TECHNICAL
REPORT

95-25

Sulphate reduction in the Äspö HRL tunnel

Marcus Laaksoharju (ed.)

GeoPoint AB, Sollentuna, Sweden

December 1995

SVENSK KÄRNBRÄNSLEHANTERING AB
SWEDISH NUCLEAR FUEL AND WASTE MANAGEMENT CO

P.O.BOX 5864 S-102 40 STOCKHOLM SWEDEN
PHONE +46 8 665 28 00 TELEX 13108 SKB
FAX +46 8 661 57 19

SULPHATE REDUCTION IN THE ÄSPÖ HRL TUNNEL

Marcus Laaksoharju (ed.)

GeoPoint AB, Sollentuna, Sweden

December 1995

Information on SKB technical reports from1977-1978 (TR 121), 1979 (TR 79-28), 1980 (TR 80-26), 1981 (TR 81-17), 1982 (TR 82-28), 1983 (TR 83-77), 1984 (TR 85-01), 1985 (TR 85-20), 1986 (TR 86-31), 1987 (TR 87-33), 1988 (TR 88-32), 1989 (TR 89-40), 1990 (TR 90-46), 1991 (TR 91-64), 1992 (TR 92-46), 1993 (TR 93-34) and 1994 (TR 94-33) is available through SKB.

SULPHATE REDUCTION IN THE ÄSPÖ HRL TUNNEL

Marcus Laaksoharju (Editor), GeoPoint AB

December 1995

Keywords: Bacterial sulphate reduction, sulphate reduction in sea bed sediments, sulphate reduction in shallow and deep groundwater, sulphate reduction in fracture minerals, sulphate reduction and water flow.

FOREWORD

This report is the result of a sub-project initiated during the construction of the Äspö HRL tunnel. A working group was formed to sort out the questions of why, where and to what extent microbial sulphate reduction was occurring. Experts in the disciplines of microbiology, geochemistry, isotope geochemistry, hydrochemistry and hydrogeology were represented. The group consisted of the following persons (in alphabetical order) whose contributions are included in the report:

Gunnar Gustafson (hydrogeology), CTH/VBB VIAK

Marcus Laaksoharju (editor, hydrochemistry), GeoPoint AB

Karsten Pedersen (microbiology), Göteborgs Universitet

Ingvar Rhén (hydrogeology), VBB Viak

Christina Skårman (multivariate statistics), GeoPoint AB

Eva-Lena Tullborg (geology, isotope chemistry), Terralogica AB

Bill Wallin (isotope chemistry), Geokema AB

Peter Wikberg (project leader), SKB

ABSTRACT

English

Sulphate reduction is a well studied process in sea bed sediments, but is less known in deep groundwaters. Sulphate-reducing bacteria can bring about the reduction of sulphate to sulphide using for example organic substances present in natural groundwater as reducing agents. Sulphide production is of particular interest for disposal of spent fuel in copper canisters because sulphide is in fact the only substance present in deep groundwater that will cause the corrosion of copper. Oxygen, another copper-corrodant is not present in deep groundwater and sulphate will not react with copper unless microbes reduce it to sulphide.

Evidence and indications of sulphate reduction based on geological, hydrogeological, groundwater, isotope and microbial data gathered in and around the Äspö Hard Rock Laboratory (HRL) tunnel have been evaluated.

This integrated investigation showed that sulphate reduction had taken place in the past but is most likely also an ongoing process. Anaerobic sulphate-reducing bacteria can live in marine sediments, in the tunnel sections under the sea and in deep groundwaters, since there is no access to oxygen. The sulphate-reducing bacteria seem to thrive when the Cl^- concentration of the groundwater is 4000-6000 mg/l. Sulphate reduction is an in situ process but the resulting hydrogen-sulphide-rich water can be transported to other locations. A more vigorous sulphate reduction takes place when the organic content in the groundwater is high (>10 mg/l, DOC) which is the case in the sediments and in the groundwaters under the sea. Some bacteria use hydrogen (geogas) as an electron donor instead of organic carbon and can therefore live in deep environments where the access to organic material is limited. The sulphate-reducing bacteria seem to adapt to changing flow situations caused by the tunnel construction relatively fast. Sulphate reduction seems to have occurred and will probably occur where conditions are favourable for the sulphate-reducing bacteria such as anaerobic brackish groundwater with dissolved sulphate and organic carbon or hydrogen.

Swedish

Sulfatreduktion är en välkänd process i marina sediment men mindre känd i djupa grundvatten. Sulfatreducerande bakterier kan reducera sulfat till sulfid genom att oxidera det organiska material som finns löst i grundvatten. Sulfidproduktionen är av stort intresse för deponeringen av utbränt bränsle i kopparkapslar, ty sulfid är det enda ämnet i djupa grundvatten som direkt orsakar korrosion av koppar. Syre, som är en annan koppar korrodant, finns inte i djupa grundvatten och sulfat reagerar inte med koppar om inte bakterier först reducerar det till sulfid.

Tecken och bevis på sulfatreduktion baserade på geologiska, hydrogeologiska, mikrobiella, grundvatten- och isotopundersökningar av Äspöberglaboratoriet (HRL) med omgivningar har utvärderats.

Den samlade undersökningen visar att sulfatreduktion sannolikt är en pågående process som även ägt rum långt tillbaka i tiden. Anaeroba sulfatreducerande bakterier kan leva i havssedimenten ovanför tunneln, i berget kring tunneldelen som går under havet och i djupa grundvatten, eftersom dessa platser är skyddade från intrång av syre. Bakterierna tycks föredra en salthalt där kloridkoncentration är 4000-6000 mg/l. Sulfatreduktion är en in situprocess, men reaktionsprodukterna löst i vatten kan transporteras till andra ställen. En kraftig sulfatreduktion uppstår då mängden löst organiskt material är hög (>10 mg/l, DOC), såsom i sedimenten och i grundvattnen under havet. Vissa bakterier använder vätgas (geogas) som elektrondonator (reduktionsmedel) i stället för organiskt material, vilket gör att de kan leva på större djup där tillgången på organiskt material är begränsad. De sulfatreducerande bakterierna tycks anpassa sig relativt snabbt (några år) till förändrade grundvattenflöden förorsakade av t.ex. ett tunnelbygge. Bakteriell sulfatreduktion tycks ha ägt rum och kommer troligen även att äga rum i anaeroba bräckta grundvatten med tillgång på löst sulfat och organiskt material eller vätgas.

CONTENTS

EXECUTIVE SUMMARY

Sulphate-Reducing Bacteria (SRB) can reduce sulphate to sulphide using organic substances in natural groundwater as reducing agents (electron donors) according to the reaction:

$$SO_4^{2-} + 2(CH_2O) + OH^- \Rightarrow HS^- + 2HCO_3^- + H_2O$$

Since sulphide can corrode a copper canister placed in a reducing 500m deep environment, a process generating sulphide has to be known in such detail that a repository can be located without the risk of extensive sulphide generation at the copper canister surface.

Sulphate reduction is indicated by the presence of SRB or by analysing the amount of sulphide generated in the groundwater. The sampling and identification of SRB cannot generally be done due to economical and practical restrictions. The reactivity of HS^- leads to special requirements for sampling and analyses. The lack of HS^- data from the boreholes in the Äspö Hard Rock Laboratory (HRL) tunnel in combination with relatively few samples of bacteria made it necessary to search for additional signs of bacterial sulphate reduction.

A task force was established to search for evidence and indications of sulphate reduction based on the integration of geology, hydrogeology, hydrochemistry, isotope chemistry (stable isotopes of carbon and sulphur) and microbiology in the HRL tunnel and in the near vicinity. The aim was to describe where, why and to what extent massive sulphate reduction occurs or has occurred in the HRL tunnel.

The difference in number, type and distribution of the sampling in both time and space made the data integration between the disciplines complicated. A further complication was the dynamism of the groundwater system due to the tunnel construction. The result from the microbial measurements was used as the only direct evidence for the presence of sulphate reduction. The results from the other disciplines were used as indications of sulphate reduction. The results from the integrated study is summarised below.

Geological indications: Sulphate reduction produces sulphide and bicarbonate which can result in the formation of carbonate minerals such as calcite and iron sulphide minerals (eg, pyrite) as fracture coatings. Calculated saturation indexes confirmed that the groundwater in the tunnel was supersaturated with respect to these minerals in most of the water samples. The geological investigations confirmed that 30% of the fracture fillings is calcite. Pyrite on the other hand was only found in small amounts in the fractures of the tunnel. This would indicate that the sulphide produced is either concentrated at other locations or that precipitated sulphides are lost as colloids. A high content of S in the colloid phase was obtained during sampling in the HRL tunnel.

Sea bed sediments consist of clay, gravel and gyttja (organic rich mud). The most interesting observation is the organic component (~30%) in gyttja which can be used for sulphate reduction. The sulphate source is either from the pore water of the sediments or from the seawater lying above. A smell of H_2S was reported during sampling which indicated an ongoing sulphate reduction in the sediments which was supported by the results from isotope, pore water and microbiological sampling of the sediments.

Hydrogeological indications: Groundwater flow modelling was used to address the question of whether sulphate reduction occurs in the water-filled rock fractures around the tunnel or if the sulphate reduction takes place in the sea bed sediments and the resulting hydrogen sulphide water is transported into the boreholes in the HRL tunnel. The calculation showed that it is possible that Baltic Sea water passes through the sea bed sediments where sulphate reduction occurs. The resulting water modified by sulphate reduction can within 100-400 days be transported into the HRL tunnel so that the portion of this water is 25%. The other water portions consist of water which probably has not undergone considerable sulphate reduction. The effect of sulphate reduction taking place in the sediments can be measured in the HRL tunnel approximately within one year, depending on the prevailing hydraulic conductivity of the fracture zones.

Groundwater chemical indications: Groundwater chemistry was used to indicate obvious sulphate reduction despite the lack of HS^- analytical data. The groundwaters in the HRL tunnel can be subdivided into a sulphate-rich water (SO_4-type) and a bicarbonate-rich water (HCO_3-type). Sulphate reduction is more likely to have occurred in the HCO_3-type of water than in the SO_4-type of groundwater, since sulphate is consumed and carbonate produced by the reaction.

In order to understand if the sulphate reduction takes place in the sampling point or if the water is transported from other locations multivariate mixing-mass balance calculations were performed. Obvious sulphate reduction in the groundwater is indicated by: abnormally low SO_4^{2-} content and abnormally high HCO_3^- content which can be defined as a mole ratio $[HCO_3^-]/[SO_4^{2-}] > 1.27$ after compensating for mass transport by mixing. The compensation is done by means of multivariate mixing calculations. The calculations showed that modern Baltic sea water, through sulphate reduction, can form the same water type found in both the sea bed sediments and in the HRL tunnel. The type of groundwater undergoing obvious sulphate reduction was named Modified Baltic Sea water. Sulphate reduction was indicated to be an ongoing process and can take place both in the sediments and also in the groundwater. The calculations indicated further that sulphate reduction occurs in the HRL boreholes at 900-1300m tunnel length and on days 350-950 of the tunnel construction. SRB were detected in this tunnel section. The potential for obvious sulphate reduction seemed to decrease with time. Sulphate reduction can then still take place but to a minor extent. A decrease due to increased influx seems to have made the environment less favourable for the SRB.

Obvious sulphate reduction seemed further to be favoured by a Cl^- concentration of 4000-6000 mg/l. This observation was consistent with laboratory experiments on SRB isolates from Äspö HRL, which show a preference for water with a similar salinity. Obvious sulphate reduction was mostly observed when the DOC (dissolved organic carbon) content was >10 mg/l in the evaluated groundwaters. Mass balance calculations showed that sulphate reduction may occur even at a low (0.6 mg/l), DOC content. Recent investigations showed high numbers of SRB in boreholes containing 2.8-4.0 mg/l DOC. These bacteria may on the other hand use hydrogen as an electron donor instead of organic carbon. They have therefore not necessarily been limited by the low content of organic carbon in the groundwater. The multivariate mixing mass balance calculations demonstrated that an understanding of the fluxes of compounds, rather than measurements of concentrations only, is necessary for modelling sulphate consumption (the electron acceptor) and carbon dioxide production by SRB. The same argument is valid for the energy sources that SRB can utilise ie, low weight organic carbon compounds and hydrogen. Prediction of SRB activity over time must also include fluxes of these compounds since they limit the rate with which SRB can grow and produce hydrogen sulphide. These fluxes have not yet been determined for these observations. It is therefore premature to establish a limit for the organic matter. This type of sulphate reduction process is however usually less vigorous than when organic carbon is consumed.

The conclusion is that there is a potential for a more vigorous sulphate reduction with a higher influx of organic carbon with the groundwater and with a salinity interval of 4000-6000 mg/l Cl. This is indicated by the decreasing DOC content of the groundwater with time during the construction of the HRL tunnel. The decrease may have lowered SRB activity and led to a less clear groundwater response.

Isotopical indications: Sulphur isotope ($\delta^{34}S$) data are used as indicators of past and present sulphate reduction. Sulphur isotopes have been analysed in sea bed sediments containing monosulphides (FeS), dissolved sulphate (SO_4^{2-}) in the pore water, fracture filling mineral pyrite (FeS_2) and in dissolved sulphate (SO_4^{2-}) of the groundwater at depths ranging from 2-1000m.

The pore water SO_4^{2-} from the upper part of the sediments displayed homogeneous $\delta^{34}S$ values of about +19.0 and +19.8‰ similar to the SO_4^{2-} in the Baltic sea water (approx. +19‰). These are typical marine signatures for the SO_4^{2-}. The FeS collected from the upper parts of the marine sediments showed $\delta^{34}S$ values between -0.3 and -2.3‰. These isotopic data indicated an ongoing bacterial sulphate reduction, with an unlimited supply of SO_4^{2-} from the Baltic sea water.

In the groundwater at Äspö and Laxemar the dissolved SO_4^{2-} of the groundwater showed no signs of increased $\delta^{34}S$ values, which is typical for the residual SO_4^{2-} in an ongoing bacterial sulphate reduction process. This suggests that there is no isotopical indication of ongoing obvious sulphate reduction in the deep groundwaters. However, abundant H_2S in the groundwater from some of the boreholes suggests that bacterial sulphate reduction is currently taking place. In addition the decrease in DOC in the groundwater at Äspö, from 28 mg/l in the shallow groundwater to generally less than 1 mg/l at 500m depth, implied that a degradation of organic carbon had taken place with depth, which may be linked to sulphate reduction.

In contrast, the $\delta^{34}S$ values of the fracture pyrite from deep boreholes at Äspö showed very low values (±0 to -26‰) at a depth of 200 to 500m. This implied that the FeS_2 observed in the basement rock at the larger depth (>200m) was formed during a sulphate reduction which to a large extent had taken place in the past. The well crystallised pyrite cubes support the conclusion that sulphate reduction had taken place in the past.

Bacterial evidence: The only direct sign of sulphate reduction in the HRL tunnel is the measured occurrence of SRB. Different techniques were used for the detection of sulphate reducers and their activity in sediments and in groundwater: enrichment cultures, viable counts in agar roller tubes, most probable number assays, sulphate reduction activity and 16S-rRNA gene sequencing. The genera found was *Desulfibrio* and *Desulfomicrobium.* The cell concentration was up to 2420 SRB ml^{-1}. The sea bed sediments had SRB ranging from approximately 5000 up to 10000 cells per cm^3 depending on sample location and depth. Sulphate assimilation and reduction showed that there was a significant sulphate reduction in all sediment samples which was greater in the upper samples of the sea bed sediments. Sulphate-reducing bacteria were also found in significant numbers in the HRL tunnel. Sulphate reduction seems to occur between 900-1300m tunnel length. Later, white mats of sulphide oxidising bacteria were observed at some places along the tunnel within this section. The observation suggested that sulphide production was present in the groundwater percolating over the observed mats. The most probable source of this sulphide is ongoing bacterial sulphate reduction in the vicinity of the HRL tunnel. The bacterial investigations strongly suggest that sulphate reduction probably is an ongoing process which takes place at many locations along the tunnel.

Conclusions: The Principal Component Analysis (PCA) plot (Figure i) was used to indicate areas with obvious sulphate reduction in the Äspö groundwaters based on the results from groundwater modelling and bacterial investigations. PCA (described in section 3.3.2.) was used to separate similar groundwater samples from dissimilar ones based on the groundwater composition. Figure i shows that the massive sulphate reduction seems to occur in samples taken in the HRL tunnel with a Modified Baltic Sea groundwater composition. This water type has undergone sulphate reduction and is obtained from boreholes which are located in the part of the tunnel that passes under the Baltic Sea and under the sea bed sediments.

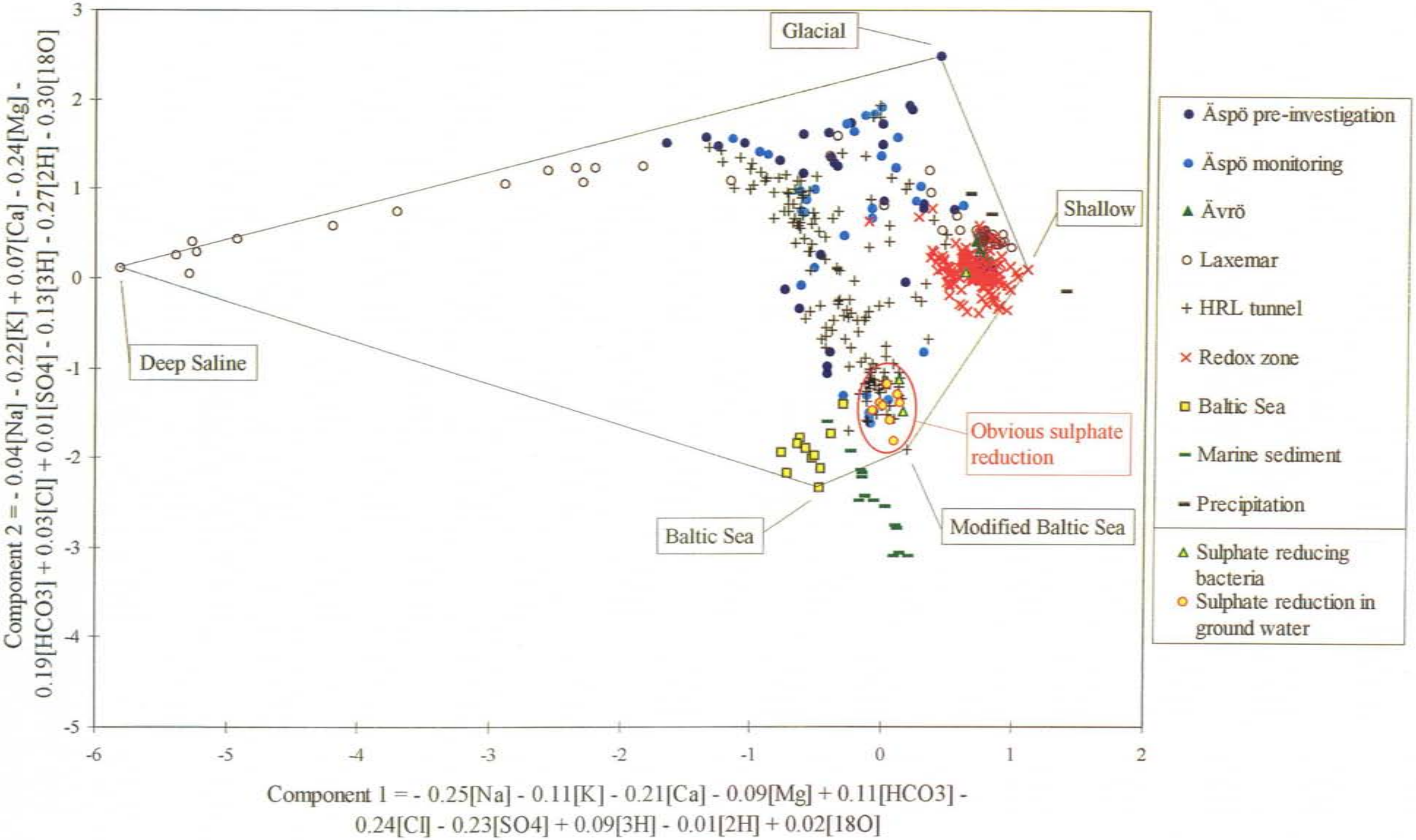


***Figure i.** The Principal Component Analysis plot is used to indicate areas of obvious sulphate reduction in the HRL tunnel. Results from groundwater modelling and bacterial investigations are included. The weight for the different elements is shown in the equations for the first and second Principal Component respectively.*

From a geological point of view, SRB have been active for a long period of time, and both past and present sulphate reduction was identified at Äspö:

Sulphate reduction in the past is indicated by the presence of pyrite with low $\delta^{34}S$ values and calcite with low $\delta^{13}C$ in fracture fillings down to depths of 500m.

Ongoing bacterial sulphate reduction was detected in the Baltic Sea sediments at 4m depth above the HRL tunnel. Furthermore, the presence of SRB in groundwaters at tunnel length 800-1400m at a depth >100m below the sediments and in the deep boreholes at Äspö indicates that sulphate reduction may also take place at depth in crystalline bed rock. However, groundwater modified by obvious sulphate reduction (low SO_4^{2-} and correspondingly high HCO_3^- after correcting for mass transport by flow) is only detected in the sediment pore water and in groundwaters from the tunnel section passing below the Baltic Sea. These modified groundwaters may have been produced in the groundwaters filling the fractures or have been transported into the tunnel from the sea bed sediments. The waters found in the tunnel boreholes with an obvious sulphate reduction cannot be explained by transport since this is compensated for. This points towards an ongoing sulphate reduction in these observations. In another location at >400m depth and at tunnel length 3100m a water not associated with the sea bed sediments contained SRB. The salinity range and the anoxic conditions were similar to the groundwater found in the earlier tunnel section but the organic content of the groundwater was <10 mg/l. These bacteria seemed to use hydrogen instead of organic carbon.

The prerequisite for bacterial sulphate reduction to take place is not quite clear. However, based on results from the present report the following comments on favourable environments for SRB can be made:

- Sulphate reducers are anaerobic bacteria; they need an oxygen-free environment.
- Brackish water with Cl between 4000 and 6000 mg/l seems to be favourable for growth of SRB.
- More vigorous sulphate reduction takes place when the influx of organic content is high (>10 mg/l), as in the sediments and groundwaters in the bedrock below the sea.
- Less vigorous sulphate reduction takes place when hydrogen (instead of organic carbon) is used as an electron donor by the bacteria. This means that sulphate reduction can also take place at depth where the access to organic material is limited.
- SRB seem to react to changing flux situations such as tunnel construction relatively fast. The increased influx of other water types lowered the available organic content <10 mg/l with time. This decrease made the reaction less obvious and the groundwater response less clear but some of the groundwaters still contained SRB.

Sulphate reduction has occurred and will probably occur where the conditions are favourable for the SRB such as anaerobic brackish groundwater with access to dissolved sulphate and organic carbon or hydrogen.

