1,97	ERR
Co	<2	<2	<2	39,5	1,95	> 0,97
V	<0,7	<0,7	<0,7	83,6	4,12	> 5,88
Mo	<1	<1	<1	58,9	2,90	> 2,90
Cd	<0,6	<0,6	<0,6	11,9	0,59	> 0,98
Ba	<2,5	<2,5	<2,5	31,3	1,54	> 0,62
Sr	12,07	12,35	12	675,7	33,29	2,77
Be	<0,1	<0,1	<0,1	85,3	4,20	> 42,03
Li	<0,5	<0,5	<0,5	<5	<0,25	ERR

Appendix 1a. Calculation of up-concentration factors for bacterial biofilm, Hvaler sample 1.

2 Here, the analysis was not rigorously related back to dry weight.

3 Filtrate after filtering biofilm to remove excess water. F = filtered, S = acidified in laboratory.

Hvaler biofilm - 2nd sample			Normalised Biofilm "mg/Kg"	Accumulation factor	Rinsed mg/Kg dry weight	Ratio rinsed/unrinsed
	Water leakage ppm	Biofilm mg/Kg dry weight				
Si	3,31	129	21,02	6,35	113	0,876
Al	1,2	1300	211,83	176,52	1500	1,154
Fe	<0,01	258200	42072,67	> 4207267	383200	1,484
Ti	0,0138	116,6	19,00	1376,78	123,9	1,063
Mg	1100	10100	1645,76	1,50	3500	0,347
Ca	929,1	17400	2835,26	3,05	15300	0,879
Na	9500	58300	9499,76	1,00	753,9	0,013
K	201	1800	293,30	1,46	180,7	0,100
Mn	2,03	1600	260,71	128,43	2100	1,313
P	<0,1	1300	211,83	> 2118,30	2000	1,538
Cu	0,003	18,8	3,06	1021,13	24	1,277
Zn	0,0134	165,9	27,03	2017,37	219,2	1,321
Pb	<0,05	<5	<0,815	ERR	<5	ERR
Ni	<0,04	<2	<0,326	ERR	<2	ERR
Co	<0,01	4,2	0,68	> 68,44	5	1,190
V	<0,005	<1	<0,163	ERR	<1	ERR
Mo	<0,01	<2	<0,326	ERR	<2	ERR
Cd	<0,01	<2	<0,326	ERR	<2	ERR
Cr	<0,01	3,7	0,60	> 60,29	4,6	1,243
Ba	0,0232	30,7	5,00	215,62	42	1,368
Sr	11,71	467	76,10	6,50	531	1,137
Zr	<0,005	75,5	12,30	> 2460,49	98,4	1,303
Ag	<0,01	<1	<0,163	ERR	<1	ERR
B	3,38	255,5	41,63	12,32	225,4	0,882
Be	<0,002	17,3	2,82	> 1409,48	24,6	1,422
Li	0,1564	2,1	0,34	2,19	1,2	0,571
Sc	<0,002	<0,5	<0,081	ERR	<0,5	ERR
Ce	<0,05	20,2	3,29	> 65,83	12,1	0,599
La	<0,01	35,5	5,78	> 578,46	48,7	1,372
Y	<0,002			ERR		

Appendix 1b. Calculation of up-concentration factors for bacterial biofilm, Hvaler sample 2.