1. INTRODUCTION

The Äspö underground experimental Hard Rock Laboratory (HRL) was initiated by SKB, the Swedish Nuclear Fuel and Waste Management Company. It is located on the island of Äspö off the Baltic coast of Sweden approximately 350km south of Stockholm. The access tunnel to the HRL proceeds from the Baltic shoreline, under the sea bed for a distance of 1km, terminating some 450m under Äspö (Figure 1-1). Anomalous high bicarbonate concentrations, low sulphate content in the groundwater and the existence of Sulphate-Reducing Bacteria (SRB) were encountered in the tunnel section 700-1475m, ie, the part of the tunnel that passes under the sea (Figure 1-2). A quick calculation indicated that several hundred mg/l of sulphate had been lost, probably reduced to sulphide. SRB can reduce sulphate to sulphide by using, for example, organic substances present in natural groundwater as reducing agents (electron donors) according to the reaction:

$$SO_4^{2-} + 2(CH_2O) + OH^- \Rightarrow HS^- + 2HCO_3^- + H_2O$$

In marine sediments containing sufficient organic matter anaerobic bacteria are known to reduce sulphate ion in the interstitial water to form sulphide species. Hydrogen sulphide (often abbreviated to sulphide) is chemically reactive, is recognised by its smell even in low concentrations (<0.2ppm), and is toxic to plants, animals and humans. These sulphide species react with iron containing minerals in the sediment alternatively with dissolved Fe^{2+} of the pore water to form different iron sulphides which often blacken the sediments. These iron sulphides are generally transformed to pyrite (FeS_2) (Drever, 1982). Sulphate reduction is believed to be the only significant process by which sulphur is incorporated into sediments. Sulphate reduction processes in the sediments have been studied eg, by Berner (1972, 1982, 1984); Goldhaber and Kaplan (1974). The sulphate reduction in groundwaters and especially in deep groundwaters has been less extensively investigated.

Since sulphide can corrode a copper canister placed in a deep reducing environment, the process generating sulphide has to be known in such detail that a repository can be located without the risk of extensive sulphide attack on the canister. The formation of copper sulphide reduces the free energy (ΔG) for the overall reaction to negative values and makes water act as an oxidant so the reaction can take place according to:

$$2Cu + H_2O + HS^- \Rightarrow Cu_2S + OH^- + H_2$$

In order to affect the copper canister, sulphide needs to be in direct contact with the canister. In normal conditions the bentonite buffer surrounding the canisters will limit the access of sulphide. Mass transport calculations are used within safety assessment for evaluating the possible effects of sulphide on the canister stability (SKB 91, 1992). However, mass transport calculations are outside the scope of this report. A comprehensive discussion of microbiology including the safety assessment aspects in groundwaters has recently been presented by Pedersen and Karlsson (1995) and has therefore not been included in this work. The aim of this report is to determine where, why and to what extent obvious sulphate reactions occur or have occurred in the HRL tunnel at Äspö. Geological, hydrogeological, groundwater, stable isotope, and microbial data were used to investigate the process of sulphate reduction in the sediments and in the groundwater outside the tunnel. Signs of sulphate reduction were divided up into indications and evidence. Indications were obtained from geology (the existence of calcite and sulphide fracture coatings), hydrogeology (flow paths), groundwater composition (HCO_3^- and SO_4^{2-} content) and stable isotopes ($\delta^{34}S$ and $\delta^{13}C$ values). Only microbial investigations provided hard evidence for the presence of sulphate reduction by providing samples of viable SRB. The results from the different investigations have been divided in the chapters below according to indications and evidence of sulphate reduction in the HRL tunnel.

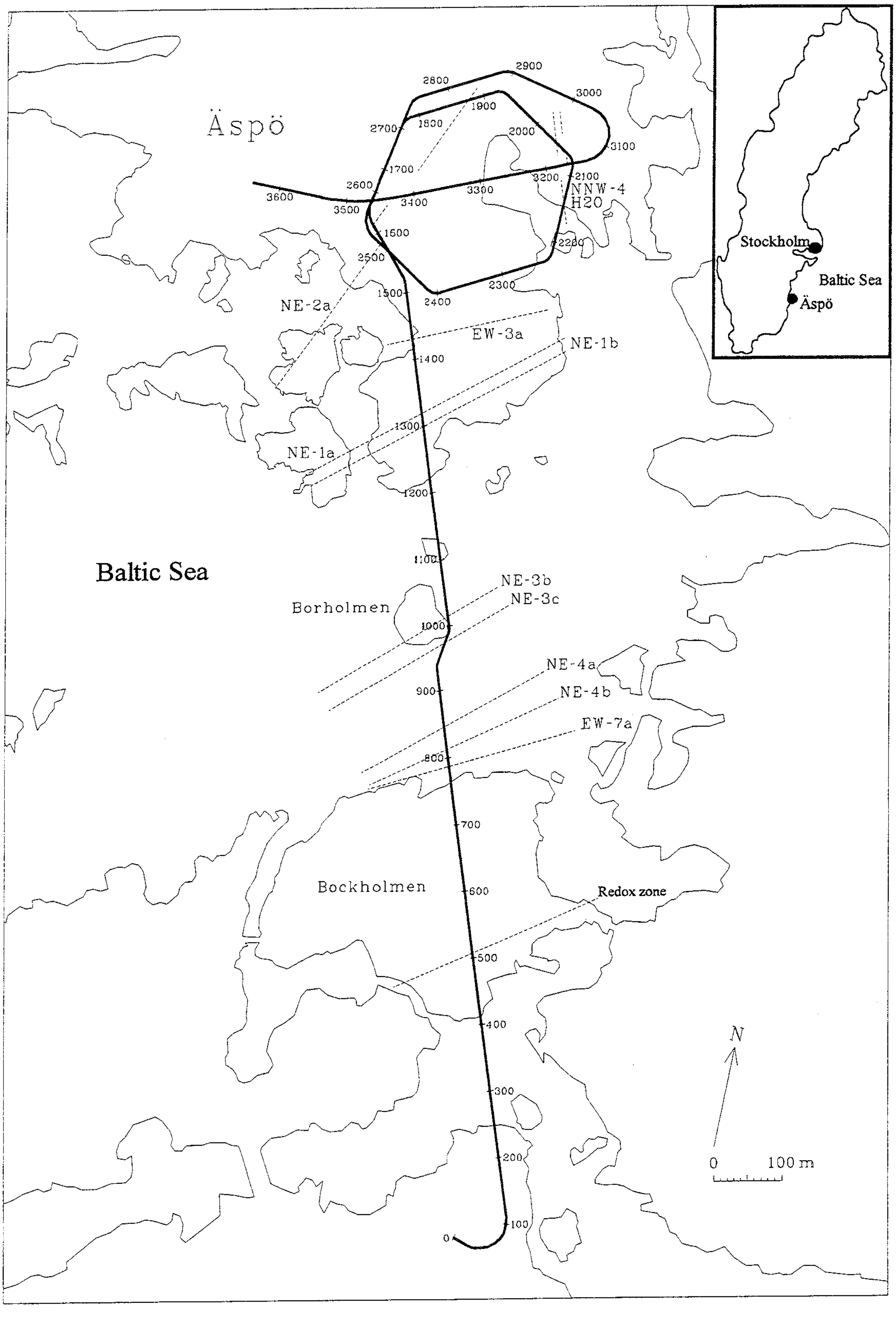


Figure 1-1. Location of Äspö (inserted map) and the location of the HRL access tunnel shown from above in relation to major fracture zones.

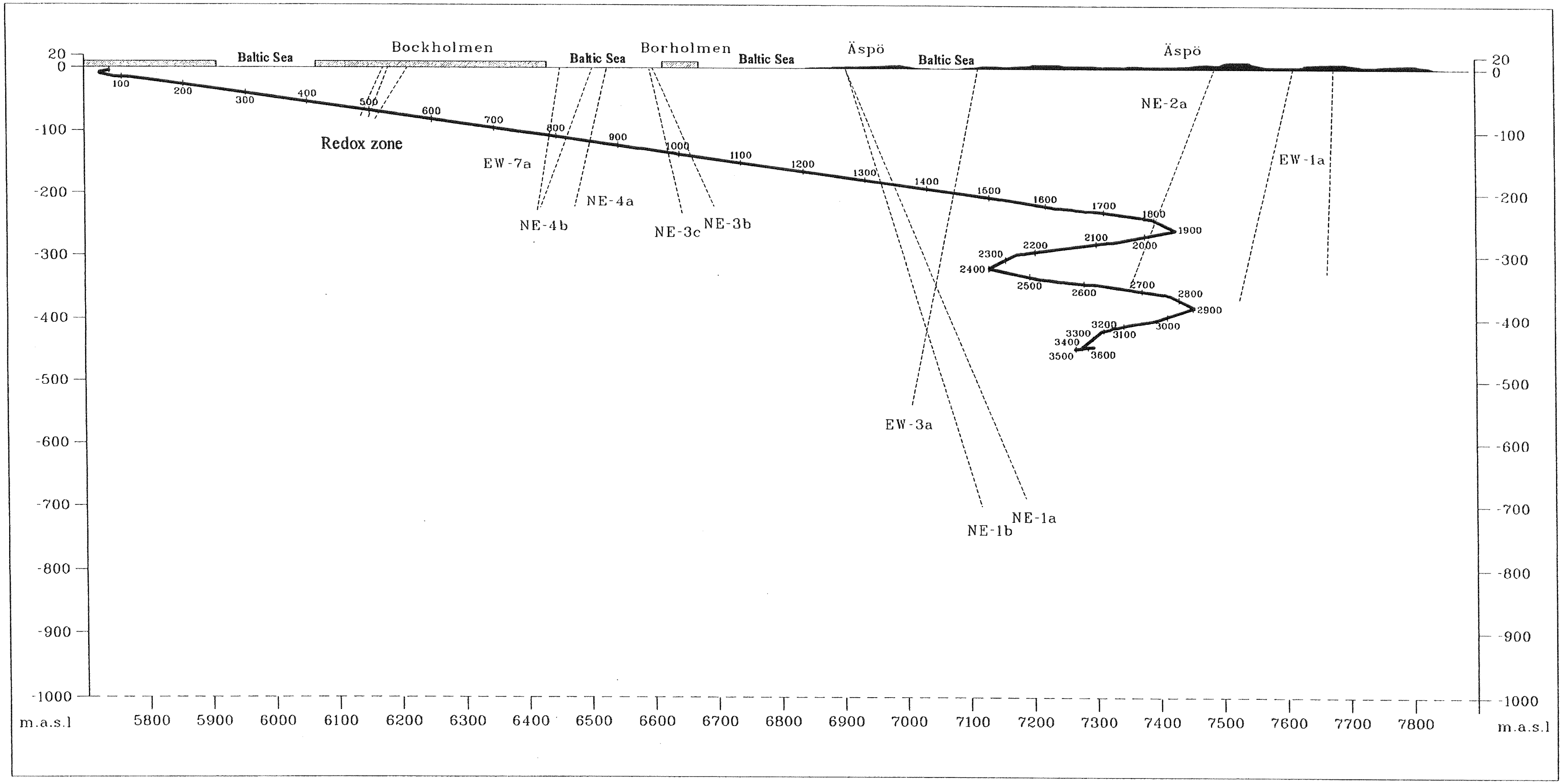


***Figure 1-2**. Location of the HRL access tunnel shown from the side in relation to major fracture zones and distribution of land and sea.*

2. SAMPLING LOCATIONS

The sampling locations for the geological, hydrogeological, groundwater, isotope, and microbial data in the Äspö HRL tunnel and in the near vicinity are shown in Figure 2-1. The sampled boreholes belong to three different types, probing holes (borehole name beginning with an S), percussion-drilled boreholes (name beginning with an H) and core-drilled boreholes (name beginning with K) (Nilsson, 1995). The sampled boreholes were the SA, KA, HA and KR boreholes in the tunnel in combination with KAS and HBH boreholes drilled from the surface. Probing holes (S) were the main part of the boreholes sampled for groundwater analyses in the HRL tunnel. Some samples were collected from the sea bed sediments above the tunnel (Stations I and II). One of the difficulties in interpreting and modelling the data was that the sampling or measurements were often performed at different times and locations. The extent of the sampling programme varied according to the methods used. This was critical since the HRL tunnel excavation enhanced and changed the groundwater flow. Thus a single sample or measurement may not reflect the dynamic change over a period of time. An interpretation of the past or present spread of sulphate reduction becomes difficult. The sampling method, sampling location, sampling day and the advancement of the tunnel construction in combination with the distribution of land and sea are depicted in Figures 2-1 and 2-2a,b,c,d,e,f and g. In order to describe the dynamism in the system the data has been divided into experimental time in relation to the start of the HRL tunnel construction. Day 0 (1990-10-14) indicates the start of the tunnel construction, day 150 represents 1991-03-13, day 350 (1991-09-29), day 550 (1992-04-16), day 750 (1992-11-02), day 950 (1993-05-21), day 1150 (1993-12-07) and day 1350 represents 1994-06-25 (the dates are in the format year-month-day). A sample taken ±100 days from the actual experimental day is set to represent that experimental day in the figures and calculations.

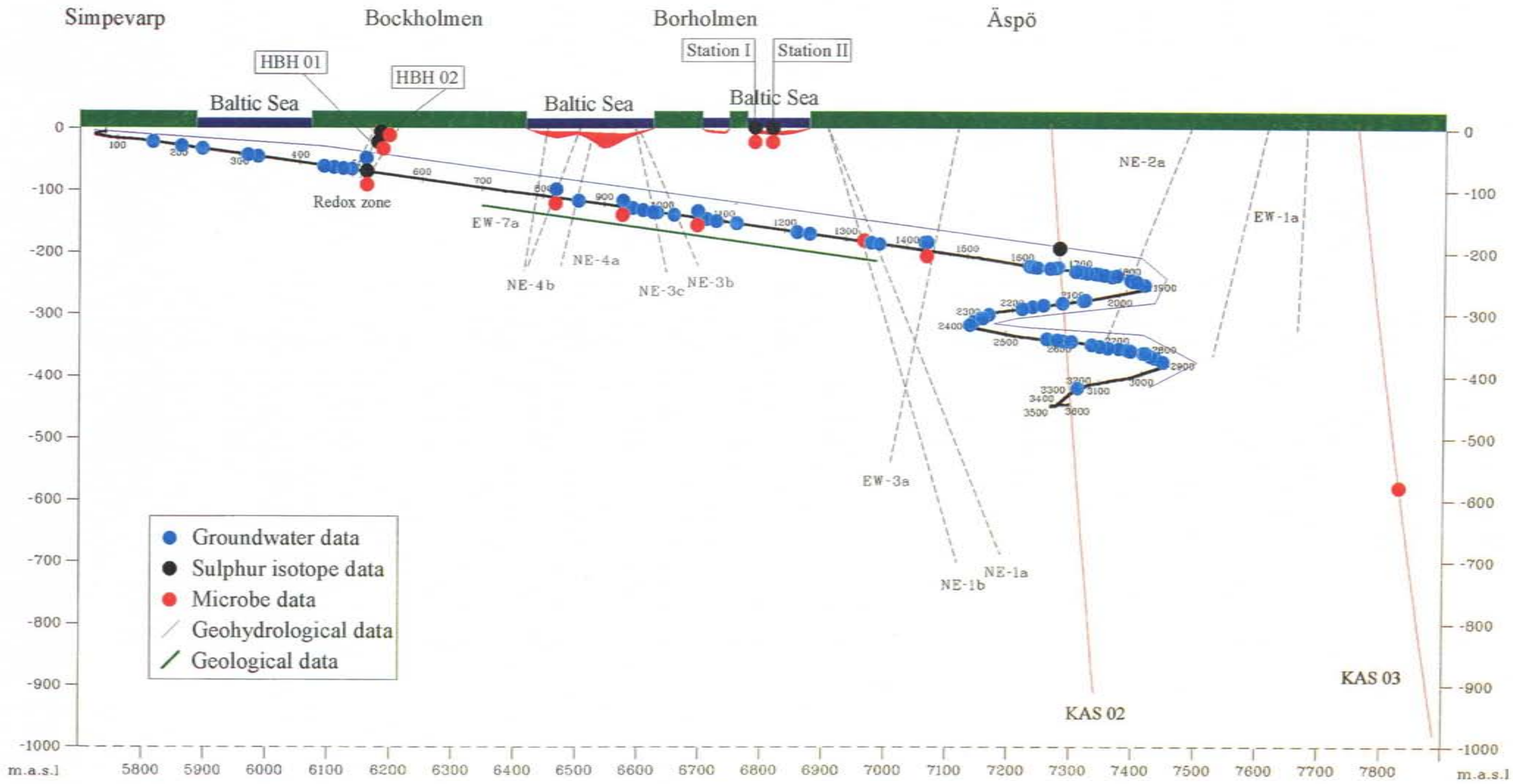


***Figure 2-1.** Sampling/mapping locations of the microbial, groundwater, isotope, hydro and geological investigations in and around the Äspö HRL tunnel which were used in the evaluation.*

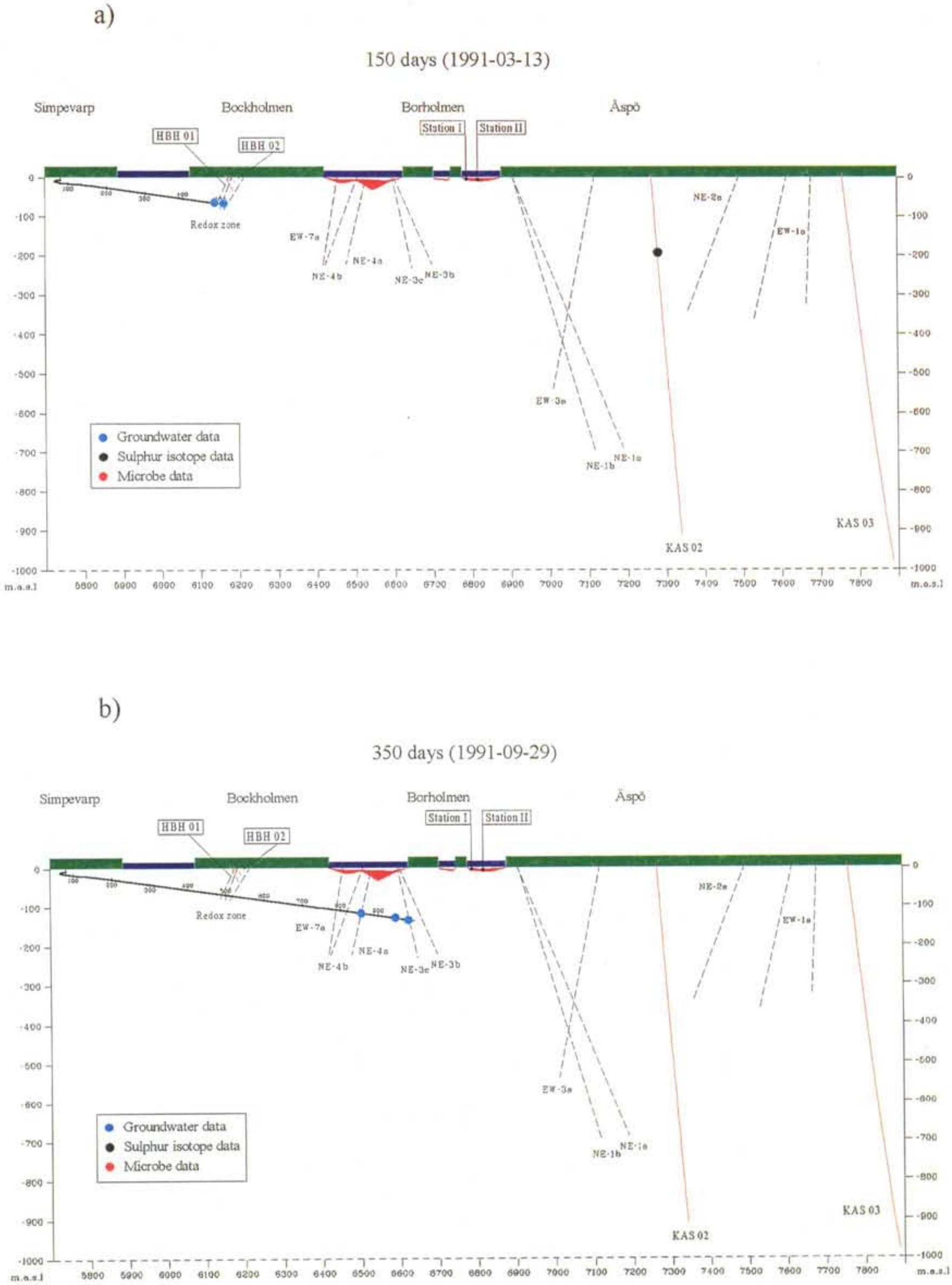
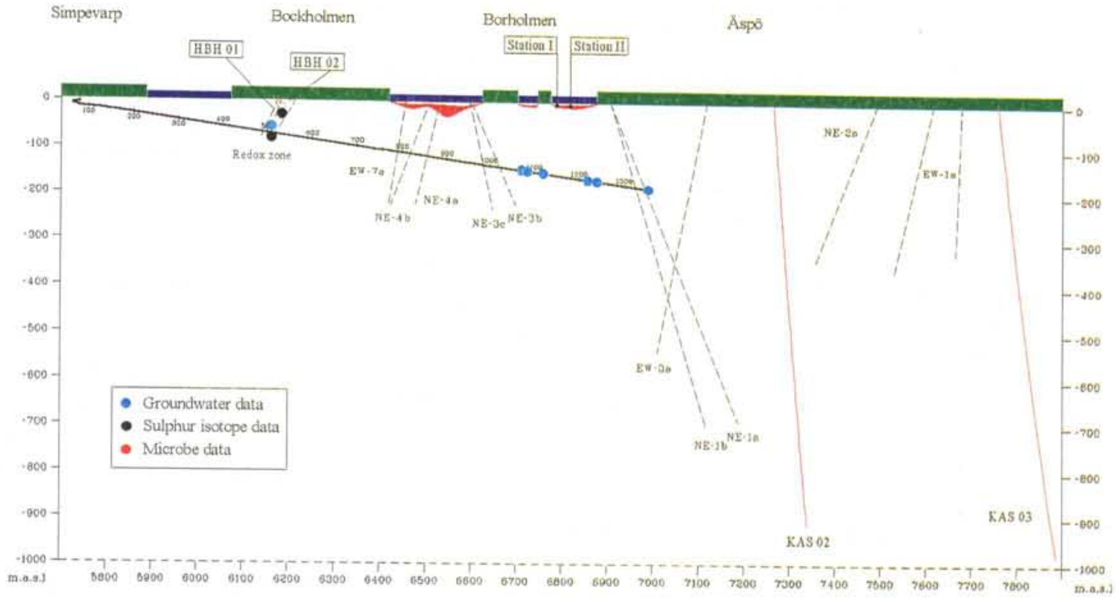


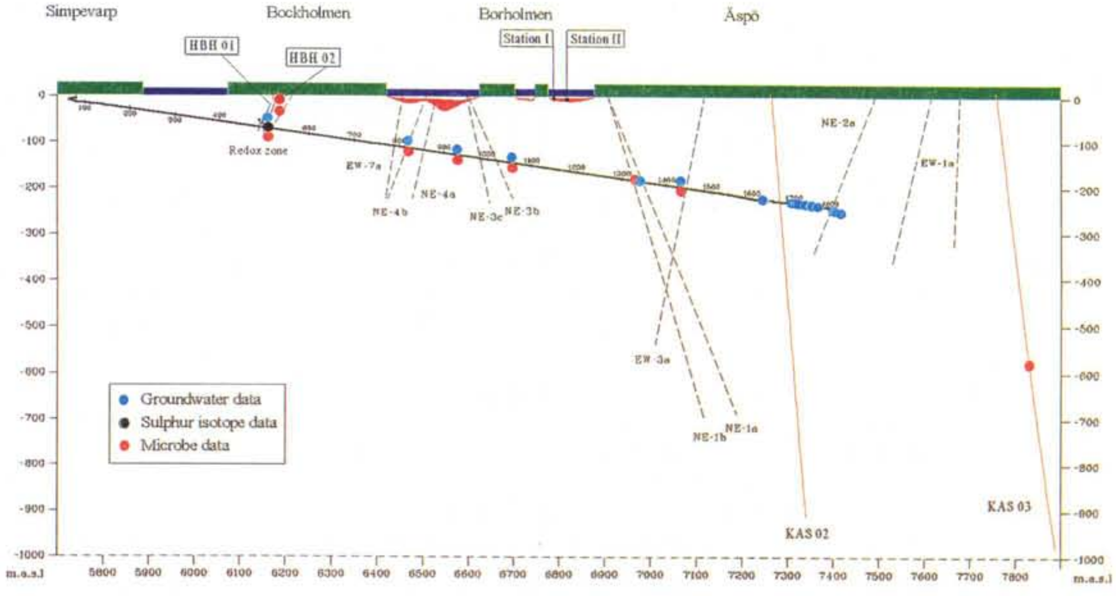
Figure 2-2a,b. Sampling location and experimental time (days 150-350 (0.4-1 year)) for the microbial, groundwater, isotope, hydro and geological data in and around the Äspö HRL tunnel. A sample taken ±100 days from the actual experimental day is set to represent that experimental day.

c)



550 days (1992-04-16)

d)

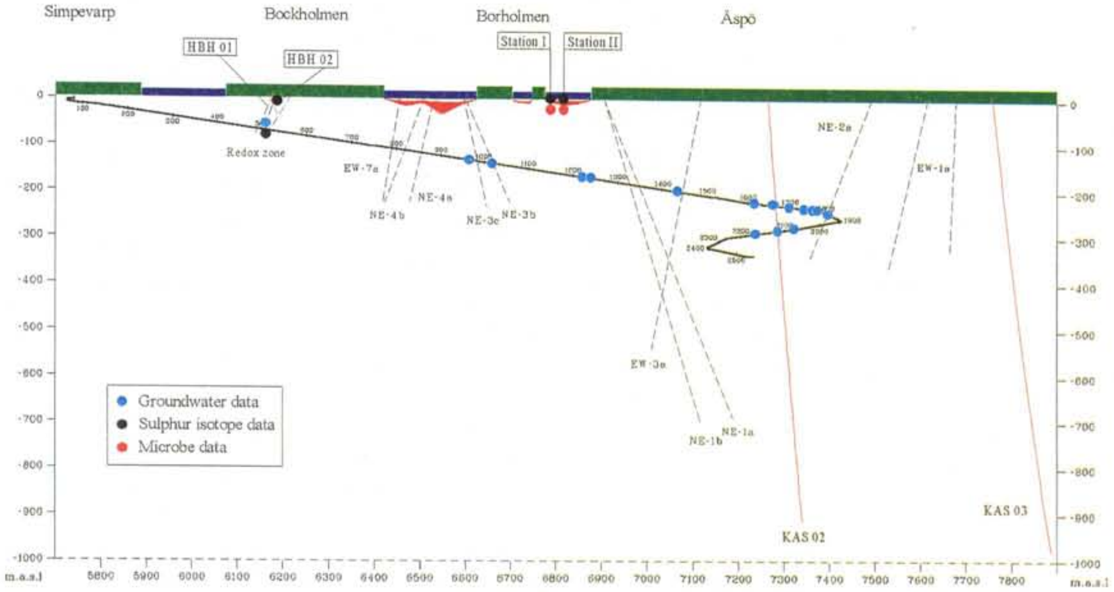


750 days (1992-11-02)

Figure2-2c,d. *Sampling location and experimental time (days 550-750 (1.5-2 years)) for the microbial, groundwater, isotope, hydro and geological data in and around the Äspö HRL tunnel. A sample taken ±100 days from the actual experimental day is set to represent that experimental day.*

e)

950 days (1993-05-21)



f)

1150 days (1993-12-07)

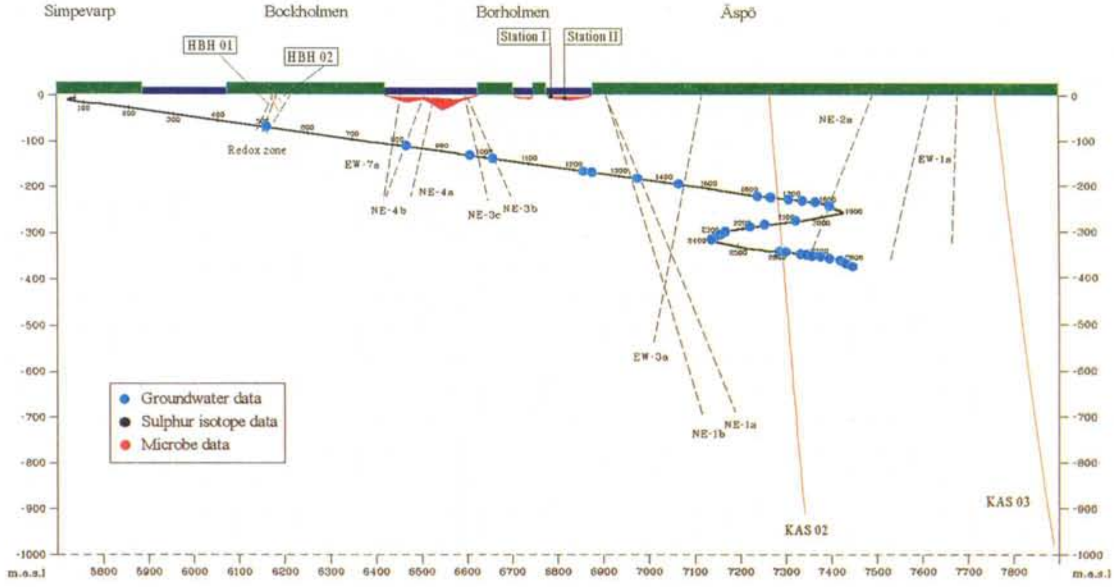


Figure 2-2e,f. *Sampling location and experimental time (days 950-1150 (2.6-3.2 years)) for the microbial, groundwater, isotope, hydro and geological data in and around the Äspö HRL tunnel. A sample taken ±100 days from the actual experimental day is set to represent that experimental day.*

g)

1350 days (1994-06-25)

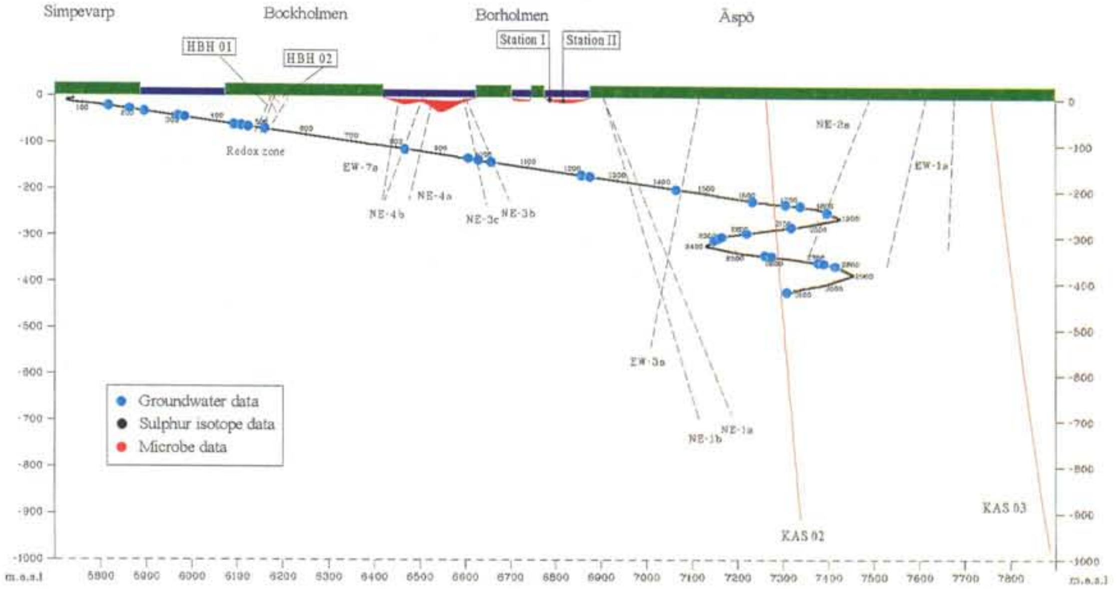


Figure 2-2g. *Sampling location and experimental time (day 1350 (3.8 years)) for the microbial, groundwater, isotope, hydro and geological data in and around the Äspö HRL tunnel. A sample taken ±100 days from the actual experimental day is set to represent that experimental day.*

3. GEOLOGICAL INDICATIONS OF SULPHATE REDUCTION

3.1 *Background and aims*

Geological indications of obvious sulphate reduction could be the precipitation of carbonate minerals and formation of iron sulphides in the fractures of the HRL tunnel. The groundwater sampled in the HRL tunnel was in contact with the rock, the fracture minerals and the sediments. The geology of the HRL tunnel has been earlier described by Stanfors et al. (1992, 1993a,b, 1994).

3.2 *Signs of sulphate reduction in the bedrock*

The geology of special interest was from fracture zone EW-7 (tunnel length ~750m) to fracture zone NE-1 (tunnel length 1320m). An overview of the bedrock geology and fracture zones found in this tunnel section is shown in Figure 3-1.

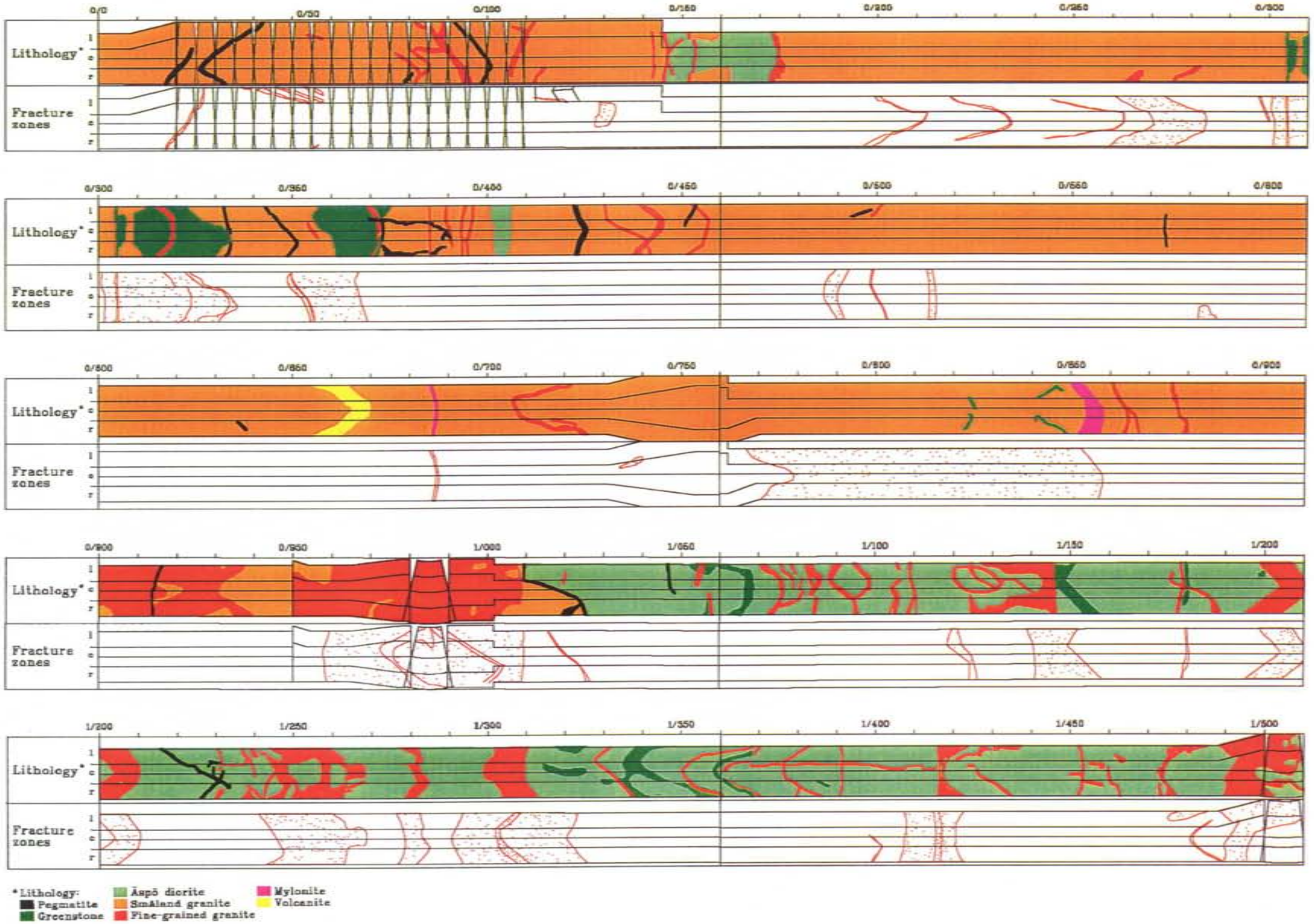


Figure 3-1. Detailed geological description of rock types and fracture zones in the tunnel section 700-1350m.

The lithologies were dominated by Småland granitoids which gradually changed from monzogranite to quartz monzodiorite (Stanfors et al., 1993a,b). The major change was detected at ~1100m and from this tunnel length towards the NE-1 fracture zone the rock type was mainly quartz monzodiorite (named Äspö diorite) whereas in the part from EW-7 to 1100m the dominating rock was monzogranite (named Småland granite). However, both the Småland granite and the Äspö diorite contained granodioritic parts.

Fine grained alkali granites occurred in the section 900 to 1000m and in lenses a few metres thick in the tunnel section 1130 to 1320m. The fine grained granite is often found in sections of major fracturing. Two of the regional water-bearing fracture zones in the tunnel section described here were situated in this type of granite (NE-3 and NE-1). Fracture zones EW-7 and NE-4 were located in the Småland granite at 750 to 850m. Minor fracture zones were recorded at 1150m (gently dipping towards NW) and at 1200 and 1250m (moderately dipping towards N). A roughly E-W trending mylonite was observed at ~850m and scattered observations of metabasites on a scale of a few metres were observed, especially in the Äspö diorite. Among the fracture coatings chlorite (43%) and calcite (30%) dominated. However, epidote (8.3%) and Fe-oxyhydroxide (4.25%) were frequently observed in the fractures of the tunnel section. The fracture coatings in the fracture zones had higher frequencies of clay minerals, epidote and Fe-oxides compared to what was found in the single fractures.

Samples from fracture zones NE-3, NE-4 and EW-7 have been used for age determination of fault movements (Maddock et al., 1994). Dating of chlorite from zone EW-7 yielded K-Ar ages of 718±17Ma. Palaeomagnetic measurements of epidote and chlorite/epidote from the same zone yielded a magnetisation age of about 1350Ma. For samples from zone NE-3 no palaeomagnetic pole was resolved whereas for samples from fracture zone NE-4 an age of 270-190Ma or alternatively 1350Ma was obtained. Gouge material (unlithified material of rock and mineral pieces) was sampled in zones NE-3 and NE-4 and from this material autogenic clay of "illite" type was separated for K-Ar dating. The ages obtained ranged from 700 to 300Ma. The finest-grained fractions had an age of 300 to 340Ma for fracture zone NE-3 and 356 to 375Ma for zone NE-4 (Maddock et al., 1994). The illite samples dated were of the same type as the clay material identified in zone NE-1 as well as in the Redox zone. This material was identified by XRD as illite dominated mixed-layer clay of allevardite type which according to Velde (1983, 1985) is formed in conditions significantly different from the present (>1km overburden and >100°C). Based on the datings (Maddock et al., 1994) and mineralogical and structural studies (Landström and Tullborg, 1993; Munier, 1993) it can be concluded that most of the large fracture zones in the tunnel section 700 to 1350m are zones of ductile deformations. The set of NE trending hydraulically conductive zones (NE-1, NE-3 and NE-4) in particular, was later geologically reactivated and this caused gouge formation. Clay mineral formation succeeding this fragmentation was thought to be ~300Ma.

Sulphate reduction due to anaerobic respiration produces sulphide and bicarbonate which can result in the formation of carbonate minerals such as calcite and iron sulphide minerals such as pyrite. Saturation index calculations using PHREEQE confirmed that the groundwater in the tunnel was supersaturated with respect to the minerals in most of the samples. Figures 3-2 and 3-3 show the calculated saturation indexes (log IAP/KT) for calcite and the partial pressures (bar) for carbon dioxide respectively. A positive value indicates supersaturation (potential for precipitation/formation), a negative value undersaturation (potential for dissolution) and a value close to zero indicates equilibrium of the water phase with respect to the actual mineral. The calcite seemed to be close to equilibrium (saturation index = 0) although there was a tendency for supersaturation (saturation index >0.5) in the first part of the tunnel (tunnel length <1500m) in samples taken during the early stage of the tunnel construction. Surprisingly the saturation index for calcite for some of the observations at the tunnel length 1600m (fracture zone NE-2a) was higher than 1. This may indicate problems with the pH measurements or calcite precipitation due to mixing. In the same tunnel section the partial pressure of carbon dioxide was higher than in the atmosphere (Log -3.5 bar) which may be partly a result of sulphate reduction. The increase of pCO_2 at the tunnel section 800-1300m cannot be explained by the increased influx of shallow water since this section is mostly situated under the Baltic Sea. Geological investigations indicated that calcite occurs as a fracture mineral. Pyrite on the other hand was generally found in small amounts in the tunnel but may exist in colloidal form indicated by high S values on colloid samples taken from the Redox Zone in the tunnel.

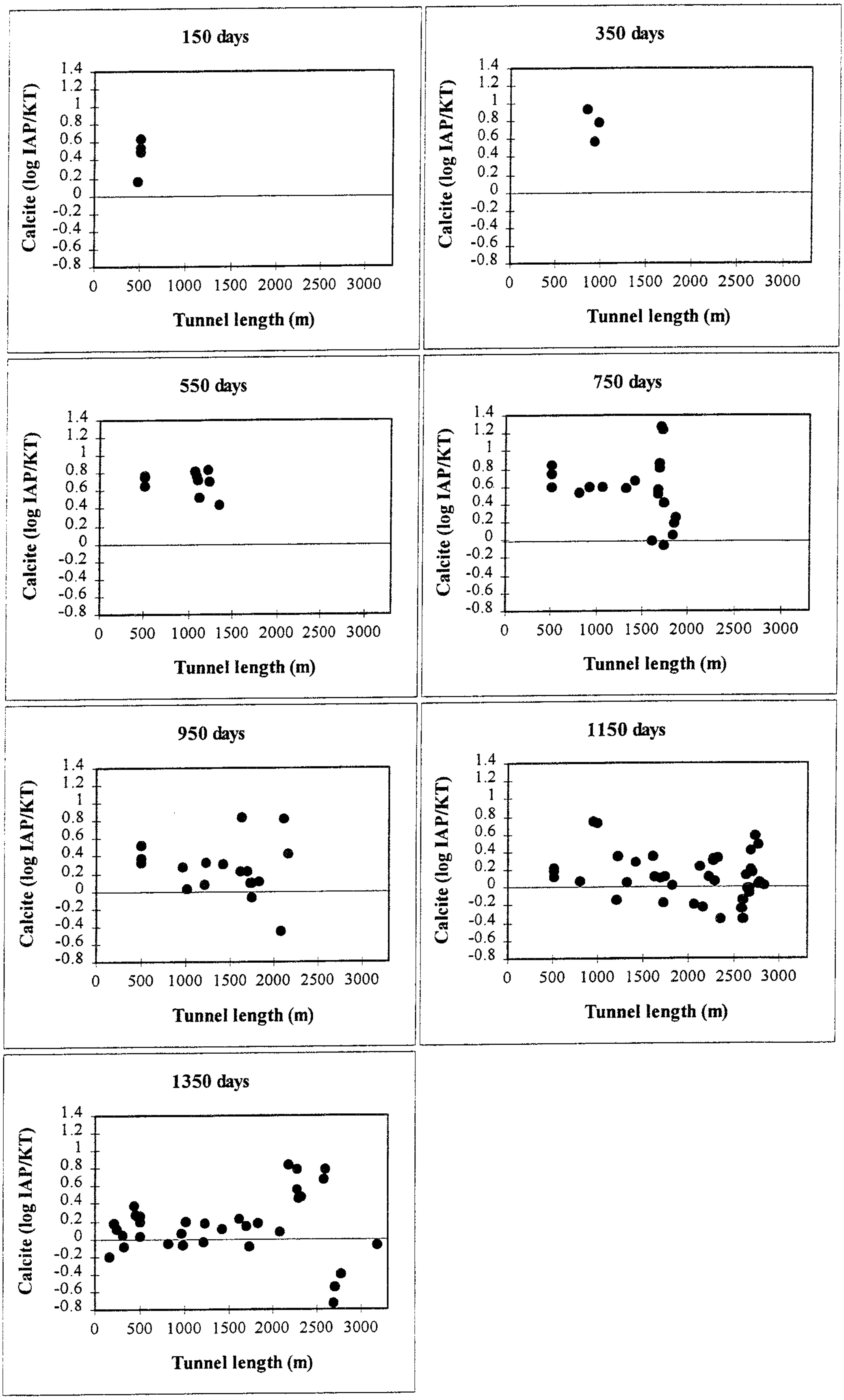


Figure 3-2*. Calculated saturation indexes (log IAP/KT) for calcite. Experimental days 150 (0.4 year), 350 (1 year), 550 (1.5 year), 750 (2 years), 950 (2.6 years), 1150 (3.2 years) and 1350 (3.8 years) are shown.*

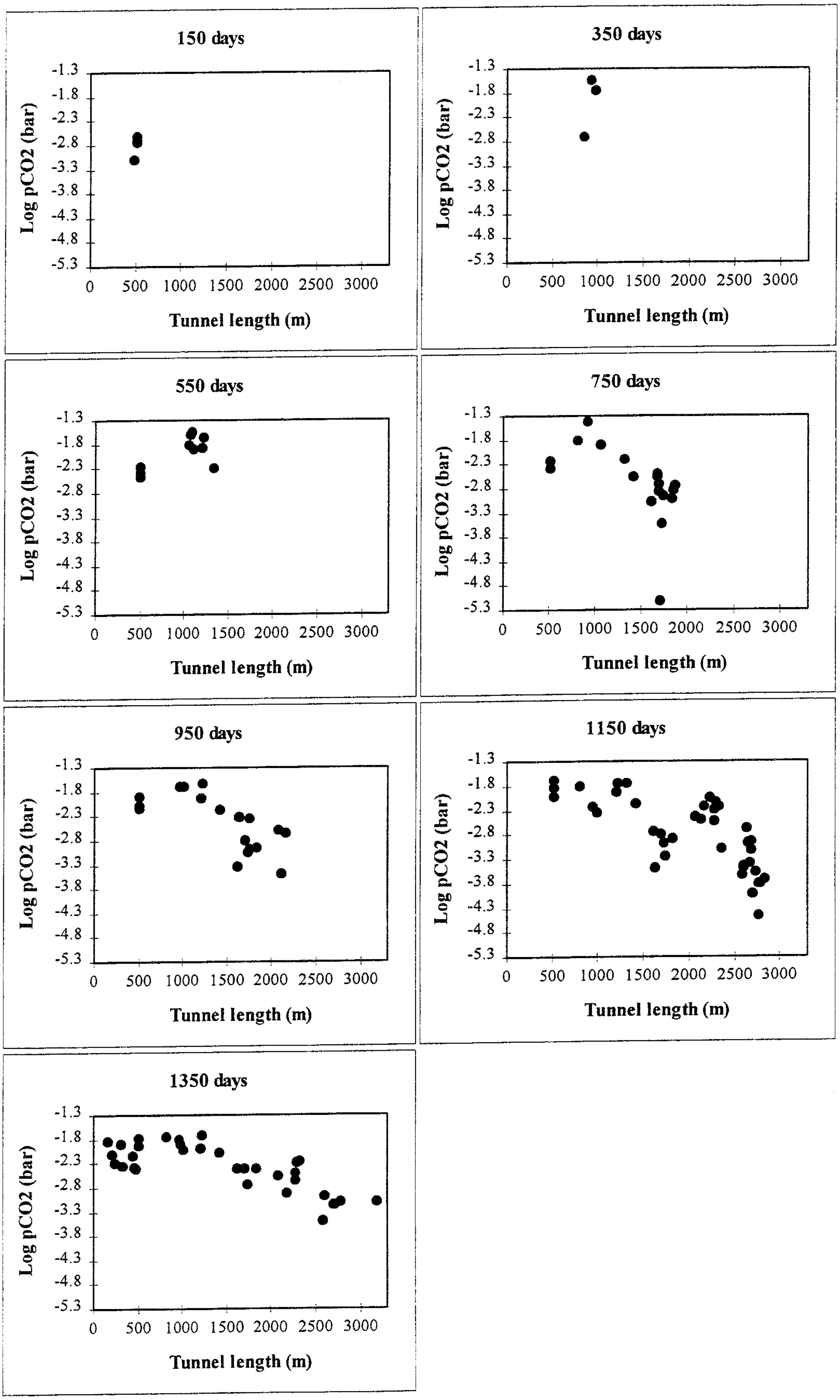


Figure 3-3. *Calculated saturation indexes for partial pressures (bar) for carbon dioxide pCO*$_2$*. The atmospheric pressure of carbon dioxide is Log -3.5 (bar). Experimental days 150 (0.4 year), 350 (1 year), 550 (1.5 year), 750 (2 years), 950 (2.6 years), 1150 (3.2 years) and 1350 (3.8 years) are shown.*

3.3 Signs of sulphate reduction in the sea bed sediments

The tunnel section studied is situated beneath the Baltic Sea where fracture zone EW-7 follows the northern shoreline of Bockholmen and fracture zone NE-1 more or less defines the SE shoreline of Äspö (Figure 3-1). The sea bed sediments consist of partly organic-rich sediments. The groundwater composition may be modified by eg, sulphate reduction when in contact with these sediments. Seismic refraction studies (Rydström and Gereben, 1989) show that the sea bed sediments consist mainly of two layers of which the upper is probably gyttja (organic-rich mud) which shows very low velocities (~600-1100 m/s) indicative of high gas content (pers. com. G. Lind, 1994) which may be a result of biological activity involving sulphate reduction. The possible methane content in the sediments was not measured. This layer is more or less continuous in the bay between Bockholmen and Äspö and at least a few metres thick. Sediments from the sea bed have been studied by Landström et al. (1994). According to their study the sediment sequence can generally be described as a bottom layer of clay followed by a thin layer of gravel and above this a several metre thick layer of gyttja. A complete core located ~40m from the Äspö shoreline was analysed for chemical and mineralogical composition. Here the gyttja layer amounted to 370cm, the gravel zone 12cm and the clay 148cm (total length of the core was 530cm). The clay horizon was subdivided into blue-grey clay and brown clay. The organic content for the gyttja was ~30% and for the gravel zone ~10% (organic content in combination with water) (pers. com. O. Landström, 1994). The organic compound in the gyttja was therefore a potential source for sulphate reduction. The sulphate source was most probably from the sea water.