Hvaler biofilm - sample 3		Biofilm mg/Kg dry weight	Normalised Biofilm "mg/Kg"	Accumulation factor	Rinsed mg/Kg dry weight	Ratio rinsed/unrinsed
	Water leakage ppm					
Si	4,06	55,6	7,93	1,95	74,1	1,33
Al	1,24	124,5	17,76	14,32	235,1	1,89
Fe	<0,01	289200	41248,88	> 4124888	468700	1,62
Ti	0,0233	9,6	1,37	58,77	21,7	2,26
Mg	1100	11100	1583,20	1,44	1600	0,14
Ca	1400	23900	3408,88	2,43	16800	0,70
Na	9000	63100	9000,01	1,00	822,4	0,013
K	161,1	1800	256,74	1,59	55,4	0,031
Mn	2,77	532,5	75,95	27,42	739,9	1,39
P	<0,1	601,8	85,84	> 858,35	949,9	1,58
Cu	0,0043	0,2	0,03	6,63	<0.2	< 1,00
Zn	0,013	62,6	8,93	686,82	91,7	1,46
Pb	<0,05	<5	< 0,713	ERR	<5	ERR
Ni	<0,04	<2	<0,285	ERR	<2	ERR
Co	<0,01	3	0,43	> 42,79	4,2	1,40
V	<0,005	<1	<0,143	ERR	<1	ERR
Mo	<0,01	<2	<0,285	ERR	<2	ERR
Cd	<0,01	<2	<0,285	ERR	<2	ERR
Cr	<0,01	<1	<0,143	ERR	<1	ERR
Ba	0,0287	30	4,28	149,09	44,6	1,49
Sr	12,87	498,5	71,10	5,52	527,6	1,06
Zr	0,0057	18,5	2,64	462,93	26,9	1,45
Ag	<0,01	<1	<0,143	ERR	<1	ERR
B	2,59	242,1	34,53	13,33	201,5	0,83
Be	<0,002	18,7	2,67	> 1333,60	26,5	1,42
Li	0,181	1,8	0,26	1,42	<0,5	< 0,28
Sc	<0,002	<0,5	<0,0713	ERR	<0,5	ERR
Ce	<0,05	<3	<0,428	ERR	<3	ERR
La	<0,01	27,6	3,94	> 393,66	31,9	1,16
Y	<0,002			ERR		ERR

Appendix 1c. Calculation of up-concentration factors for bacterial biofilm, Hvaler sample 3.

SINTEF Biofilms		**Biofilm 1**	Normalised Biofilm	Accumulation Factor	Rinsed Biofilm	Ratio rinsed / unrinsed	**Biofilm 2**	Normalised Biofilm	Accumulation Factor	Rinsed Biofilm	Ratio rinsed / unrinsed
	Water leakage										
	ppm	mg / Kg dry weight	"mg / Kg"		mg / Kg dry weight		mg / Kg dry weight	"mg / Kg"		mg / Kg dry weight	
Si	2,88	3100	95,238	33,07	1800	0,58	2700	140,333	48,73	3900	1,44
Al	0,0264	6700	205,837	7796,86	9400	1,40	7300	379,418	14371,89	6000	0,82
Fe	2,38	124500	3824,885	1607,09	89700	0,72	144300	7500,000	3151,26	194100	1,35
Ti	<0,01	1400	43,011	>4301,08	2200	1,57	1400	72,765	>7276,51	1400	1,00
Mg	12,91	8000	245,776	19,04	10300	1,29	7700	400,208	31,00	6400	0,83
Ca	51,75	29700	912,442	17,63	31300	1,05	28400	1476,091	28,52	22700	0,80
Na	67,58	2200	67,588	1,00	708,8	0,32	1300	67,568	1,00	448,4	0,34
K	5,2	<40	<1,229	<0,24	<40	ERR	<40	<2,079	<0,40	<40	ERR
Mn	1,43	1100	33,794	23,63	950,8	0,86	1200	62,370	43,62	1100	0,92
P	<0,1	541,6	16,639	>166,39	558,4	1,03	513,2	26,674	>266,74	553,4	1,08
Cu	<0,002	21,5	0,661	>330,26	18,2	0,85	22,5	1,169	>584,72	16,8	0,75
Zn	<0,005	69,9	2,147	>429,49	82,4	1,18	113,3	5,889	>1177,75	90,3	0,80
Pb	<0,05	<10	<0,307	ERR	12,1	>1,21	77,7	4,038	>80,77	18,4	0,24
Ni	<0,04	36,9	1,134	>28,34	36	0,98	40,2	2,089	>52,23	19,7	0,49
Co	<0,01	3,2	0,098	>9,83	10,5	3,28	5	0,260	>25,99	<2	<0,40
V	<0,005	87,2	2,679	>535,79	93,7	1,07	85,3	4,433	>886,69	83,1	0,97