4. HYDROGEOLOGICAL INDICATIONS OF SULPHATE REDUCTION

4.1 Background and aim

Hydrogeological modelling was used to describe the flow system around the HRL tunnel. Groundwater flow modelling can be used to address questions such as whether the sulphate reduction is an in situ process or an ongoing one ie, in the sea bed sediments lying above the HRL tunnel. The extent of the hydrogeological investigations along the HRL tunnel is depicted in Figure 2-1. The hydrogeology of the HRL tunnel has been earlier described by Rhén et al. (1993, 1994); Rhén and Stanfors (1993).

4.2 Hydrogeological calculations of the inflow to the tunnel

4.2.1 Inflow to the tunnel

Before the tunnel was constructed the groundwater flow was small due to the small gradients caused by natural water levels and the hydraulic head distribution at deeper levels. When the tunnel was constructed the hydraulic gradients increased considerably in the vicinity of the tunnel causing a different flow pattern. Figure 4-1 shows the flow pattern and hydraulic head distribution of a hypothetical homogeneous case. In the modelling the sea level was set at 0m depth and the tunnel at 200m below sea level. No hydraulic resistance was assumed around the tunnel. Even though the rock was definitely not a homogeneous porous medium the water flow into the tunnel can still be illustrated by this flow model.

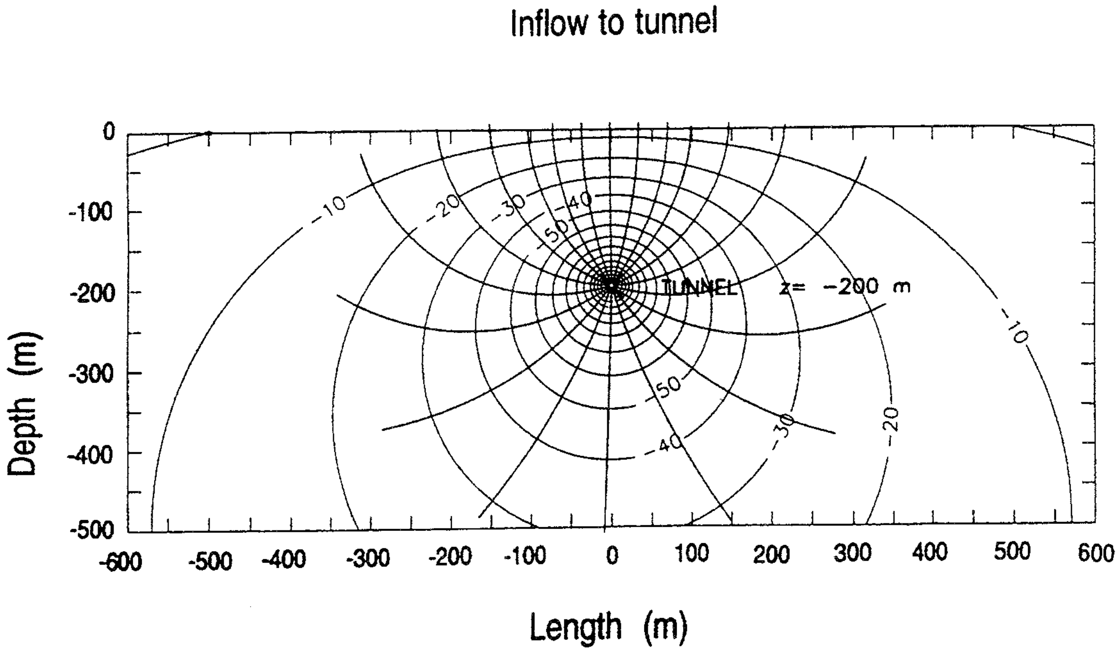


Figure 4-1. Calculated hydraulic head distribution and flow lines around the HRL tunnel. Modelled hydraulic head distribution (m) shown as isobars around the HRL tunnel when the tunnel passes 200m below sea level. No hydraulic resistance around the tunnel is assumed. The flow lines for particle traces (backtracked from the tunnel) are evenly distributed around the tunnel.

The flow lines in Figure 4-1 are regular because of the assumed homogeneity. In reality the flow path will be irregular, but on a scale greater than some 50m the flow lines will most probably have approximately the same pattern as in the simplified model. Considering the flow lines in Figure 4-1 about 50% of the inflow to the tunnel will come from an area ±200m on both sides of the tunnel at some time after the tunnel excavation. For the tunnel part below the sea this 50% should be sea water and below Äspö and Bockholmen a great deal of the 50% should be fresh water. Between Bockholmen and Äspö the sea covers much more than ±200m on both sides of the tunnel and the flow lines indicate that with time about 70% of the flow into the tunnel may be sea water and the rest a mixture of deeper waters. The flow lines indicate that it should be possible to distinguish different water types around the tunnel, because the flow lines are evenly spread around the tunnel. The sulphate reduction may take place in the sea bed sediments and the resulting water may be transported to the tunnel. The heterogeneity of the flow pattern will cause dispersion, which causes a mixture of waters of different origins.

The flow lines in Figure 4-1 are calculated particle traces (the calculated flow path for a particle transported by the flow) which all have about the same transport time from the starting point to the tunnel, except for the flow lines in the middle which have a somewhat shorter transport time. In this modelling case all particle traces from the Baltic Sea will sooner or later reach the tunnel. The stream tubes (between a pair of flow lines) in Figure 4-1 represent 5% of the total flow into the tunnel. By back-tracking the particles from the tunnel to the surface the transport time for each flow line can be estimated from the model, considering specified values for hydraulic conductivity (K), flow porosity (n_e) and hydraulic head difference (dh) between the upper surface of the model and the tunnel. This means that it is possible to approximate the mean transport time from surface to tunnel for the water within a stream tube, and thus also estimate the proportion (q) of surface water or Baltic water entering the tunnel as a function of a relative time period (t_R).

4.2.2 Inflow to sampling points in or close to NE-1

The inflow time of say 25% and 30% of modern Baltic Sea water to sampling points in or close to the NE-l fracture zone has been modelled. In this case a reference point outside the tunnel has been used when dh was estimated. The reason is that the zone had been grouted close to the tunnel and the estimated K below represents the natural properties of the zone, which means that the estimated hydraulic head for the tunnel has to be outside the grouted part, if the simple homogenous model above is to be used. This also means that the transport time through the grouted part is neglected. The error is however probably rather small, since the water samples in the boreholes were taken outside the tunnel periphery. The transport time (t) through NE-1 can be calculated with a equation called the Hydrogeological-model using the given hydrogeological properties of the fracture zone (Rhén and Stanfors, 1993) and the relative time and influx proportions below:

$$t = \frac{n_e}{K \bullet dh} \bullet t_R$$

Transport time:	t	
Transmissivity:	$T_g = 4.5 \bullet 10^{-4}$ m^2/s (geometric mean)	
Hydraulic cond:	$K = T_g/w$	
Width of NE-1:	w = 30m (estimate)	
Flow porosity:	n_e = 0.0006–0.022 (based on tracer test)	
	n_e = 0.003–0.01 (mapping of grouted fractures close to the HRL tunnel)	
Head difference:	dh = 10–20m (outside or partly outside the grouting part of NE-1)	
Relative time and water influx proportions:	t_R ($m^2 \cdot$days/s)	q (%)
	0.46	5
	1.0	15
	1.2	25
	1.5	35
	3.0	45
	4.0	55
	8.0	65
	20.0	75

Assuming a porosity of 0.01 and a hydraulic head of 10m, a breakthrough time for a portion of 25% and 30% respectively of Baltic sea water was calculated by using the above Hydrogeological-model (see Table 4-1). The rock around the groundwater sampling points ±100m outside the NE-1 fracture zone probably has a lower K and n_e value. The Multivariate Mixing Model can be used to calculate a breakthrough time for Baltic Sea water based on changes in the measured groundwater composition in boreholes ±100m from NE-1. Within this model a multivariate technique (PCA) is used to describe the observations on a two dimensional plot. The plot is used as a phase diagram from which the mixing portions are calculated (see chapter 5.2). It is important to note that this model is purely chemical and does not contain any hydraulic parameters. The results of the calculations using the two models are shown in Table 4-1.

Table 4-1. The Hydrogeological-model and the Multivariate Mixing model are used to predict at what time 25% alternatively 30% of Baltic sea water will arrive at a borehole in or close to NE-1. Case 1 = 25% influx of Baltic sea water, Case 2 = 30% of influx of Baltic sea water.

	Hydrogeological-model	**Multivariate Mixing model**
Case 1: Influx of 25% Baltic Sea water	80 days (to a sampling point in the NE-1 zone)	376 days (to a sampling point close (±100m) to the NE-1 zone)
Case 2: Influx of 30% Baltic Sea water	93 days (to a sampling point in the NE-1 zone)	574 days (to a sampling point close (±100m) to the NE-1 zone)

The calculations based on the Hydrogeological-model in Table 4-1 predicted that 25% of Baltic sea water should be present at the sampling points after 80 days; and 30% of Baltic Sea water should be present at the sampling points after 90 days. As expected the hydrogeological-model predicted a shorter breakthrough time for the Baltic Sea water into the zone than the multivariate mixing model predicted for the same water type to reach the sampling points outside the fracture zone. The hydraulic conductivity is most probably lower outside than within NE-1. The probable heterogeneity and complexity of fracture zone NE-1 is shown in Figure 4-2. The modelling in the figure was based on inverse modelling of pressure build-up tests performed in boreholes drilled in NE-1. The average of the estimated values of the conductivity field has been used to estimate the transmissivity distribution around the tunnel. The conclusion is that the local flow lines must be irregular due to the heterogeneity of the fracture zone. In any case, the above calculations indicate that 25% Baltic Sea water can be transported within 100 to 400 days into the tunnel section under the Baltic Sea where sulphate reduction may take place. Alternatively, if the sulphate reduction takes place in the sediments the "sulphate reduced" water can be leached and transported into the HRL tunnel within that time frame.

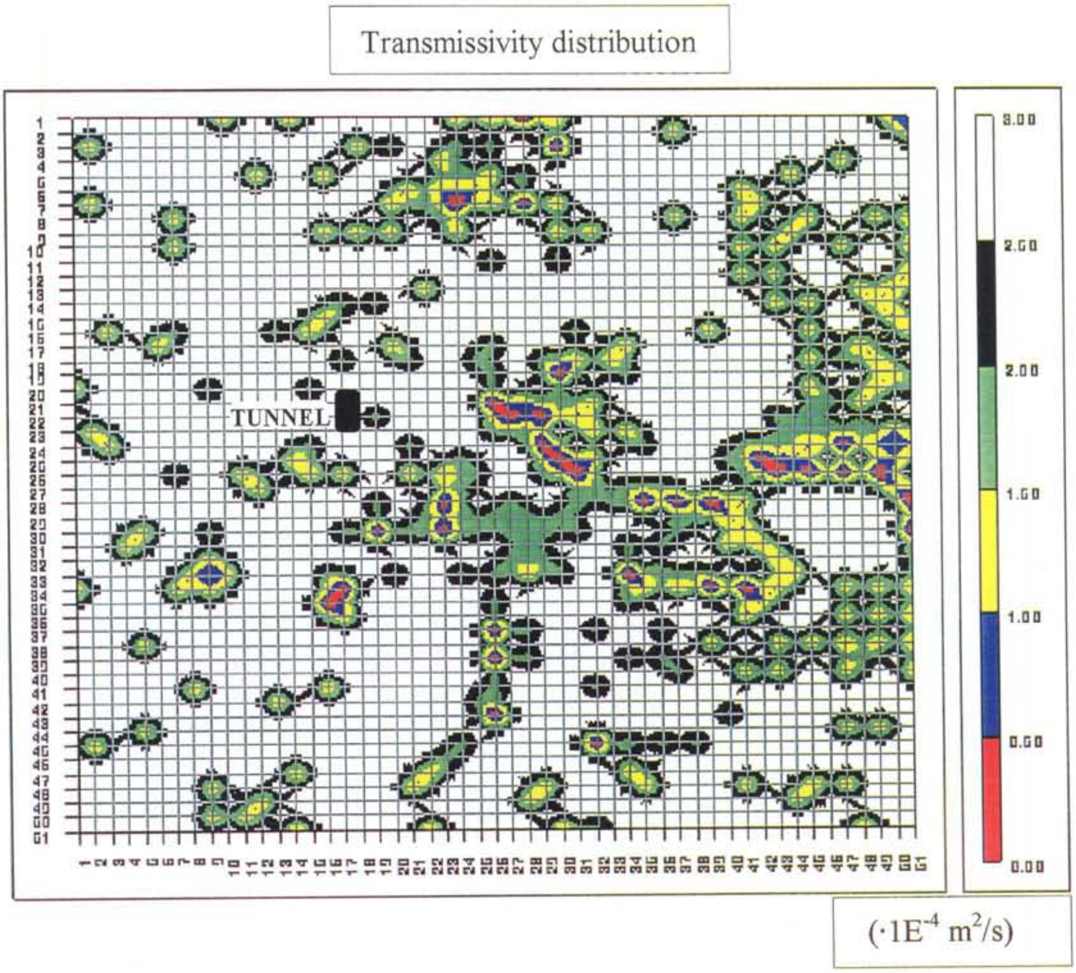


Figure 4-2. Estimated transmissivity distribution around the tunnel in fracture zone NE-1 (Alm, 1994).

4.2.3 Measured groundwater flow and heterogeneity of the inflow to the tunnel

In order to further demonstrate the heterogeneity of groundwater flow into the tunnel, flow measurements can be used. The flow into the tunnel was measured with weirs along the tunnel (Rhén et al., 1994). The results of the measurements are shown in Table 4-2. In the tunnel section 700-1475m 70% is assumed to come from one or more fracture zones and in the tunnel section 1475-2265m only 1.0-20% was estimated to come from one or more distinct fracture zones. The weir at tunnel length 1030m captured the inflow from EW-7, NE-4 and NE-3. The weir at tunnel length 1370m captured the inflow from NE-1. As weirs were not located upstream and downstream of each fracture zone, the inflow from each fracture zone could only be estimated roughly. The flow distribution between the fracture zones was based on the mapped quantities leaking in to the tunnel. The inflow rates of the different fracture zones are shown in chapter 5, Figures 5-1 to 5-7. The measurements show how complex the flow situation is in the tunnel and the sulphate reduction process can be inhibited or accelerated depending on the prevailing flow situation.

Table 4-2. Estimated inflow from the fracture zones into the HRL tunnel. In the tunnel section 700-1475m 70% was assumed to come from one or more fracture zones and in the tunnel section 1475-2265m between 1.0-20% was assumed to come from one or more fracture zones.

Weir at chainage (m)	Flow rate at weir (m^3/s) $*10^{-3}$	Fracture zone	Percentage of inflow at weir %	Inflow rate from fracture zone (m^3/s) $*10^{-3}$	Comment
		EW-7	1	0.1	
		NE-4	16	1.2	
		NE-3	53	3.9	
1030	7.42				
1232	2.75				
		NE-1	70	5.0	
1372	7.1				
1584	1.18	EW-3	70	0.8	
1745	0.32	NE-2a	1.0	0.003	uncertain
1883	0.50	NE-2b	1.0	0.003	uncertain
2028	0.47	NNW4a	20	0.06	
2178	0.79	NNW4b	15	0.12	

5. HYDROCHEMICAL INDICATIONS OF SULPHATE REDUCTION

5.1 *Background, aims and classification of the groundwaters*

5.1.1 Indicators of sulphate reduction

Sulphate reduction in the groundwater is indicated by abnormally low SO_4^{2-} content and abnormally high HCO_3^- content. The effect from mass transport by mixing of different groundwaters which can also give a similar SO_4^{2-}/HCO_3^- ratio has first to be compensated for. After this compensation by using mixing calculations the observations which have undergone or undergo obvious sulphate reduction can be identified. The discussion below starts by major component identification, classification, multivariate mixing-mass balance calculations used to identify obvious sulphate reduction in the groundwaters. The many figures are necessary to show the dynamic changes of the tunnel chemistry with time.

5.1.2 Groundwater data

The groundwater chemistry in the tunnel reflects the dynamics in the system which is driven by changing hydraulic properties due to the tunnel construction. The advantage of the groundwater sampling programme is that it encompasses the area and duration of the HRL tunnel construction (Figure 2-2a,b,c,d,e,f and g). The groundwater chemistry sampled in boreholes (SA, KA, HA and KR) along the Äspö HRL tunnel has been evaluated up to tunnel length 3191m. A more comprehensive description of the tunnel chemistry is given by Laaksoharju and Skårman (1995).

The groundwater observations from the HRL tunnel stored in GEOTAB, the SKB database, have been sorted according to where and when the sampling was conducted. The data has been divided into 200-day time intervals named experimental days where day 0 (1990-10-14) indicates the start of the tunnel construction, day 150 is 1991-03-13, day 350 (1991-09-29), day 550 (1992-04-16), day 750 (1992-11-02), day 950 (1993-05-21), day 1150 (1993-12-07) and day 1350 is 1994-06-25. In order to gather as complete a data set as possible for each time period a sample collected with a tolerance of ±100 days from the actual experimental day was set to represent that day (see Figures 2-2a,b,c,d,e,f and g). The analytical methods, laboratories and detection limits used when sampling and analysing the groundwaters are reported by Nilsson (1995) and Laaksoharju and Skårman (1995). The important groundwater components when evaluating sulphate reduction turned out to be: Cl, HCO_3, SO_4, and DOC. Concentrations of these elements are shown along the tunnel length as snapshots based on experimental days (Figures 5-1,5-2,5-3 and 5-4).

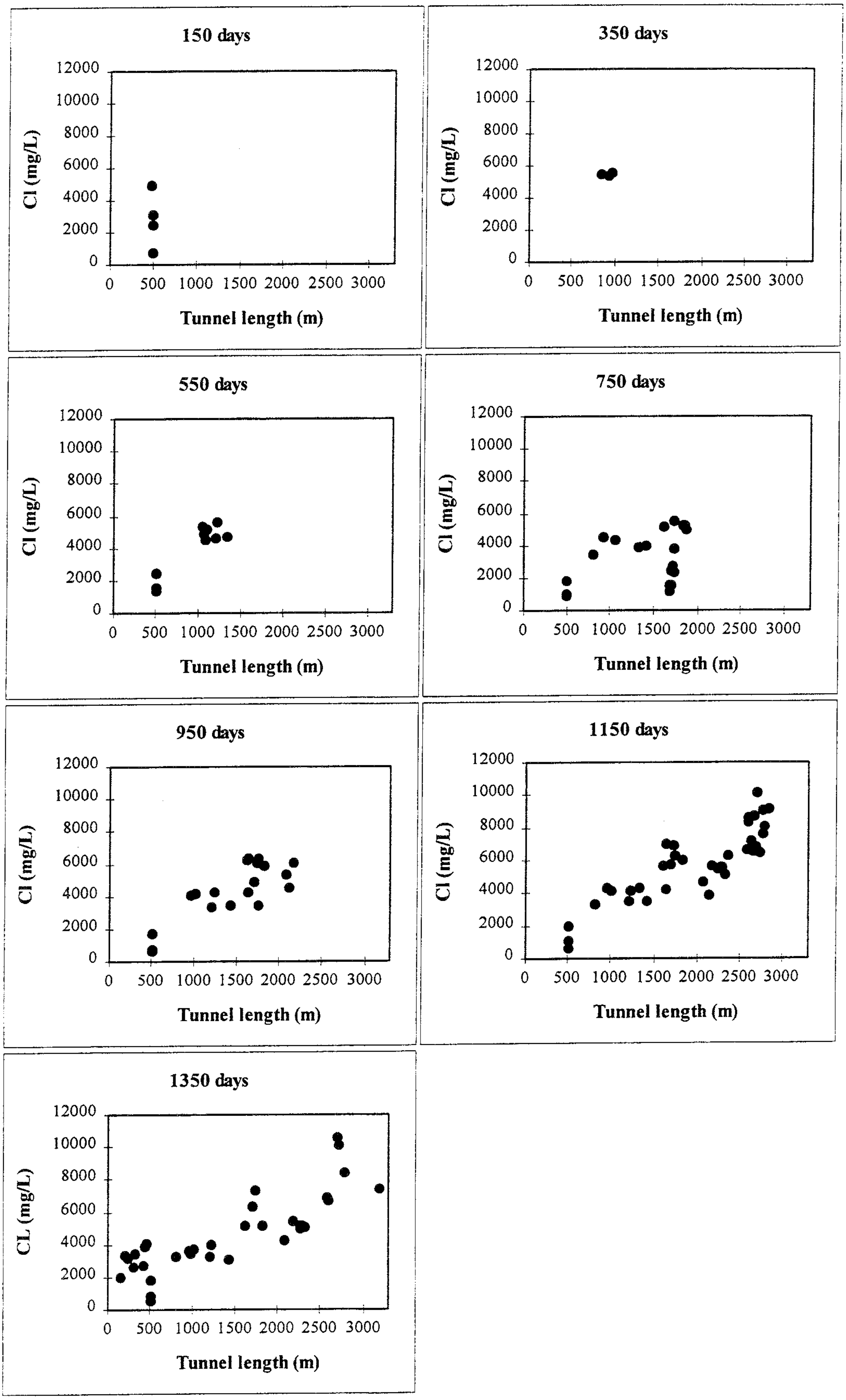


Figure 5-1. *Cl$^-$ content of the water versus the tunnel length of the HRL tunnel. Experimental days 150 (0.4 year), 350 (1 year), 550 (1.5 year), 750 (2 years), 950 (2.6 years), 1150 (3.2 years and 1350 (3.8 years) are shown.*

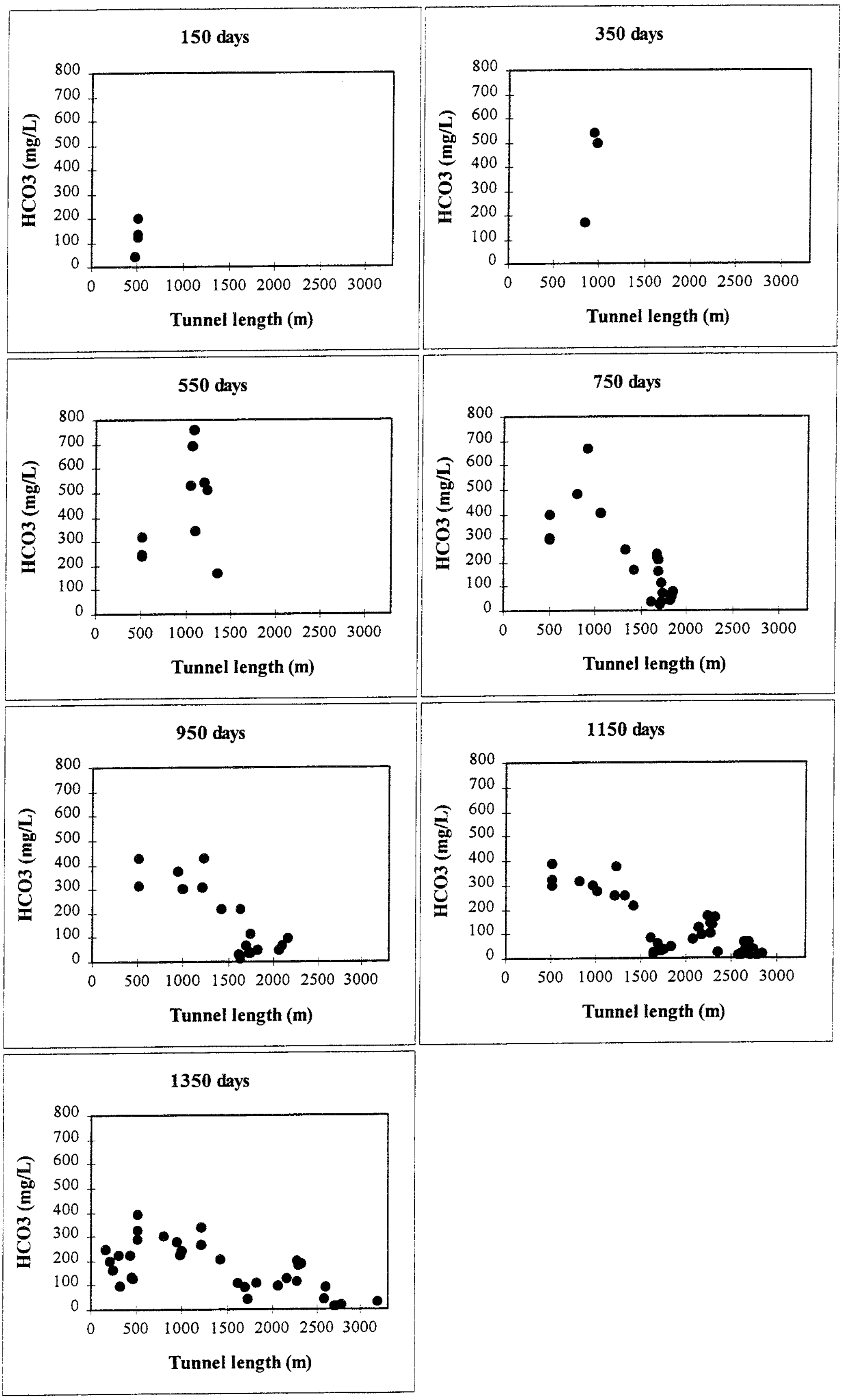


Figure 5-2. *HCO_3^- content of the water versus the tunnel length of the HRL tunnel. Experimental days 150 (0.4 year), 350 (1 year), 550 (1.5 year), 750 (2 years), 950 (2.6 years), 1150 (3.2 years) and 1350 (3.8 years) are shown.*

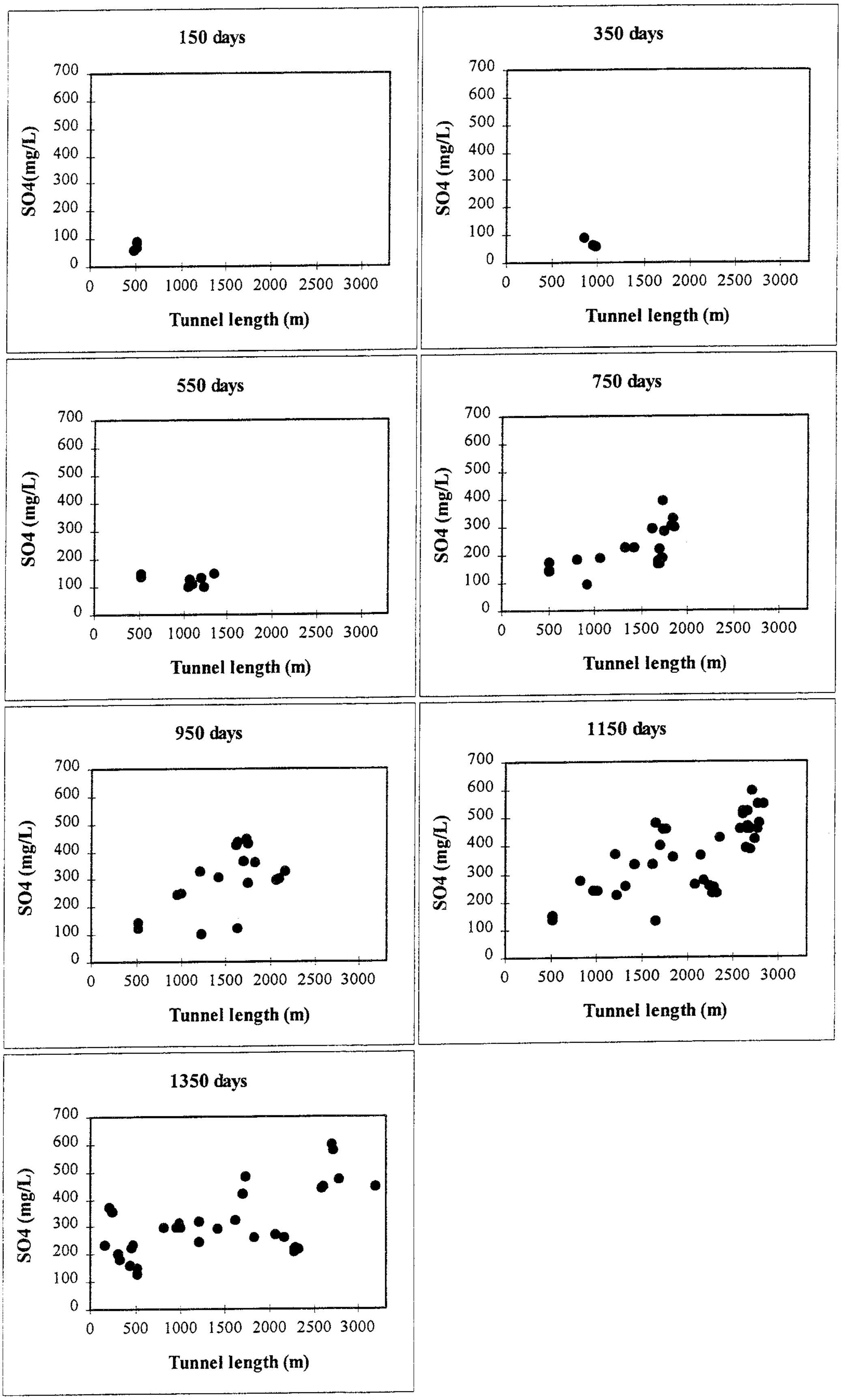


Figure 5-3. *SO_4^{2-} content of the water versus the tunnel length of the HRL tunnel. Experimental days 150 (0.4 year), 350 (1 year), 550 (1.5 year), 750 (2 years), 950 (2.6 years), 1150 (3.2 years) and 1350 (3.8 years) are shown.*

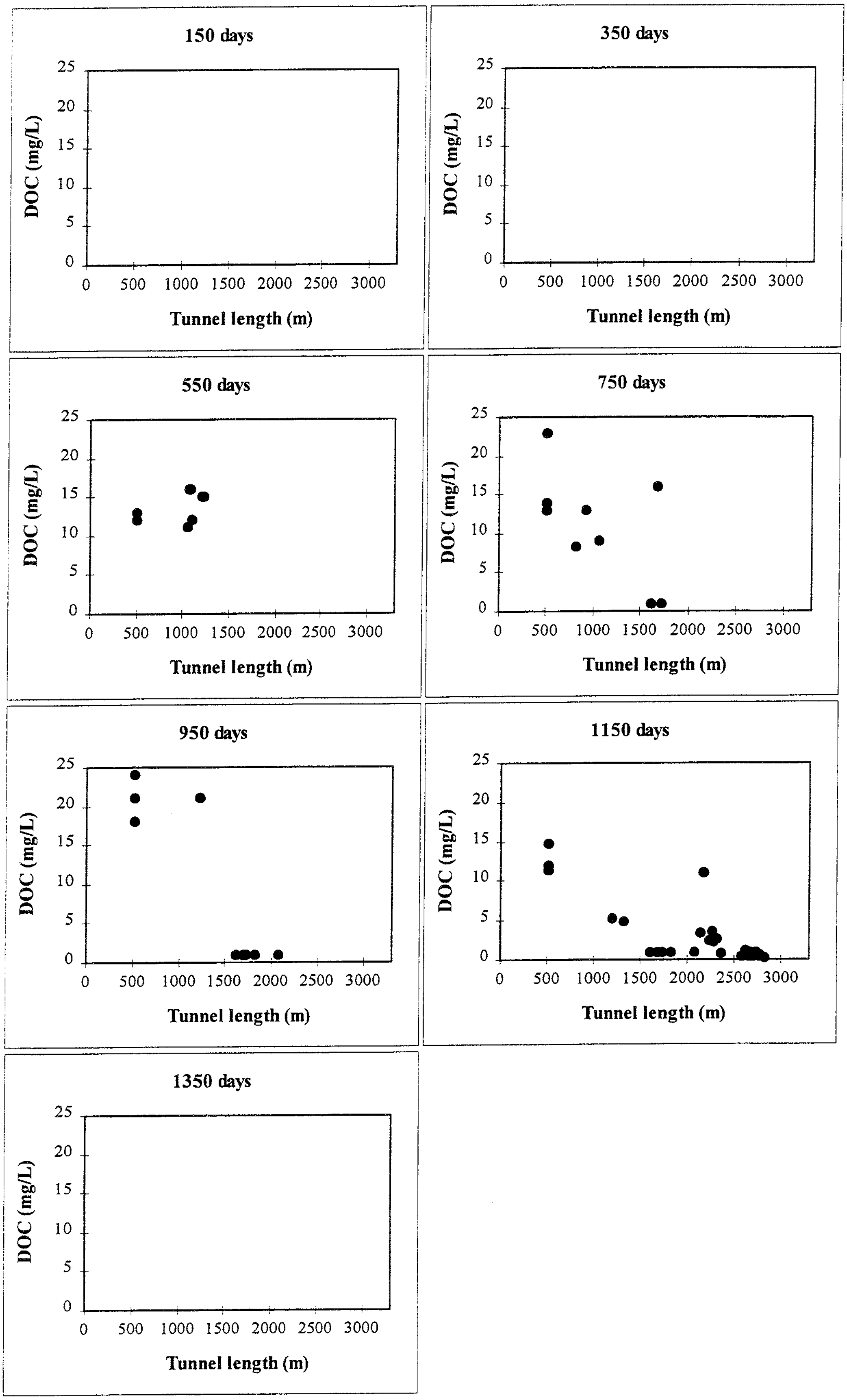


Figure 5-4. DOC content of the water versus the tunnel length of the HRL tunnel. Experimental days 150 (0.4 year), 350 (1 year), 550 (1.5 year), 750 (2 years), 950 (2.6 years), 1150 (3.2 years) and 1350 (3.8 years) are shown. During the experimental days 150, 350 and 1350 no DOC analyses were performed.

The general trend in Figure 5-1 is that Cl^- increases from 500 to 10592 mg/l with the tunnel length but is fairly stable with time (experimental days). However, a slight decrease in the Cl^- content from around 6000 mg/l to about 4000 mg/l can be detected in observations at the tunnel length 1000-1500m. The behaviour of the other elements followed expected correlation behaviour with Cl ie, Na and Ca had a similar trend (Laaksoharju and Skårman, 1995). For HCO_3^- a general decrease during the same experimental time was obtained along the tunnel (Figure 5-2). For SO_4^{2-} a general increase was seen on days 550-1350 along the whole tunnel length (Figure 5-3). Generally, high DOC values were recorded during the experimental time in the first part ie, at <1600m of the tunnel (Figure 5-4). It is important to note that the number of DOC analyses are fewer than for the other elements, which makes the interpretation of this compound behaviour less certain.

5.1.3 Distribution of the main components along the tunnel

The major ion composition (Cl, Na, Ca, HCO_3 and SO_4) of the groundwater of the HRL tunnel waters is shown in Figure 5-5. The plot shows that at 500m tunnel length (Redox zone) the major components (in descending order) showed a change from a Cl-Ca-Na-HCO_3-SO_4 water to a Cl-Na-Ca-HCO_3-SO_4 rich water on experimental days 150-550. On experimental days 750-1350 a Cl-Na-HCO_3-Ca-SO_4 water was obtained. Observations at tunnel length 1000-1500m showed a Cl-Na-Ca-HCO_3-SO_4 water on experimental days 550-750. On days 950-1350 a Cl-Na-Ca-SO_4-HCO_3 water dominated.. Observations at tunnel length 1500-3200m (days 750-1350) showed a Cl-Ca-Na-SO_4-HCO_3 water during the tunnel construction (Laaksoharju and Skårman, 1995).

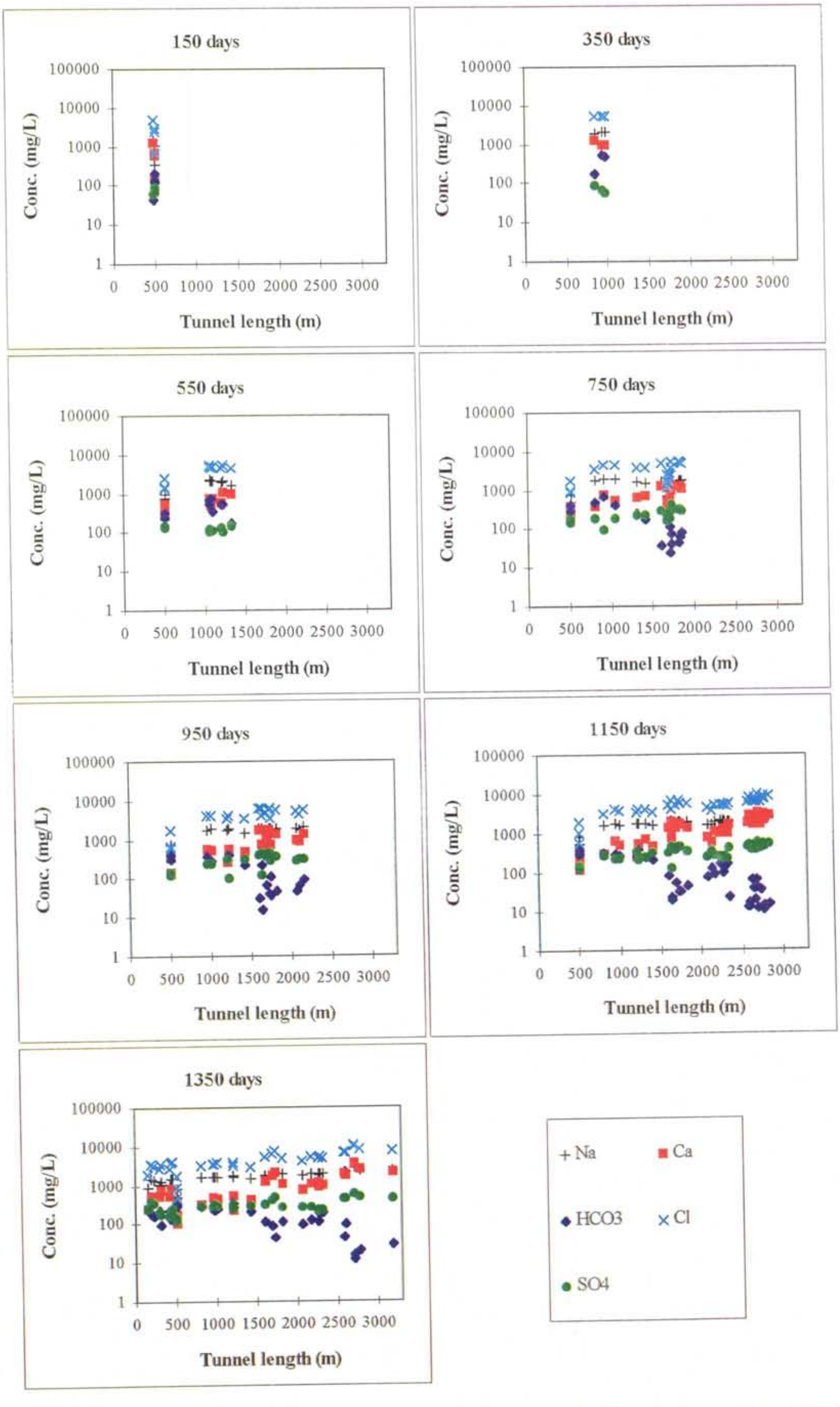


Figure 5-5. *Major groundwater ion trends along the HRL tunnel. Experimental days 150 (0.4 year), 350 (1 year), 550 (1.5 year), 750 (2 years), 950 (2.6 years), 1150 (3.2 years) and 1350 (3.8 years) are shown.*

5.1.4 Subdivision of sulphate water and chloride water

Piper plots (Piper, 1953) are widely used to present and classify major ion groundwater data. Standard trilinear Piper plots were used based on Davis and De Wiest (1967). The major variables used were Cl, Na, Ca, HCO_3, Mg, K and SO_4. In addition a classification system developed by Morgan and Winner (1962), and Back (1966) was used where the water types were classified according to the domain in which they occurred on the diagram segments. The HRL samples form a group of a sodium-chloride type [Na-Ca:Cl-SO_4-HCO_3] or a [Na-Ca:Cl-HCO_3-SO_4] type of water. The waters can therefore be subdivided into a sulphate-rich water (SO_4-type) and a bicarbonate-rich water (HCO_3-type). Based on this subdivision the occurrence of these two water types are shown in Figure 5-6. At this point it can be assumed that sulphate reduction is more likely to have occurred in the HCO_3-type of water than in the SO_4-type of groundwater, since sulphate is consumed and carbonate produced by the reaction.

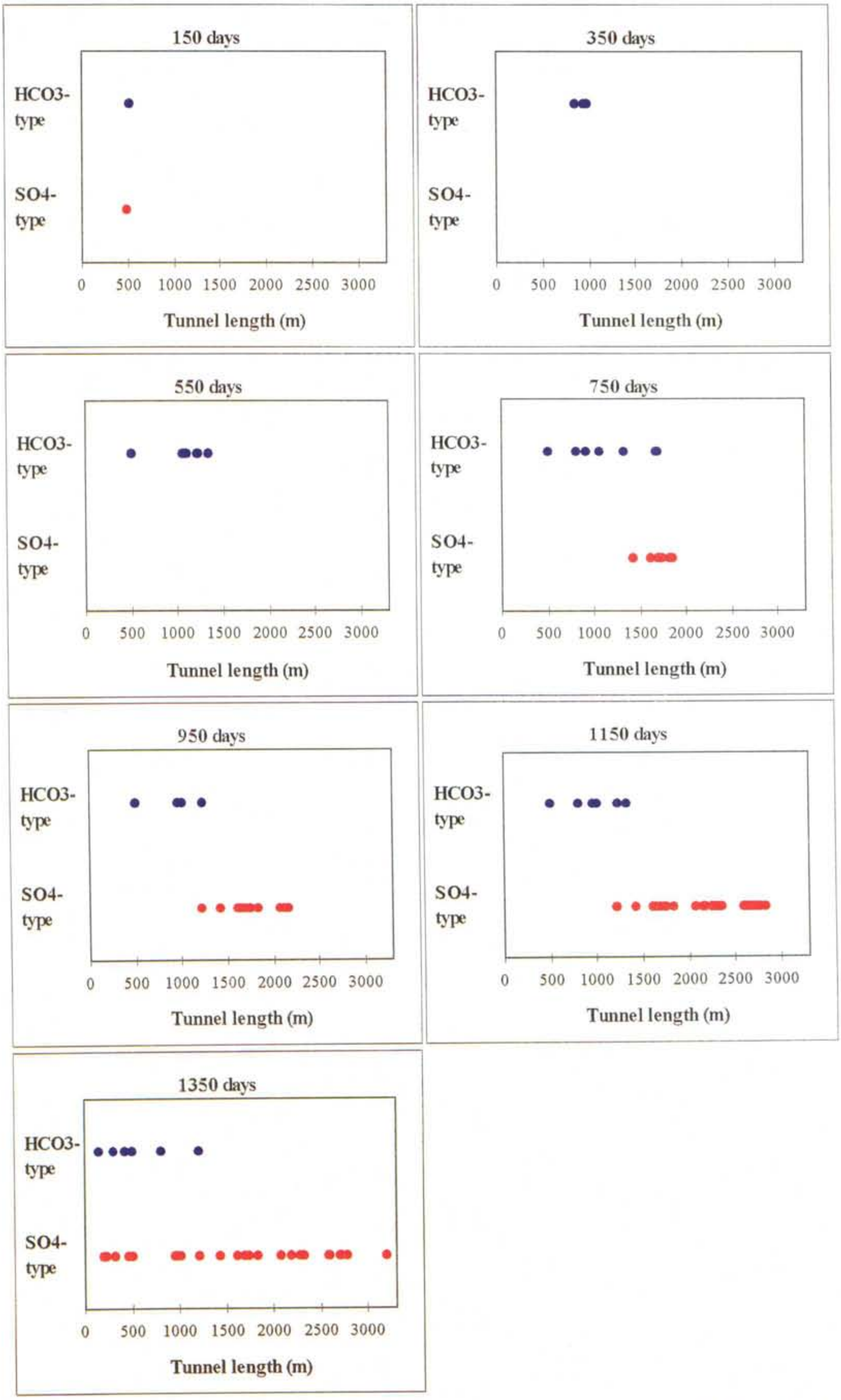


Figure 5-6. *The waters along the HRL tunnel have been subdivided into a sulphate rich water (SO_4-type) and a bicarbonate rich water (HCO_3-type). Obvious sulphate reduction is more likely to occur in the HCO_3-type of water. Experimental days 150 (0.4 year), 350 (1 year), 550 (1.5 year), 750 (2 years), 950 (2.6 years), 1150 (3.2 years) and 1350 (3.8 years) are shown.*

5.2 *Multivariate mixing-mass balance calculations*

Some observations of the groundwater composition in the HRL tunnel showed abnormally high HCO_3^- in combination with a low SO_4^{2-} content. In order to understand if this is due to mixing or is a result of in situ sulphate reduction, multivariate mixing mass balance calculations were performed according to Laaksoharju and Skårman (1995). The procedure was done in two steps: multivariate mixing calculations followed by mass balance calculations. Mixing calculations were performed to establish feasible mixing ratios that can describe the groundwater composition obtained. The mass balance calculations describe the mass transfer included in the reactions needed to describe the water composition not accounted for by mixing.