Mo	<0,01	43	1,321	>132,10	25,8	0,60	54	2,807	>280,67	56,4	1,04
Cd	<0,01	<4	<0,123	ERR	<4	ERR	<4	<0,208	ERR	<4	ERR
Cr	<0,01	29	0,891	>89,09	42,9	1,48	31,2	1,622	>162,16	26	0,83
Ba	0,0776	707,4	21,733	280,06	520,8	0,74	503,8	26,185	337,44	598,2	1,19
Sr	0,1823	94	2,888	15,84	70,8	0,75	89,5	4,652	25,52	91,9	1,03
Zr	<0,005	16,3	0,501	>100,15	12,8	0,79	16,9	0,878	>175,68	21,4	1,27
Ag	<0,01	<2	<0,0614	ERR	<2	ERR	<2	<0,104	ERR	<2	ERR
B	0,0663	7,2	0,221	3,34	5,4	0,75	69,5	3,612	54,48	<2	<0,03
Be	<0,002	<1	<0,0307	ERR	1,6	>1,60	<1	<0,052	ERR	5	>5,00
Li	<0,002	2,2	0,068	>33,79	3,1	1,41	3,1	0,161	>80,56	2,1	0,68
Sc	<0,002	11,4	0,350	>175,12	11	0,96	11,3	0,587	>293,66	12,5	1,11
Ce	<0,05	15,8	0,485	>9,71	<6	<0,38	34,6	1,798	>35,97	<6	<0,17
La	<0,01	4,9	0,151	>15,05	7,4	1,51	<1	<0,052	ERR	12,9	>12,90
Y	<0,002		0,000	ERR		ERR			ERR		ERR

Appendix 1d. Calculation of up-concentration factors for bacterial biofilms. Trondheim (SINTEF) samples 1 & 2.

Gråkallen	Biofilm	Rinsed	Ratio Rinsed/Unrinsed
	mg/Kg dry weight	mg/Kg dry weight	
Si	<2	<2	ERR
Al	2000	2400	1,20
Fe	343800	383400	1,12
Ti	751	924,4	1,23
Mg	1400	1800	1,29
Ca	520,9	536,7	1,03
Na	302,2	364,8	1,21
K	771,2	1000	1,30
Mn	107,7	113,3	1,05
P	1200	1400	1,17
Cu	367,6	419,2	1,14
Zn	189,9	120,5	0,63
Pb	98,7	101,3	1,03
Ni	5,1	<2	<0,39
Co	<1	<1	ERR
V	20,5	20,1	0,98
Mo	98,2	110,7	1,13
Cd	<2	<2	ERR
Cr	8,6	4,6	0,53
Ba	60,5	57,8	0,96
Sr	13,4	16,3	1,22
Zr	68,3	83,4	1,22
Ag	<1	4,1	>4,10
B	<1	<1	ERR
Be	8,7	1,1	0,13
Li	1,3	1,9	1,46
Sc	0,9	1,1	1,22
Ce	69,6	61,6	0,89
La	2,9	2	0,69
Y			ERR

Appendix 1e. Calculation of up-concentration factors for iron incrustation, Gråkallen.

Arekilen	Water	Incrustation	Normalised	Accumulation Factor	Rinsed Incrustation	Ratio rinsed/unrinsed
	ppm	mg/Kg dry weight	mg/Kg		mg/Kg dry weight	
Si	6,87	<20	<1,93	<0,28	<20	
Al	<0,02	<20	<1,93		<20	
Fe	0,1273	504200	48741,41	382886,14	582100	1,15
Ti	<0,01	5,2	0,50	>50,27	5,3	1,02
Mg	7,88	211,7	20,47	2,60	89,8	0,42
Ca	101,8	13900	1343,72	13,20	11600	0,83
Na	23,52	243,3	23,52	1,00	162,9	0,67
K	7,4	<200	<19,33	<2,61	<200	
Mn	<0,002	845,8	81,76	>40882,07	889,3	1,05
P	<0,1	324,4	31,36	>313,60	332,5	1,02
Cu	0,0028	<2	<0,19	<69,05	<2	
Zn	<0,005	375,9	36,34	>7267,71	411,9	1,10
Pb	<0,05	<50	<4,83		<50	
Ni	<0,04	37,3	3,61	>90,15	25,8	0,69
Co	<0,01	<10	<0,97		<10	
V	<0,005	<10	<0,97		10,9	>1,09
Mo	<0,01	157,8	15,25	>1525,46	178,2	1,13
Cd	<0,01	<20	<1,93		<20	
Cr	<0,01	<10	<0,97		<10	
Ba	0,0131	207	20,01	1527,55	227,3	1,10
Sr	0,5367	171,6	16,59	30,91	165,5	0,96
Zr	<0,005	20,1	1,94	>388,62	22,2	1,10
Ag	<0,01	<10	<0,97		<10	
B	0,0623	25,5	2,47	39,57	12,8	0,50
Be	<0,002	13,2	1,28	>638,03	12,9	0,98
Li	<0,002	<5	<0,48		<5	
Sc	<0,002	<5	<0,48		<5	
Ce	<0,05	78,4	7,58	>151,58	102,2	1,30
La	<0,01	<5	<0,48		<5	
Y	<0,002					ERR