5.2.1 Principal Component Analysis

A simple two component mixing mass balance model has been described by Laaksoharju (1990), Smellie and Laaksoharju (1992) and Banwart et al. (1994). The main aim of the model was to differentiate between what is due to mixing and what is due to water/rock interaction by using a conservative tracer in the groundwater. Conservative tracers such as Cl^- may have more than one source ie, modern Baltic Sea, old sea water and deep saline groundwater in the tunnel. If Cl^- is used as a conservative tracer, erroneous predictions for the mixing ratios and the mass balance calculations may occur. The same applies to other conservative tracers such as $\delta^{18}O$ and δ^2H which may also have a signature caused by mixtures from several groundwater sources. To try to circumvent these disadvantages a standard multivariate technique called Principal Component Analysis (PCA) has been used. The method is generally used for classification, simplification of the data information and finding the most important variables in a data set etc. (Chatfield and Collins, 1989). This PCA analysis consists of a mathematical linear transformation to new variables which are called Principal Components derived in decreasing order of importance. Generally the first few components will account for most of the variation in the original data so that the effective dimensionality of the data can be reduced and hence the information be simplified. The strength of this approach is that several or all variables in a data matrix can be examined simultaneously. Greater resolution is possible and the character of the data in a general data matrix is therefore more easily identified than using univariate analysis, where only one variable is compared at a time (Wold, 1987).

PCA has been used earlier at Äspö to classify and compare groundwater data (Laaksoharju and Nilsson, 1989; Laaksoharju, 1990; Smellie and Laaksoharju, 1992). The major components Cl, Ca, Na, Mg, K, SO_4 HCO_3 and the isotopes δ^2H, $\delta^{18}O$ and ^3H were used in the PCA (Laaksoharju and Skårman, 1995). It is known that these components describe most of the variability or the information in the groundwater data and are widely used in groundwater classification and modelling.

The PCA has been used to sort similar groundwater observations from dissimilar ones and to identify end-members at Äspö. PCA has now been applied in multivariate mixing calculations (Laaksoharju and Skårman, 1995). The distance in the PCA plot is used to calculate mixing ratios for the groundwaters. It is important to note that both conservative and non-conservative elements were used to enhance the resolution in the multivariate mixing calculations. This is possible since conservative or non-conservative behaviour in one or several of the variables can be tracked by PCA. The samples from the Äspö HRL tunnel were compared with samples taken during the pre-investigation of Äspö and the monitoring phases during tunnel construction. In addition samples from Ävrö, Laxemar, the Redox Zone, sediments, rain (precipitation) and Baltic sea were included. The results from the PCA analysis and the identified end-members are shown in Figure 5-7.

The result of the modelling of two Baltic sea water samples undergoing sulphate reduction are also shown in Figure 5-7, according to the reaction:

$$SO_4^{2-} + 2(CH_2O) + OH^- \Rightarrow HS^- + 2HCO_3^- + H_2O$$

The simulated progressive sulphate reduction consumed the measured SO_4^{2-} in stages from 0% to 100% producing HCO_3^-. The calculations demonstrate that modern Baltic sea water can develop the groundwater composition found in the HRL tunnel (case A in Figure 5-7) through sulphate reduction. Sulphate reduction can also develop the pore water composition found in the sea bed sediments by reducing Baltic Sea water (case B in Figure 5-7). It is therefore concluded that sulphate reduction can take place both in the sediments and in the groundwater found in the tunnel.

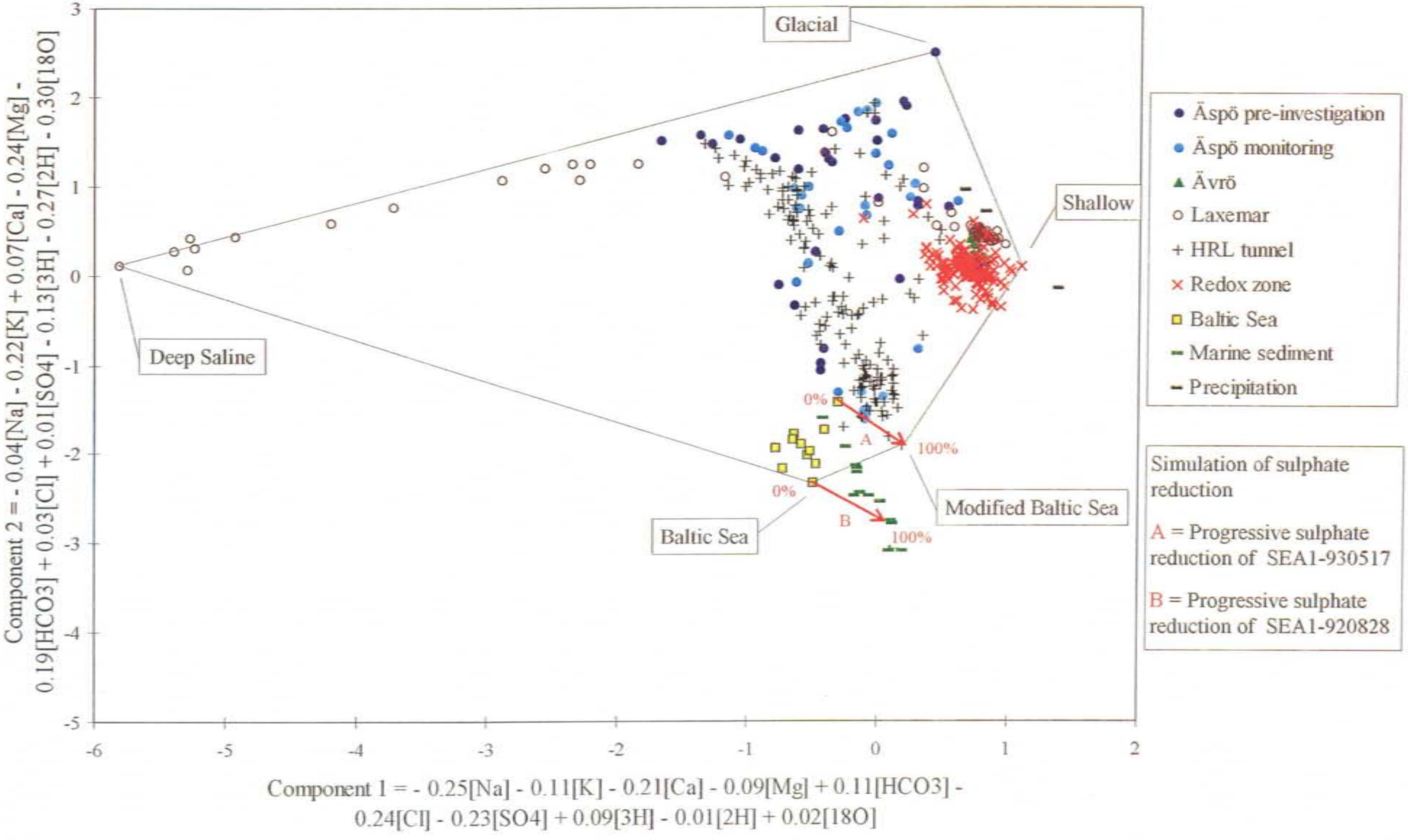


Figure 5-7. *Principal Component plot based on the major components and stable isotopes and tritium values from the HRL tunnel in relation to the main groundwater types found in the Äspö area. The weight for the different elements is shown in the equations for the first and second Principal Component respectively. These components together account for 70% of the variability, or the information in the data. Two Baltic sea water samples undergoing sulphate reduction in stages (from 0% to 100%) are modelled. The calculations show that modern Baltic sea water, through sulphate reduction can form water found in the HRL tunnel (case A) and sediment pore water (case B).*

5.2.2 Selected end-members used to explain the Äspö groundwater

The selected end-members in Figure 5-7 represent extreme waters found in the Äspö area. The **Glacial** end-member has been determined as an old glacial meltwater based on the stable isotope values which indicate cold climate recharge. The water from KAS03:129-134m shows a $\delta^{18}O$ = -15.8 SMOW and $\delta^{2}H$ = -124.8 SMOW in combination with an apparent ^{14}C age of 31365 years (Smellie and Laaksoharju, 1992). In the Baltic region these meltwaters are most likely to be mixed with the young marine stages of the Baltic Sea producing saline waters with negative $\delta^{18}O$. These types of waters are observed at Äspö today at various depths in the basement (Wallin and Peterman, 1994). **Deep Saline** water represents the brine type of water found in KLX02:1631-1681m, **Baltic Sea** water represents modern Baltic sea water (SEA01). **Modified Baltic Sea** water represents Baltic sea water which has been modified by some chemical process such as sulphate reduction according to the modelling shown in Figure 5-7. **Shallow** waters are represented by the water found in HBH02:7.5m. The selected end-members are believed to best represent waters that interact in different degrees and portions in groundwaters sampled from the whole area. The HRL tunnel groundwaters seem to be a result of mixing and water rock interaction along a Baltic Sea (modern/modified) - Glacial evolution path (Figure 5-7). An input from shallow groundwater is obtained when the tunnel passes under land. When the tunnel reaches deeper parts (>300m depth) of Äspö input of older water ie, glacial groundwater occurs.

5.2.3 Influence of glaciation on the groundwater at Äspö

The history behind the different water types is believed to reflect many complex events such as pre-glacial, glacial and post-glacial events in combination with water rock interaction. It is known that water rock interactions need a long time span (thousands or millions of years) at low temperatures (10-25°C) (pers. com. C. Bruton, 1994). Fast reactions such as ion exchange, redox reactions and reactions of the carbonate system may have taken place and altered the element concentrations relatively quickly. It is therefore important to describe the post-glacial events which may have affected the water composition on Äspö and which made the environment favourable for sulphate reduction.

When the continental ice was melting and retreating, glacial meltwaters are believed to have been injected into the basement of Äspö. The first stage of the Baltic Sea known as the Baltic Ice Lake, started to form about 14000 years ago during the Older Dryas period. During 10000 before present (BP) the Baltic Ice Lake covered the island. The fresh water probably did not affect the glacial water in the basement since the density was similar and there was no hydraulic pressure difference since the island was under water. During the next phase the Yoldia Sea (10000-9000 BP) covered the island. The brackish-marine water could have affected the water in the more conductive parts of the basement by density driven turnover (buoyancy flow).

The Ancylys Lake (9000-7000 BP) which was a freshwater lake probably did not affect the water in the basement. The next stage was the Litorina Sea (7000-2000 BP) which according to Kankainen (1986) provided saline waters which reached salinities of about 6000-7000 mg/l. Density turnover is believed to have been an important process during this period. The glacial water was replaced and mixed with the sea water. During 4000 BP the Äspö area rose above the sea level. A shallow water aquifer started to form which created hydraulic pressure differences and started to wash out the sea water from the more shallow parts of the basement. The denser sea water could mix with this fresh water and sink into the basement. Previous works (Frape and Fritz, 1987; Gascoyne et al., 1987; Nurmi and Kukkonen, 1986; Fritz et al., 1989; Wallin, 1990) have shown that groundwaters in the crystalline basement on the Canadian and Baltic shields may have been formed by marine water intrusions. In the Baltic Shield area the highly saline coastal groundwaters are only found below the highest limit of the postglacial Litorina Sea (Wallin, 1995). In Figure 5-8 the predicted C^{14} ages for the groundwaters in combination with possible influx of Litorina ($Cl = 6500$ mg/l) at 7000BP and modern Baltic Sea water ($Cl = 3760$ mg/l) is shown. The modelling shows that the older groundwaters found in the tunnel and at depths of 100-350m at Äspö may have been affected by the Litorina Sea. The Litorina Sea water had a higher penetration potential since its density was higher compared to modern Baltic Sea water. Baltic Sea water seems to have affected observations at a more shallow depth (<100m) in the HRL tunnel. The main driving force for the influx of modern sea water is the increased hydraulic gradients caused by the tunnel construction.

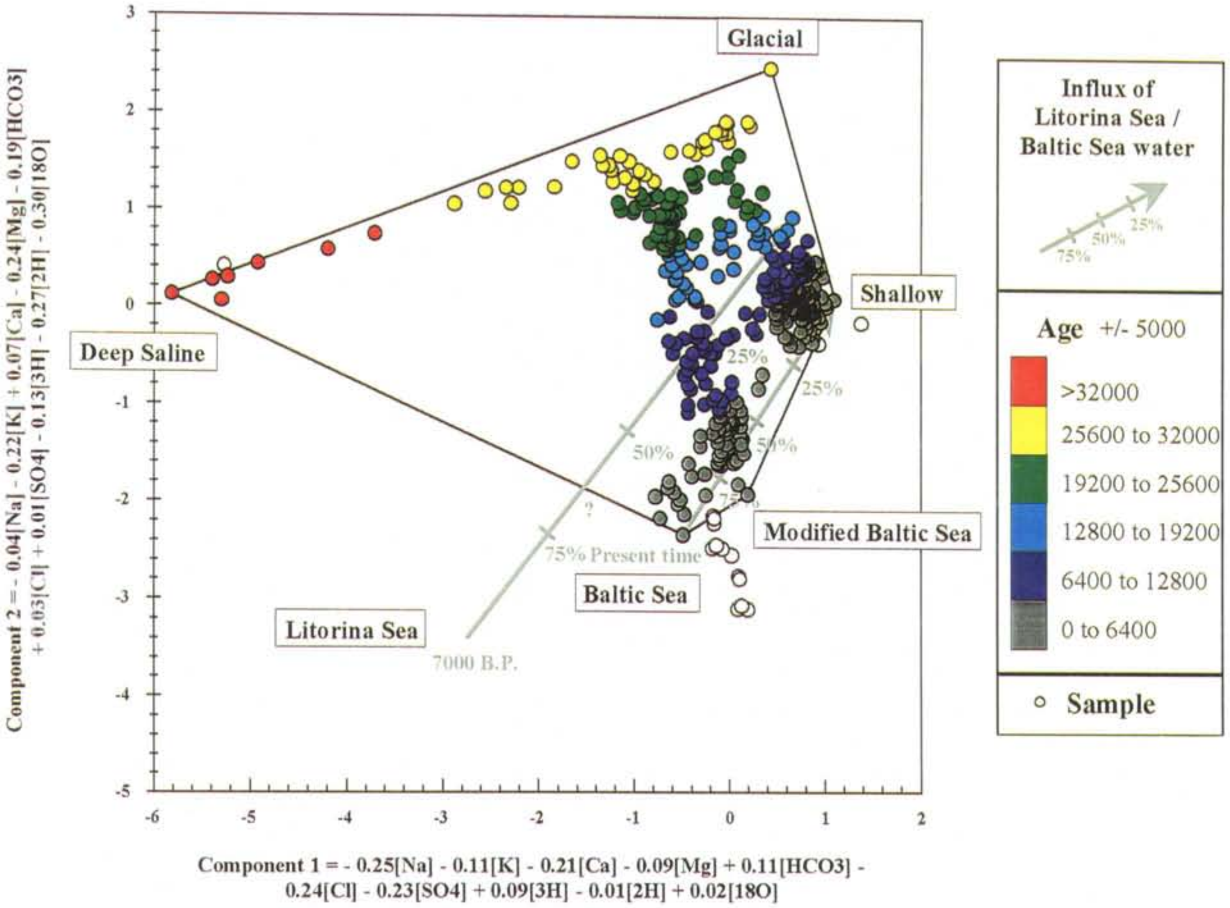


Figure 5-8. *Principal component plot is used to show the predicted C^{14} ages for the groundwaters. The possible influx of Litorina-Modern Baltic Sea water is shown.*

5.2.4 Mixing calculations applied to the tunnel groundwater

The end-members identified in the PCA can be used to explain the composition of the water entering the tunnel both during and after its construction. The five end-members in Figure 5-7 form a pentagon which is used like a phase diagram. The observations within the pentagon can by definition be described by the selected end-members. The co-ordinates for the observations are given by the first and second Principal Component respectively (Component 1 and Component 2) in Figure 5-7. The distance from any observation to the five end-members can be calculated by using simple trigonometrical functions. The distance is equivalent to the mixing ratios of any water observed in the system. The calculated mixing ratios needed to explain the groundwater during the tunnel construction are shown in Figure 5-9; mixing ratios <10% are regarded as uncertain and can be due to uncertainty in the model.

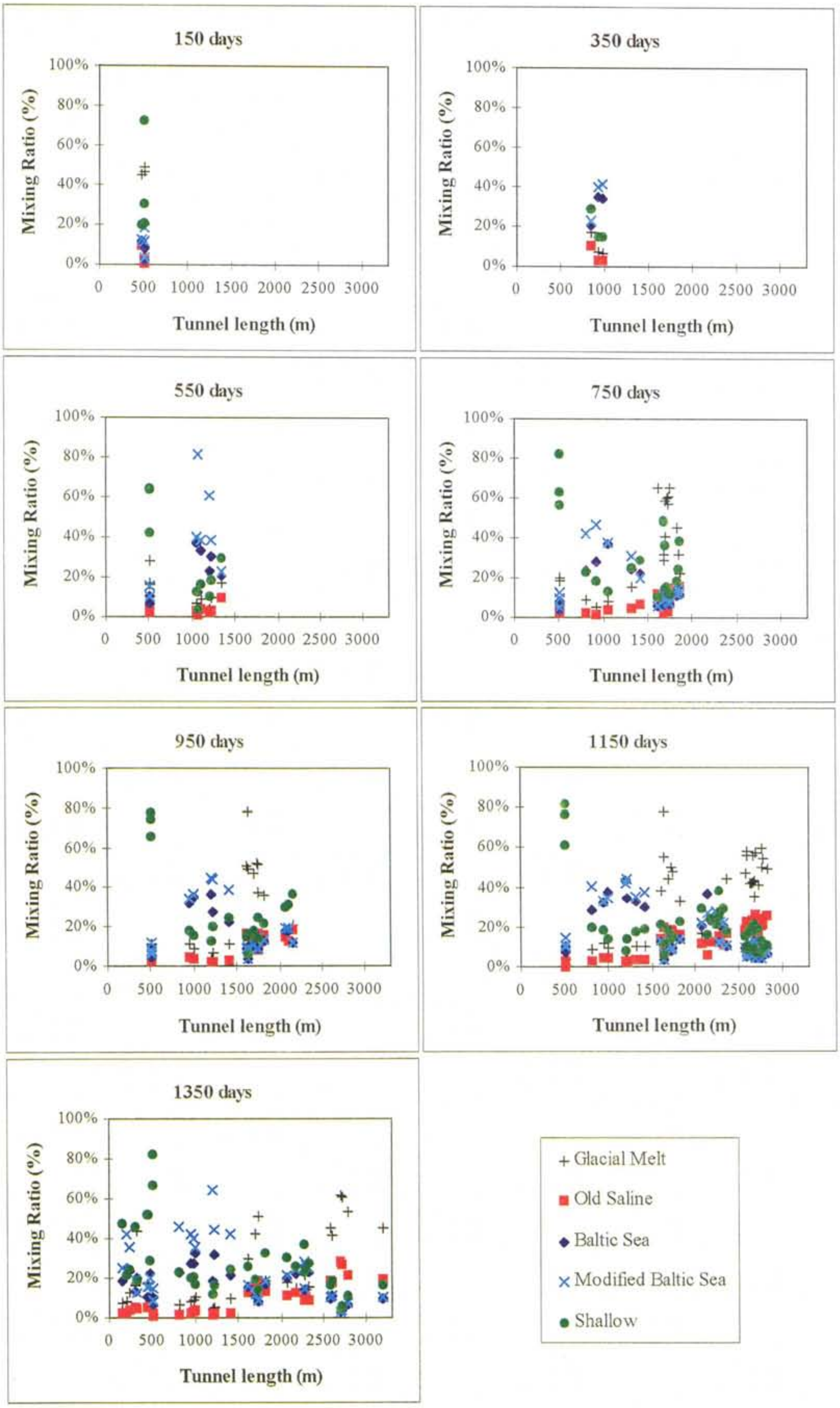


Figure 5-9. Calculated mixing ratios (%) of glacial, deep saline, Baltic Sea, modified Baltic Sea and shallow groundwaters obtained in boreholes along the HRL tunnel. The mixing ratios are calculated from the PCA analysis and add up to 100% at each tunnel length. A mixing portion <10 % is regarded as uncertain. Experimental days 150 (0.4 year), 350 (1 year), 550 (1.5 year), 750 (2 years), 950 (2.6 years), 1150 (3.2 years) and 1350 (3.8 years) are shown.

The above mixing ratio calculations describe the portions of water needed from the end-members to achieve a measured water composition in the tunnel. In the samples from the Redox zone (tunnel position 513m) a high portion of shallow water is found. At tunnel length 700-1600m Modified Baltic sea water and modern Baltic Sea water dominate. At tunnel length >1600m post glacial water and shallow water dominate. In the deepest part of the tunnel ie, tunnel length >2500m post glacial and deep saline water seems to be the major water type. Interestingly a significant portion of modern Baltic sea water is found in observations at tunnel length 2000-2300m where the tunnel passes under the Baltic Sea (see Figure 1-1 for orientation). A general feature of the tunnel water is that the location (ie, under sea, under land) in combination with depth determines the changes in the mixing proportions with time. Therefore, the groundwater flowing to a tunnel under the Baltic sea at a moderate depth (<300m) contains modern Baltic Sea water with time. Inflow to a tunnel under land, at a moderate depth, contains shallow water with time. Inflow at greater depths is more dominated by older water types. The changes in the groundwater chemistry are dependent on the prevailing hydrogeological situation; they may take place within a time frame of days to years.

5.2.5 Mass balance calculations as an indicator of sulphate reduction

Mass balance calculations were used to indicate that sulphate reduction had occurred in the groundwater. When performing mass balance calculations the end-member composition and the mixing ratios (shown in Figure 5-9) were used to predict new values for important elements for sulphate reduction such as HCO_3^- and SO_4. The predicted values were then compared to the measured ones (Figure 5-10). Little (<10%) or no deviation (0%) from the model predictions indicates that the concentration can be adequately explained by mixing; large deviations indicate sources or sinks for a particular element that are not accounted for by the mixing model. The reason for the deviation are reactions that influence the mass balance. The outcome of the calculations may be affected by the number or type of selected end-members. In any case tests showed that the relative <u>strength</u> of the predicted gain or loss for a particular element is more sensitive than the predicted element <u>behaviour</u> itself (Laaksoharju et al., 1995 in Banwart et. al., 1995). When the potential for sulphate reduction is calculated the strength is of importance. This means that there is a risk that observations containing sulphate reduction are masked by the selected end-members.

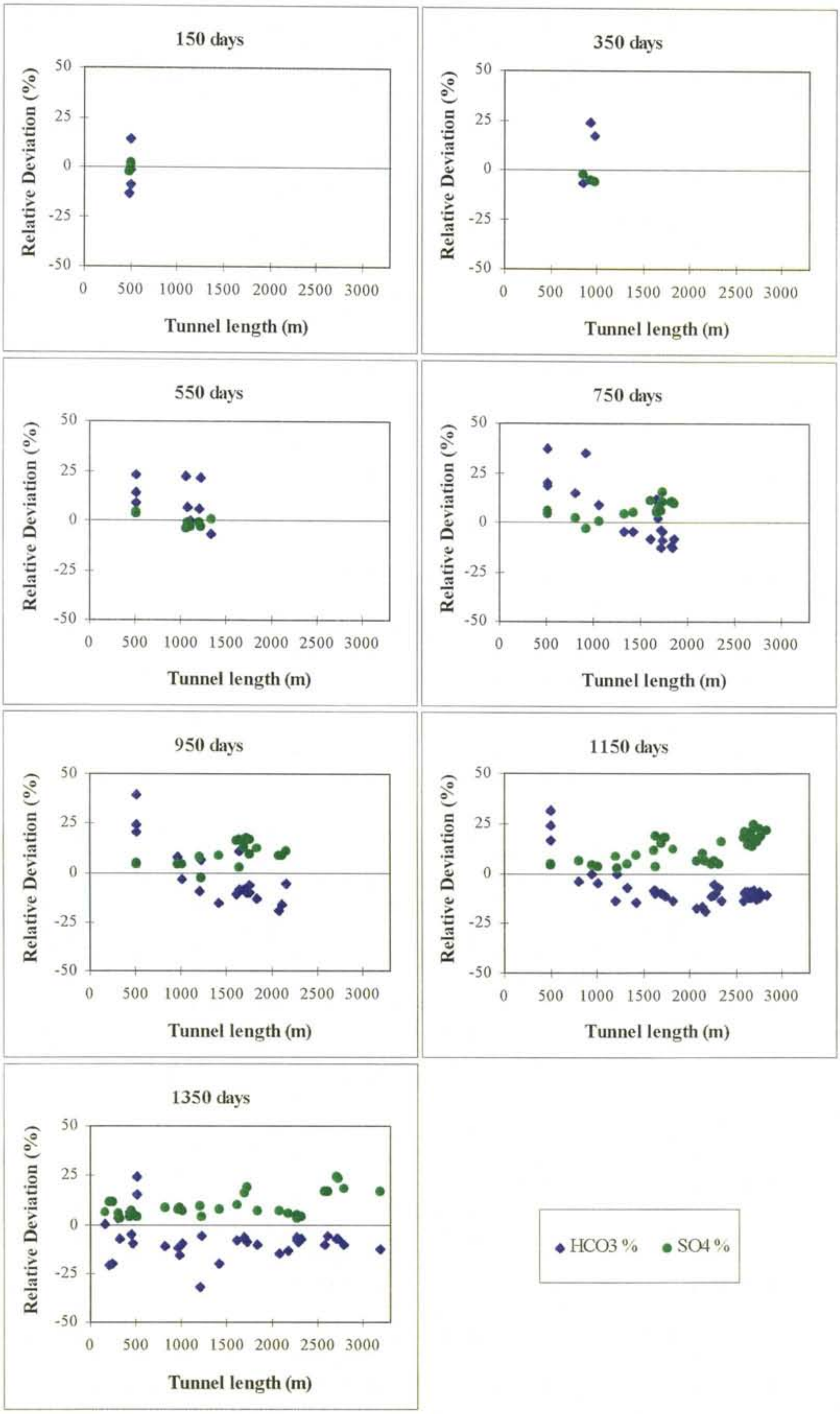


Figure 5-10. *Results of the mass balance calculations show the deviation of the predicted HCO_3^- and SO_4^{2-} content compared to observed tunnel water composition with time. A deviation from the observed element content indicates mass balance reactions. A negative value indicates loss of and a positive value gain of that particular constituent which cannot be explained by mixing. Experimental days 150 (0.4 year), 350 (1 year), 550 (1.5 year), 750 (2 years), 950 (2.6 years), 1150 (3.2 years) and 1350 (3.8 years) are shown.*

The mass balance calculations (Figure 5-10) show that the samples taken from the section under the Baltic Sea (700-1600m tunnel section) have fewer observations where HCO_3^- is gained and SO_4^{2-} is lost during tunnel construction. This is interpreted as a higher potential for sulphate reduction of samples taken during the earlier stages of the tunnel construction and taken from tunnel length <1500m. A high potential for sulphate reduction is a sample which has undergone sulphate reduction which cannot be explained by mixing and mass transport of water from other locations. In order to ascertain which samples have the highest potential for sulphate reaction the following boundary conditions for a clear sign of sulphate reduction were established. The reaction

$$SO_4^{2-} + 2(CH_2O) + OH^- \Rightarrow HS^- + 2HCO_3^- + H_2O$$

shows that one mole of SO_4^{2-} (96g SO_4^{2-}) yields 2 moles HCO_3^- (2 * 61 = 122g HCO_3^-). Thus 96 mg/l SO_4^{2-} yields 122 mg/l HCO_3^- and the ratio HCO_3^-/SO_4^{2-} is 1.27. So when the deviation ratio in Figure 5-10 between bicarbonate and sulphate is larger than 1.27 expressed as mole ratio ($\Delta[HCO_3^-]/\Delta[SO_4^{2-}] > 1.27$), and the SO_4^{2-} deviation is negative there is a large probability that obvious sulphate reduction had taken place.

The samples from SA0940B (91-11-08), SA0976B (91-10-15), SA1062B (92-04-23), SA1077A (92-04-23). SA1210A (92-05-07), SA0923A (92-12-02), and SA1229A (92-05-07 and 93-06-23) with their calculated potential for obvious sulphate reduction are shown in Figure 5-11. The figure shows that obvious sulphate reduction may occur at tunnel length 900-1300m and on experimental days 350-950. The potential for obvious sulphate reduction seems to decrease with time which means that the response of high bicarbonate and low sulphate seems to vanish. The decrease cannot be explained by a changed flow situation since the flow is compensated for in the calculations. The probable explanation is that sulphate reduction decreases with time.

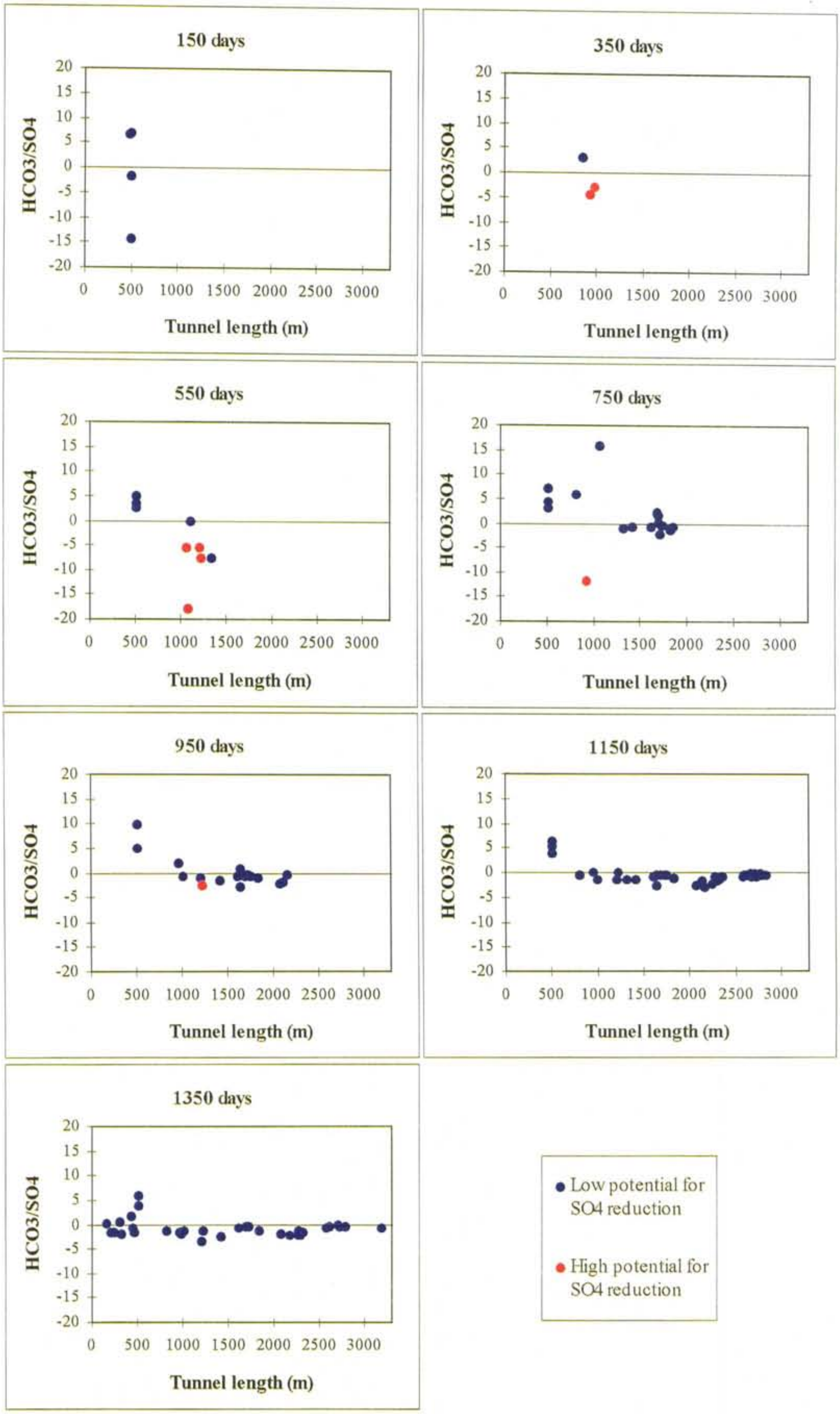


Figure5-11. *Samples with a calculated potential for obvious sulphate reduction along the HRL tunnel. Red dot = high potential for obvious sulphate reduction, blue dot = low potential for obvious sulphate reduction. Experimental days 150 (0.4 year), 350 (1 year), 550 (1.5 year), 750 (2 years), 950 (2.6 years), 1150 (3.2 years) and 1350 (3.8 years) are shown.*

Sulphate reduction and chloride concentration

The potential for sulphate reduction in the tunnel showed no direct correlation with the influx portions of Baltic Sea water, Glacial water, Shallow water or Deep Saline water. The only water type which showed a correlation was Modified Baltic Sea water (Figure 5-12). The higher the percentage of modified Baltic Sea water the higher the potential for obvious sulphate reduction. Obvious sulphate reduction seems to require a distinct Cl^- concentration of 4000-6000 mg/l (Figure 5-13). This observation is in agreement with laboratory experiments on microbes, which show a preference for water with a similar salinity.

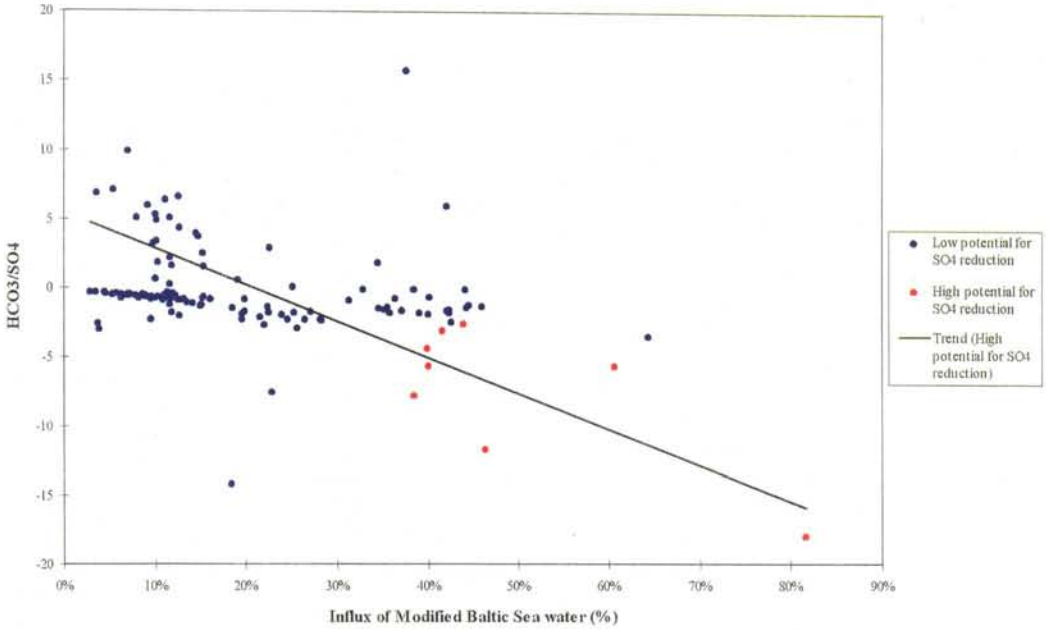


Figure 5-12. *The higher the percentage of Modified Baltic sea water the higher the potential (more negative value) for obvious sulphate reduction. The linear regression analysis is based on the groundwaters with high potential for sulphate reduction (red dots).*

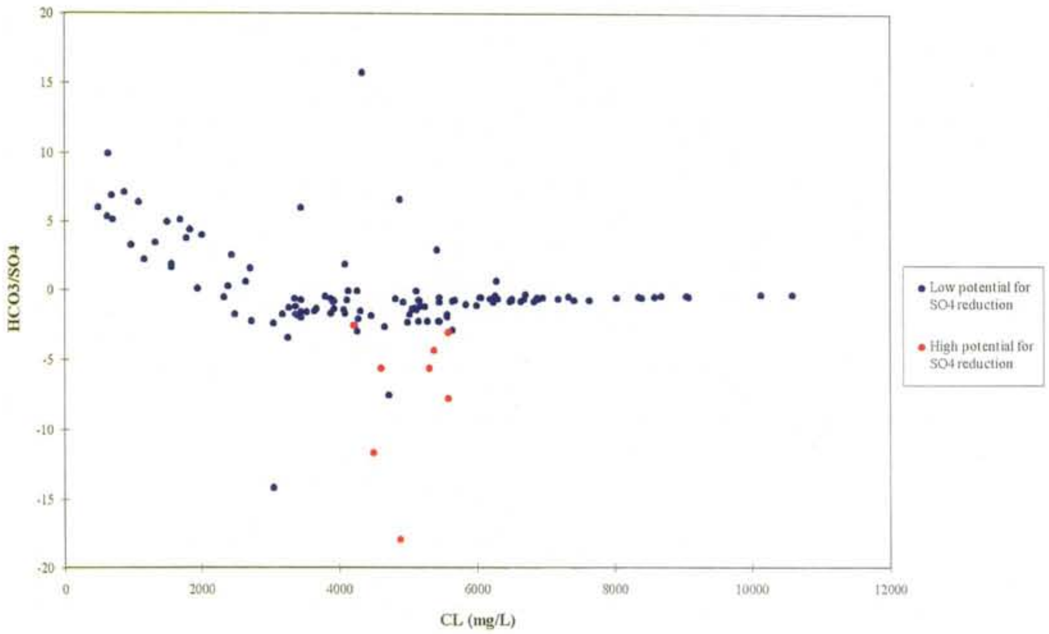


Figure 5-13. *Extensive sulphate reduction seems to require a distinct* Cl^- *concentration of 4000-6000 mg/l. Observations with a calculated high potential for sulphate reduction are shown as red dots.*

Sulphate reduction and DOC

Obvious sulphate reduction seemed to require a >10 mg/l DOC content in the evaluated groundwaters (Figure 5-14). However, recent investigations in the front part of the tunnel showed large numbers of SRB (up to 75 SRB per ml) in the boreholes at tunnel lengths 3105m, 3110m and 3250m. These boreholes contained 2.8-4.0 mg/l DOC which is lower than the 10 mg/l DOC limit found in the observations in the first part of the HRL tunnel. However, these bacteria seemed to also use hydrogen as an electron donor instead of organic carbon. Therefore they may not have been totally limited by the organic carbon content in the groundwater. Although the effect from mass transport due to mixing has not yet been evaluated in these boreholes. This sulphate reduction process is, however, usually less vigorous than when organic carbon is consumed.

Mass balance calculations showed that a theoretical lower boundary condition for extensive sulphate reduction to take place occurs when CH_2O = 0.6 mg/l, SO_4^{2-} = 1 mg/l, HCO_3^- = 1.27 mg/l and the HS^- content is 0.3 mg/l. These calculations also indicated that an upper limit of 121 mg/l HS^- might have been produced assuming that the measured 122 mg/l HCO_3^- in sample SA1229A resulted purely from sulphate reduction. There are no observations of such high concentrations of sulphide and in practice the high reactivity of HS^- will keep it at low levels. The highest concentration measured in the groundwater at the Äspö site and in water which had undergone sulphate reduction was around 1 mg/l HS^-. The sulphide produced reacts with iron and other metals, which form insoluble sulphide phases, and thus decreases the concentration in the groundwater.

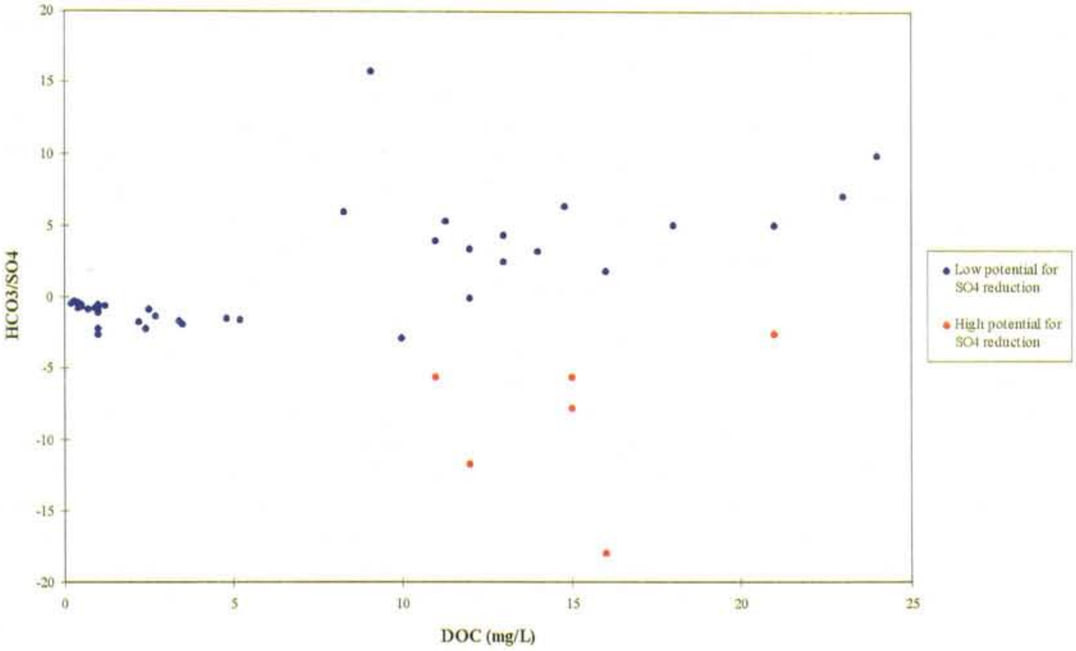


Figure 5-14. *Extensive sulphate reduction seems to require a > 10mg/l DOC content in the evaluated groundwaters. Observations with a calculated high potential for sulphate reduction are shown as red dots.*

The above-mentioned favourable conditions for sulphate reduction were met during the first phase of the tunnel construction (days 250-950) in observations from tunnel length 900-1400m. Sulphate and organic matter found in the sea water as well as sediments form the necessary conditions for obvious sulphate reduction. Increased hydraulic flow due to the tunnel construction, which resulted in an increase of influx of Modern Baltic Sea water, Glacial water, Shallow water and Deep Saline water may have made conditions less favourable for sulphate reduction. The hypothesis is that sulphate reduction is still going on but the increased influx of other water types lowered the available organic content <10 mg/l with time. This is indicated by the DOC analyses (Figure 5-4) of the groundwaters. This decrease will make the reaction less obvious and the groundwater response less clear.

6. INDICATIONS OF SULPHATE REDUCTION FROM ISOTOPES

6.1 *Background and aims*

The available sulphur isotope ($\delta^{34}S$) data has been used as an indicator of past and present sulphate reduction. Sulphur isotopes have been analysed in monosulphides (FeS) found in Baltic sea bed sediments and in dissolved sulphate (SO_4^{2-}) in the pore water of the sediments. They have also been analysed in the fracture filling mineral pyrite (FeS_2) and in dissolved sulphate (SO_4^{2-}) of the groundwater at depths ranging from 2-1000m (Figure 2-1 and 2-2a,b,c,d,e,f and g). Unfortunately there are insufficient observations from the HRL tunnel below the Baltic sea. An open system for the sulphate reduction discussed below refers to a system with a large unlimited supply of SO_4^{2-}, whereas a closed system refers to a system with a limited supply of SO_4^{2-}.

6.2 *Methods used for sampling and evaluation*

Groundwater was collected in plastic containers washed in acid. The samples were filtered, acidified to a pH of 1 and SO_4^{2-} was collected as a barium sulphate precipitate using $BaCl_2$. The precipitate was carefully washed in distilled water and removed by decanting and drying. Trace amounts of H_2S in the water may have been oxidised during the sampling procedure, although these should not have affected the $\delta^{34}S$ values of the SO_4^{2-} and were therefore considered negligible.

Preparation for the sulphur isotope analyses was done according to a technique where the $BaSO_4$ was mixed with vanadium pentoxide (P_2O_5), silica (not all samples, only those of small amounts, (pers. com. Krouse, 1993) and pure copper and placed in a quartz glass boat and preheated in an oven at 450°C for 30 minutes. Then the sample was burned in an oven at 900°C for another 30 minutes. The SO_2 gas generated was collected in a pentane trap using liquid nitrogen in order to remove the excess CO_2 gas. The SO_2 gas was then transferred to a sample vessel for analysis.

Sulphur isotope measurements were performed with a Finnigan Mat Delta E, double inlet mass spectrometer at Energiteknikk at Kjeller, Oslo, Norway. The instruments were calibrated with sulphide standards and the results are related to Canyon Diablo Troilite (CDT). Isotopic analyses of the dissolved SO_4^{2-} and sulphides (FeS, FeS_2) are expressed in per mil (‰) deviations (δ values) from the standards:

$$\delta = [(R_{sample}/R_{standard}) - 1] * 1000$$

and R is $^{34}S/^{32}S$. The relative accuracy of the $\delta^{34}S$ ratios is equal to or better than 0.01 percent of the δ values. This corresponds to 0.1 per mil in $\delta^{34}S$.

6.3 Indications from the $\delta^{34}S$ values in the sea bed sediments

Sea bed sediments and pore water have been sampled in Stations I and II situated above the HRL tunnel (Figure 2-1) and were analysed for their chemical composition and for sulphur isotopes in the upper part of the sediments (8-10cm). In the same pore water SRB were also identified. The sediments were black because of the large concentration of monosulphides, dominated by iron sulphides such as troilite (FeS), and had a typical smell of H_2S.

The SO_4^{2-} pore water from the upper 5cm displayed homogeneous $\delta^{34}S$ values of about +19.0 and +19.8‰ (Table 6-1 below) similar to the SO_4^{2-} in the Baltic sea water (approx. +19‰). These are typical marine signatures for the $\delta^{34}S$. The FeS collected from the corresponding sediments showed $\delta^{34}S$ values between -0.3 and -2.3‰ (as shown in Table 6-2). These isotopic data strongly indicate an ongoing bacterial sulphate reduction.