Appendix 1f. Calculation of up-concentration factors for iron incrustation, Arekilen well.

Fisosen	Water	Biomass	Normalised Biomass	Accumulation Factor
	ppm	mg/Kg dry weight	"mg/Kg"	
Si	2,56	2000	1016,59	397
Al	0,0455	15600	7929,39	174272
Fe	0,0152	2800	1423,22	93633
Ti	<0,01	100	50,83	>5083
Mg	39,38	6100	3100,59	79
Ca	151	23000	11690,76	77
Na	864,1	1700	864,10	1
K	43,99	4200	2134,84	49
Mn	0,0432	100	50,83	1177
P	1,24	2300	1169,08	943
Cu	0,0024	34	17,28	7201
Zn	<0,005	145	73,70	>14741
Pb	<0,05	42	21,35	>427
Ni	<0,04	31	15,76	>394
Co	<0,01	10	5,08	>508
V	<0,005	37	18,81	>3761
Mo	<0,01	6	3,05	>305
Cd	<0,01			
Cr	<0,01	21	10,67	>1067
Ba	0,3658	2200	1118,25	3057
Sr	6,08	469	238,39	39
Zr	<0,005			
Ag	<0,01			
B	0,9434	70	35,58	38
Be	<0,002			
Li	1,11			
Sc	<0,002			
Ce	<0,05			
La	<0,01			
Y	<0,002			

Appendix 1g. Calculation of up-concentration factors for biomass, Fisosen spring.

Appendix 2. Analytical results, Arekilen well groundwater

Resultat: Prøvemrk.	Ledn.evne µS/cm	pH	Alkalitet mmol/l
"Arekilen"	662	7.58	3.77

Prøve nr	F^-	Cl^-	NO_2^-	Br^-	NO_3^-	PO_4^{3-}	SO_4^{2-}
1	532 ppb	46.7ppm	<500 ppb	144 ppb	20.5ppm	<200 ppb	70.6ppm

	AREKILEN	AREKI. S
Si	6.87 ppm	6.79 ppm
Al	<20.0 ppb	27.0 ppb
Fe	127.3 ppb	3.12 ppm
Ti	<10.0 ppb	<10.0 ppb
Mg	7.88 ppm	8.20 ppm
Ca	101.8 ppm	106.1 ppm
Na	23.52 ppm	23.64 ppm
K	7.40 ppm	6.93 ppm
Mn	< 2.0 ppb	15.2 ppb
P	<100.0 ppb	160.7 ppb
Cu	2.8 ppb	6.4 ppb
Zn	< 5.0 ppb	16.9 ppb
Pb	<50.0 ppb	<50.0 ppb
Ni	<40.0 ppb	<40.0 ppb
Co	<10.0 ppb	<10.0 ppb
V	< 5.0 ppb	< 5.0 ppb
Mo	<10.0 ppb	<10.0 ppb
Cd	<10.0 ppb	<10.0 ppb
Cr	<10.0 ppb	<10.0 ppb
Ba	13.1 ppb	17.1 ppb
Sr	536.7 ppb	561.5 ppb
Zr	< 5.0 ppb	5.2 ppb
Ag	<10.0 ppb	<10.0 ppb
B	62.3 ppb	55.7 ppb
Be	< 2.0 ppb	< 2.0 ppb
Li	< 2.0 ppb	< 2.0 ppb
Sc	< 2.0 ppb	< 2.0 ppb
Ce	<50.0 ppb	<50.0 ppb
La	<10.0 ppb	<10.0 ppb
Y	< 2.0 ppb	< 2.0 ppb

Neither of samples was filtered in field. AREKI.S is acidified in lab. This may lead to exaggerated results for some parameters. The unacidified results are thus used in the context of this report.

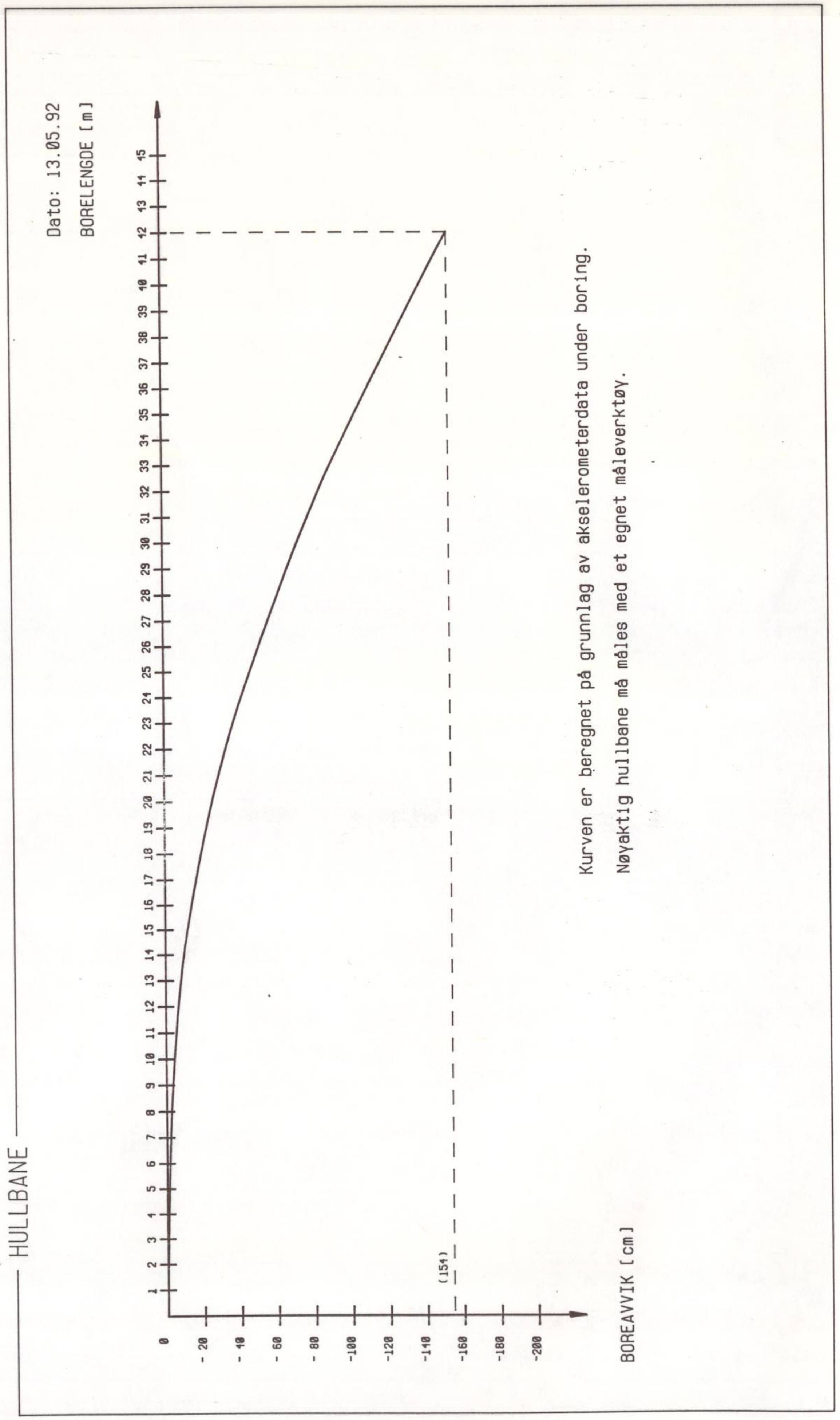
Appendix 3. Analytical results, Fisosen groundwater

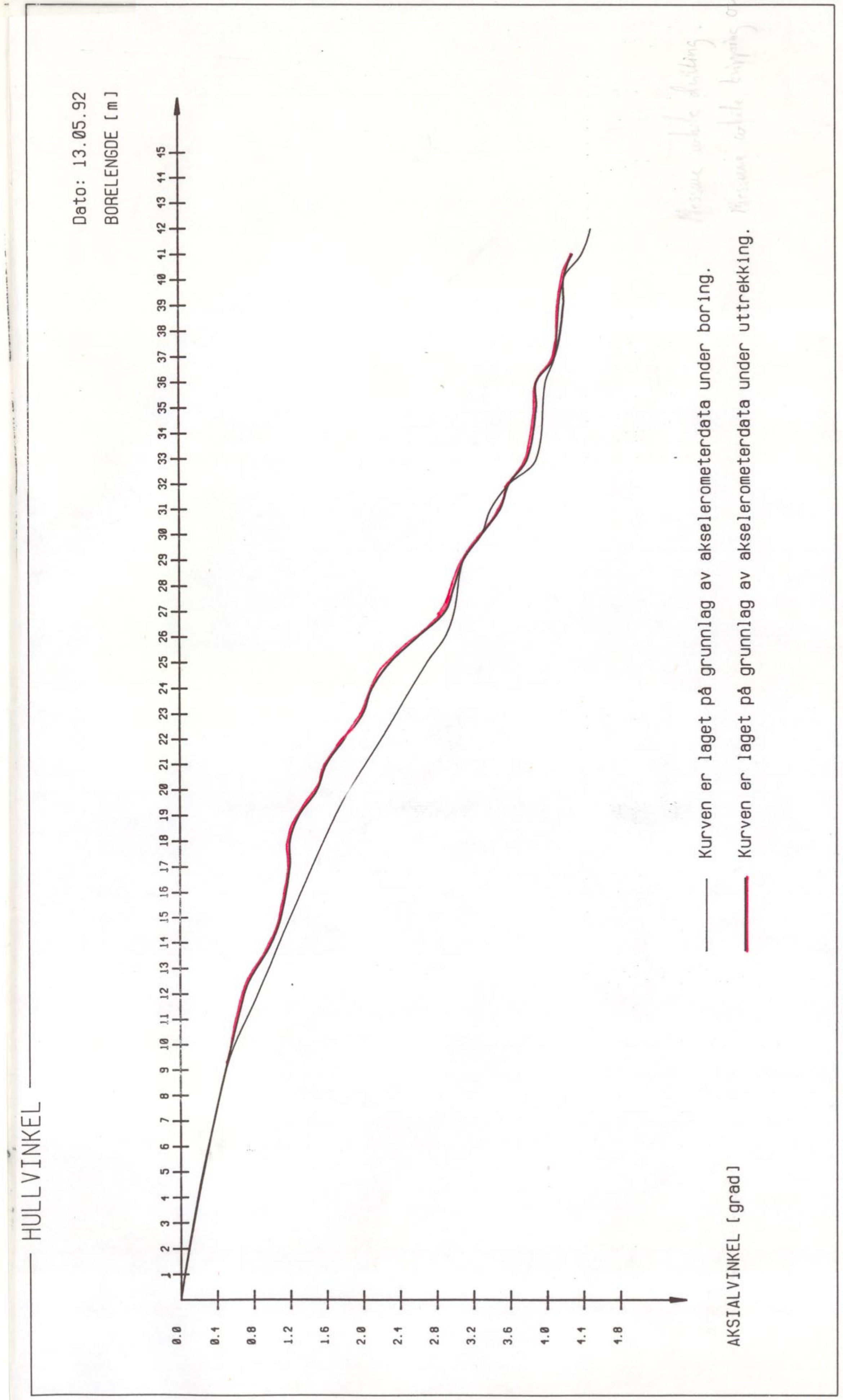
Prøve nr	F^-	Cl^-	NO_2^-	Br^-	NO_3^-	PO_4^{3-}	SO_4^{2-}
1	<500 ppb	0.16 %	<25.0ppm	4.04ppm	<500 ppb	2.51ppm	114 ppm

	FISOSEN
Si	2.56 ppm
Al	45.5 ppb
Fe	15.2 ppb
Ti	<10.0 ppb
Mg	39.38 ppm
Ca	151.0 ppm
Na	864.1 ppm
K	43.99 ppm
Mn	43.2 ppb
P	1.24 ppm
Cu	2.4 ppb
Zn	< 5.0 ppb
Pb	<50.0 ppb
Ni	<40.0 ppb
Co	<10.0 ppb
V	< 5.0 ppb
Mo	<10.0 ppb
Cd	<10.0 ppb
Cr	<10.0 ppb
Ba	365.8 ppb
Sr	6.08 ppm
Zr	< 5.0 ppb
Ag	<10.0 ppb
B	943.4 ppb
Be	< 2.0 ppb
Li	1.11 ppm
Sc	< 2.0 ppb
Ce	<50.0 ppb
La	<10.0 ppb
Y	< 2.0 ppb

Sample for ICP neither filtered nor acidified.

Appendix 4. Details of contruction - SINTEF borehole.





Appendix 5. Analytical results - SINTEF borehole.

Anmerkninger : *) Det inneholder endel uløste fragmenter, og da blir den oppgitte alkalitet noe usikker.

Utført : v/TB. 19.11.92

Resultat: Prøvemrk.	Ledn.evne µS/cm	pH	Alkalitet mmol/l
Sintef 1	662	7.32	3.47 *

Prøve nr	F^-	Cl^-	NO_2^-	Br^-	NO_3^-	PO_4^{3-}	SO_4^{2-}
1	<50.0ppb	110 ppm	<1.00ppm	306 ppb	<50.0ppb	<200 ppb	18.3ppm

SINTEF 1-FS

Si	2.88	ppm
Al	26.4	ppb
Fe	2.38	ppm
Ti	<10.0	ppb
Mg	12.91	ppm
Ca	51.75	ppm
Na	67.58	ppm
K	5.20	ppm
Mn	1.43	ppm
P	<100.0	ppb
Cu	< 2.0	ppb
Zn	< 5.0	ppb
Pb	<50.0	ppb
Ni	<40.0	ppb
Co	<10.0	ppb
V	< 5.0	ppb
Mo	<10.0	ppb
Cd	<10.0	ppb
Cr	<10.0	ppb
Ba	77.6	ppb
Sr	182.3	ppb
Zr	< 5.0	ppb
Ag	<10.0	ppb
B	66.3	ppb
Be	< 2.0	ppb
Li	< 2.0	ppb
Sc	< 2.0	ppb
Ce	<50.0	ppb
La	<10.0	ppb
Y	< 2.0	ppb

Sample for ICP field filtered and acidified.

Appendix 6. Analytical reference numbers

Internal analytical reference numbers at NGU

	Sample no.	Job no.
Hvaler biofilm 1, filtrate	1F, 1FS (acidified)	59/90[4]
Hvaler biofilm 1	BIOF	59/90
Hvaler biofilm 1, groundwater	2	59/90[4]
Hvaler biofilm 2	91-8	104/91
Hvaler biofilm 2, rinsed	91-8R	104/91
Hvaler biofilm 2, groundwater	91-8F (filtered and acidified)	105/91
Hvaler biofilm 3	91-9	104/91
Hvaler biofilm 3, rinsed	91-9R	104-91
Hvaler biofilm 3, groundwater	91-9F (filtered and acidified)	105/91
SINTEF, groundwater	SINTEF1 [-FS (filtered + acidified)]	155/92
SINTEF, biofilm sample 1	SINTEF2	155/92
SINTEF, biofilm sample 1, rinsed	SINTEF2R	155/92
SINTEF, biofilm sample 2	SINTEF3	155/92
SINTEF, biofilm sample 2, rinsed	SINTEF3R	155/92
Gråkallen, incrustation	GRÅK-1	37/92
Gråkallen, incrustation, rinsed	GRÅK-1-R	37/92
Arekilen, groundwater	Arekilen, Areki-S (acidified)	80/92
Arekilen, incrustation	AREBIO-U	80A/92
Arekilen, incrustation, rinsed	AREBIO-R	80A/92
Fisosen, groundwater	FISOSEN (not acidified)	117/93
Fisosen, biomass		117/93

[4] For 1F, 1FS and 2, the content of Fe, Mn and Si was determined seperately:

	1F	1FS	2
Fe	66 ppb	77 ppb	298 ppb
Mn	3.1 ppm	3.1 ppm	2.8 ppm
Si	4.08 ppm	4.00 ppm	4.52 ppm