Table 6-1. $\delta^{34}S$-values from pore water sulphate in sea bed sediments from Stations I and II above the HRL tunnel (for orientation see Figure 2-1).

Station	Depth (cm)	$\delta^{34}S$ (‰)	Station	Depth (cm)	$\delta^{34}S$ (‰)
I	0-2	+19.2	II	0-2	-
I	4-5	+19.0	II	4-5	+19.8
I	8-10	-	II	8-10	-

In an open system the FeS formed by SRB will attain $\delta^{34}S$ values around ±0‰, which is consistent with the observations in the FeS in the sea bed sediments. The reason for the approximately 20‰ lighter $\delta^{34}S$ signature in the FeS compared to the initial SO_4^{2-} is due to the fact that the reducing bacteria starts to use the light isotopes because of a more favourable thermodynamic reaction. An initial value of around +19‰ in the SO_4^{2-} in the Baltic sea water will therefore result in a δ value close to 0‰ in the FeS. Hence, in conclusion the observed $\delta^{34}S$ values of the FeS and the dissolved SO_4^{2-} pore water from the sea bed sediments indicate that there is an open system for bacterial sulphate reduction, at least in the upper part of the sediment, with an unlimited supply of SO_4^{2-} from the Baltic sea water. It is known that since sulphate reduction removes sulphate from the pore water of the sediments, it will create a concentration gradient for sulphate resulting in diffusion of sulphate into the sediment from the sea water directly above (Berner, 1972, 1982, 1984; Goldhaber and Kaplan, 1974). The higher HCO_3^- concentrations (715 mg/l) and correspondingly low SO_4^{2-} concentrations (16 mg/l) observed further down (6m) in the sediment imply a transition from an open to a closed system for the ongoing sulphate reduction. This is supported by the fact that a closed system signifies a limited supply of SO_4^{2-}.

Table 6-2. δ^{34}S-values from monosulphides (FeS) in sea bed sediments from Stations I and II above the HRL tunnel.

Station	Depth (cm)	δ^{34}S (‰)	Station	Depth (cm)	δ^{34}S (‰)
I	0-2	-0.3	II	0-2	-1.4
I	4-5	-2.2	II	4-5	-2.0
I	8-10	-1.8	II	8-10	-2.3

6.4 Indications from the δ^{34}S values in the shallow groundwater

The δ^{34}S values from dissolved SO_4^{2-} in shallow groundwater are only available from boreholes KR0012B, KR0013B and KR0015B in the HRL tunnel (Redox fracture zone see Figure 2-1). The isotope signatures varied between +21 to +17‰, indicating a mixed sulphur source as seen in Table 6-3 below. High pCO_2 as well as HCO_3^- readings were observed in boreholes KR0012B, KR0013B and KR0015B. In the Redox zone both SO_4^{2-} and HCO_3^- in the groundwater increased with time. If leaching of marine sediments in the near vicinity was responsible for the increase in SO_4^{2-} in the KR0012B, KR0013B and KR0015B boreholes then the δ^{34}S values should have remained around +19 and +20‰. However, there was a significant relative decrease of the δ^{34}S values of the dissolved sulphate in the discharge area of the Redox zone of slightly more than two per mil. Hence, the decrease in δ^{34}S values of the Redox zone can be due to oxidation of monosulphides and pyrite which may have lowered the values. It is important to note that there is no chemical, isotopic or microbial indication of ongoing sulphate reduction in the Redox zone.

Table 6-3. δ^{34}S values from the Redox Zone in the Äspö HRL tunnel.

Borehole	Date	d^{34}S
HBH01	920228	+19.16
HBH01	920228	+17.4
KR0012B-1	920422	+17.8
KR0012B-2	920422	+19.3
KR0013B-1	920422	+20.5
KR0013B-2	920422	+20.5
KR0013B-1	920422	+19.6
KR0013B-2	920422	+19.6
HBH01	920422	+17.3
HBH01	920423	+17.5
KR0012B	920506	+19.6
KR0013B	920506	+21.2
KR0015B	920506	+19.4
KR0012B-1	920626	+18.6
KR0012B-2	920626	+18.2
KR0013B-1	920626	+19.7
KR0013B-2	920626	+19.8
KR0015B-1	920626	+17.7
KR0015B-1	920826	+17.4
KR0015B-2	920826	+17.5
KR0012B	930207	+17.5
KR0013B	930207	+17.7
KR0015B	930207	+17.2
HBH02	930207	+17.1

6.5 *Indications from the δ^{34}S values in the deep groundwater at Äspö and Laxemar*

Sulphate in groundwaters sampled from boreholes KAS02, KAS03, KAS06 and KLX01, showed δ^{34}S values ranging from +20‰ in the upper (<150m depth) bedrock to about +10‰ in the deep saline groundwater (Figure 6-1) (Wallin, 1993; Wallin and Peterman, 1994). The data revealed an overall decrease in δ^{34}S values with depth. However, one exception was a water sample from KAS06 (465m), which attained +20‰ ie, similar to the shallower groundwaters.

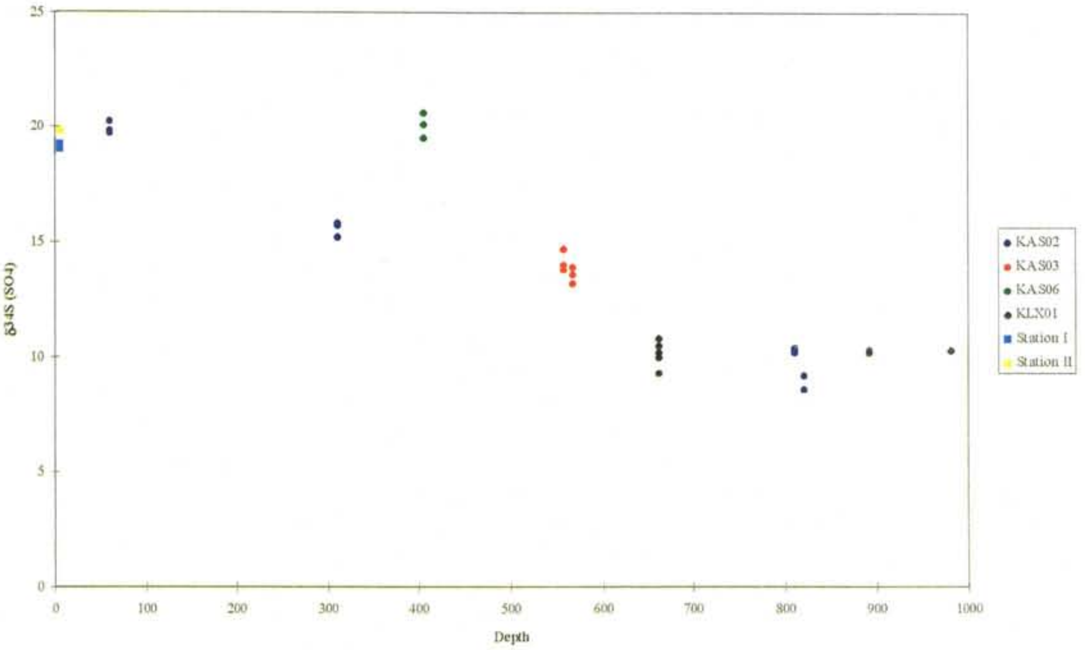


Figure 6-1. *$\delta^{34}S$ values from dissolved SO_4^{2-} in the KAS boreholes and SO_4^{2-} in sea bed sediments plotted versus depth.*

During a bacterial sulphate reduction the SO_4^{2-} is consumed by the bacteria, which is easily observed in a closed system. Hence, if an extensive bacterial sulphate reduction had taken place in the present groundwater at Äspö, assuming a closed system, very low SO_4^{2-} concentrations would be expected. This is not the case in the groundwater at Äspö and Laxemar. Moreover, the SO_4^{2-} showed no signs of increased $\delta^{34}S$ values, which is typical for the remaining SO_4^{2-} in an ongoing bacterial sulphate reduction system. This strongly suggests that there is no obvious sulphate reduction taking place today in the deep groundwater. Accordingly, the anomalous $\delta^{34}S$ value (+20‰) of SO_4^{2-} observed in borehole KAS06 (465m), which is relatively high in SO_4^{2-} concentration and not enriched in $\delta^{34}S$, cannot therefore be connected to a sulphate reduction. Instead this SO_4^{2-} with its high $\delta^{34}S$ value (Figure 6-1) reflects a marine signature, possibly a Litorina sea water signature.

In contrast, the $\delta^{34}S$ values of the fracture pyrite from KAS02, KAS03 and KAS06 are very low (±0 to -26‰) from a depth of 200 to 500m (Wallin, 1993; Tullborg et al., 1995). This is illustrated in Figure 6-2. This implies that the FeS_2 observed in the basement rock at the lower depth (>200m) was formed during a sulphate reduction which to a large extent had taken place in the past. The well crystallised pyrite cubes' appearance also supports the conclusion that a sulphate reduction had taken place in the past (Figure 6-3). The wide span observed in the $\delta^{34}S$ values and the very low values may indicate a low rate of reduction (Trudinger and Chamber, 1973) or a complex assembly of sulphate reducers (Hallberg, 1984).

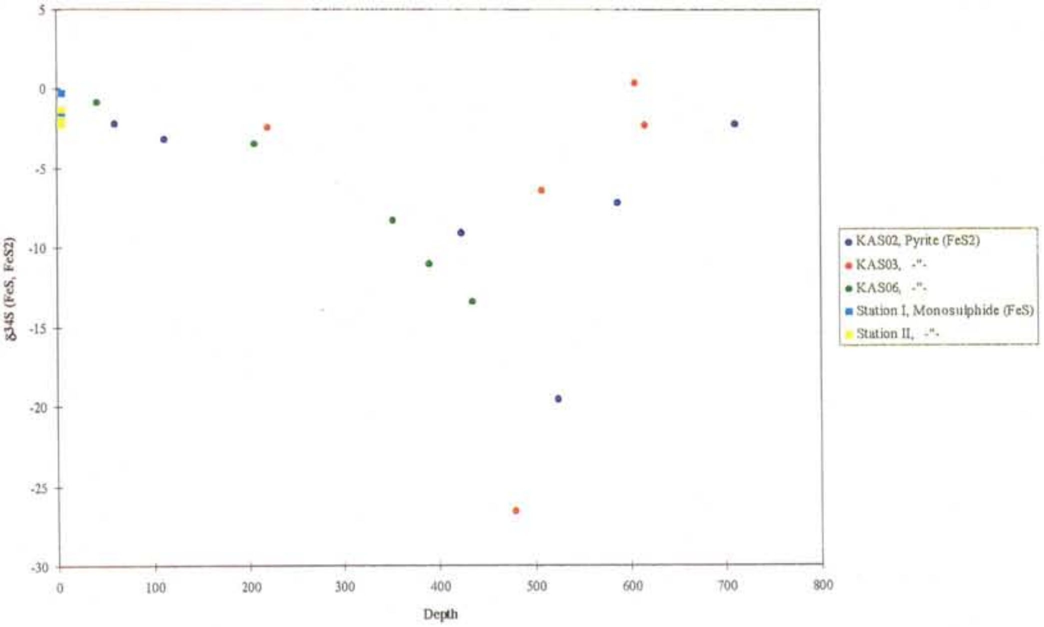


Figure 6-2. *$\delta^{34}S$ values from FeS_2 in the KAS boreholes at Äspö and FeS in sea bed sediments plotted versus depth.*

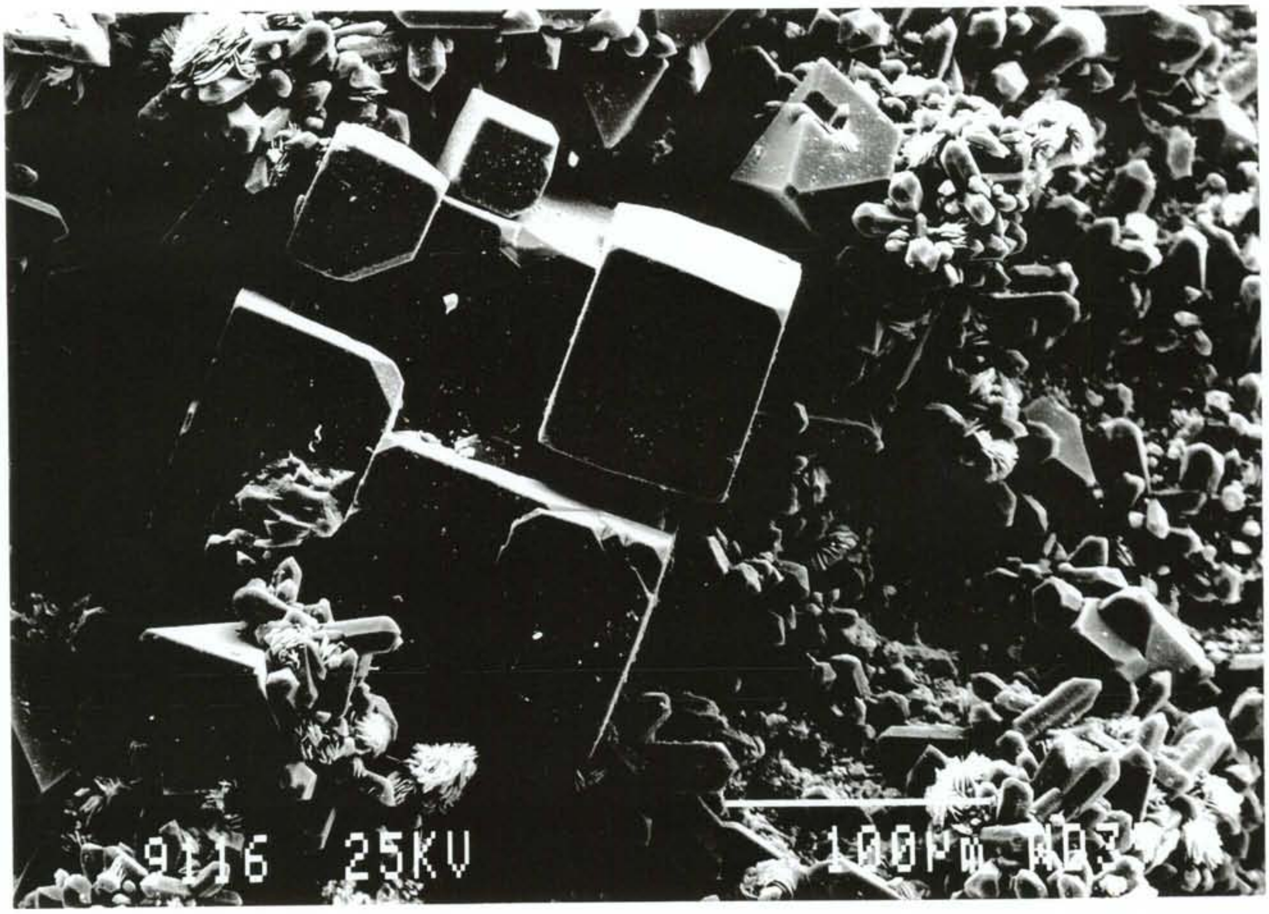


Figure 6-3. *SEM micrograph of pyrite (FeS_2) crystal in a fracture in drillcore KAS03 at a depth of 534m which is used as an indicator of possible sulphate reduction by bacteria taken place in the past.*

Calcite and pyrite from a subvertical fracture (KAS02:207m) have been investigated in detail (pers. com. E-L. Tullborg et al., 1995). The drillcore has a 0.7m-long exposure of the fracture. Fifteen calcite samples from the fracture coating were analysed for oxygen and carbon isotopes ($\delta^{18}O$ -7.2 to -15.9‰ and $\delta^{13}C$ -7.0 to -46.5‰). Most of the samples showed $\delta^{18}O$ values around -10‰ indicating equilibrium with the present groundwater. The large variation in $\delta^{13}C$ values were interpreted to be due to bacterial degradation of organic material which resulted in low $\delta^{13}C$ (HCO_3^-). Three pyrite samples from the fracture filling were analysed for $\delta^{34}S$. Values between -2 to -16‰ were recorded. The low $\delta^{13}C$ values in the calcite as well as the low $\delta^{34}S$ values in the pyrite support the hypothesis that SRB may have been active in this fracture in the past. It is, however, not possible to determine whether this is an ongoing process or not.

From the results above it can be concluded that sulphate reduction had taken place at depth down to about 500m at Äspö in the past, but there is no sign of any obvious bacterial sulphate reduction in the isotope signature in present day deep groundwater. However, abundant H_2S in the groundwater from boreholes KAS02 and KAS03 suggests that possible bacterial sulphate reduction is currently taking place although to a minor extent. In addition the decrease in DOC in the groundwater at Äspö, from 28 mg/l in the shallow groundwater to about 1 mg/l at 500m depth, implies that a degradation of C(org) is taking place, which may be linked to sulphate reduction.

7. MICROBIAL EVIDENCE OF THE PRESENCE OF SULPHATE REDUCTION

7.1 Background and aims

The only direct sign of the presence of sulphate reduction in the HRL tunnel is the measured occurrence of sulphate-reducing bacteria. They are noted for their end product, hydrogen sulphide. Sulphate-reducing bacteria thrive outside the aerobic environment, in niches to which oxygen has no access. The SRB in groundwater from boreholes in the HRL tunnel and outside the tunnel have been enriched and identified. The sampling locations for the microbes are shown in Figure 2-1 and the sampling in relation to other sampling activities and the tunnel construction is shown in Figure 2-2a,b,c,d,e, f and g.

With the isolation of a sulphate-reducing bacterium, Beijerinck clearly demonstrated the correlation of the observed process to a special kind of bacteria which he called *Spirillum desulfuricans* (Beijerinck, 1885). The isolated type of sulphate reducer, which had curved motile cells, was known for a while as *Vibrio desulfuricans* and then finally named *Desulfovibrio desulfuricans*. The genus designation *Desulfovibrio* was maintained for non-sporing sulphate reducers usually having curved motile cells growing on a relatively limited range of organic substrates; preferred substrates are lactate or pyruvate, which are incompletely oxidised to acetate. *Desulfovibrio* species are still the best known sulphate reducers. Sporing SRB with a similar metabolism were classified within the genus *Desulfotomaculum*. Later, additional types of sulphate reducers were described, several of which differed markedly, both physiologically and morphologically, from the known *Desulfovibrio* and *Desulfotomaculum* species. Hence, the term SRB describes a rather heterogeneous assemblage of bacteria having just the dissimilatory sulphate-reducing metabolism and obligate anaerobism in common (shown in Table 7-1).

Not all types of sulphate reducers have been classified to date. Some of the species isolated from Äspö groundwaters are indicated by 16S-rRNA gene comparisons to be new species. Very little is known about the range of termophilic sulphate reducers, or about types that grow very slowly and yield low cell densities under laboratory conditions.

Table 7-1. Some properties of selected classified SRB (from Widdel, 1988)

Species	Electron donors	Autotrophic growth (CO_2 as carbon source)	Temperature optimum °C	oxidation of lactate
Desulfotomaculum				
nigrificans	hydrogen, formate, lactate, ethanol	-	55 (max 70)	incomplete
antarcticum	lactate	-	20-30	incomplete
orientis	hydrogen, formate, lactate, ethanol	yes	37	incomplete
Desulfovibrio				
desulfuricans	hydrogen, formate, lactate, ethanol	-	30-36	incomplete
vulgaris	hydrogen, formate, lactate, ethanol	-	30-36	incomplete
salexigens	hydrogen, formate, lactate, ethanol	-	30-36	incomplete
thermophilus	hydrogen, formate, lactate	-	65 (max 85)	incomplete
Desulfomaculum				
baculatum	(hydrogen), formate, lactate	-	30-36	incomplete
Thermodesulfobacterium				
commune	hydrogen, lactate	-	70 (max 85)	incomplete
Desulfolobulbus				
propionicus	hydrogen, lactate, ethanol	-	28-39	incomplete
Desulfobacter				
hydrogeniphilus	hydrogen, acetate	yes	28-32	complete
Desulfobacterium				
autotrophicum	hydrogen, formate, lactate, ethanol	yes	20-26	complete
Desulfococcus				
niacini	hydrogen, formate, ethanol, acetate	yes	29	complete
Desulfosarccina				
variabilis	hydrogen, formate, lactate, ethanol	yes	33	complete
Desulfonema				
limicola	hydrogen, formate, lactate, acetate	yes	30	complete

Lactate (or pyruvate) has been used for a long time as an excellent organic substrate for enrichment, isolation and cultivation of incompletely lactate oxidising *Desulfovibrio* and *Desulfotomaculum* species. Lactate is also oxidised by several complete lactate oxidising sulphate reducers; *Desulfobacter, Desulfococcus, Desulfosarccina, Desulfobacterium* and *Desulfonema.*

Incomplete oxidation of lactate to acetate, hydrogen sulphide and carbon dioxide:

$$2CH_3CHOHCOO^- + SO_4^{2-} \Rightarrow 2CH_3COO^- + 2HCO_3^- + HS^- + H^+$$
$$\Delta G^{o\prime} = -160.1 \text{ kJ}$$

Complete oxidation of lactate to hydrogen sulphide and carbon dioxide:

$$2CH_3CHOHCOO^- + 3SO_4^{2-} \Rightarrow 6\ HCO_3^- + 3HS^- + H^+$$
$$\Delta G^{o\prime} = -255.3 \text{ kJ}$$

Incomplete oxidising sulphate reducers using lactate are usually able to grow just as well with hydrogen as the electron donor. *Desulfovibrio* species may grow rather fast on hydrogen, which is, therefore, an almost unfailing electron donor for their enrichment. The utilisation of hydrogen by *Desulfovibrio* species was the first hint from nutrition physiology that sulphate reducers can conserve energy solely by electron transport phosphorylation.

$$4H_2 + SO_4^{2-} + H^+ \Rightarrow 4\ H_2O + HS^-$$
$$\Delta G^{o\prime} = -152.2 \text{ kJ}$$

Hydrogen is also used by several slow-growing, completely oxidising SRB that may grow autotrophically (CO_2 is used as a single carbon source). After the classical *Desulfovibrio* species had been shown to be chemolitoheterotrophic (growth with hydrogen as electron donor and organic carbon as carbon source), autotrophic growth of hydrogen-using sulphate reducers was reported for *Desulfosarccina variabilis, Desulfonema limicola,* and *Desulfococcus niacini*; however, growth was rather slow. Later many other sulphate reducers have been shown to grow autotrophically (Table 7-1).

7.2 Methods for sampling and identification of bacteria

Several sampling and identification methods were used. A more detailed description of the methodology is described in Pedersen and Karlsson (1995). A short description of the methods used is given below.

Acridine orange stained direct counts (AODC) were used to determine the total numbers of unattached bacteria, as described by Pedersen and Ekendahl (1990). Sulphate-reducing bacteria grow under anoxic, reducing conditions. For cultivation it is therefore necessary to exclude air as far as possible, even during preparation of the medium. Therefore, the preparation of all media was done using a pressure-proof device suggested by Widdel and Bak (1991). Most gram-negative mesophilic sulphate reducers known so far grow in a defined medium without complex nutrients such as yeast extract or peptone.

The medium composition used here followed the defined multipurpose medium described by Widdel and Bak (1991) adjusted to a salinity of 1%. Samples of 10ml were taken with sterile syringes directly on the tubings from boreholes and transferred in or above the tunnel into 100ml Hungate bottles with 50ml of the medium in a nitrogen atmosphere. Bottles with hydrogen sulphide production were registered as positive for SRB. A total of 19 different colonies appearing in the enrichment cultures were picked and isolated via roller tube agar. The widest variety of colony morphologies possible was chosen. The 16S-rRNA genes of the isolates were subsequently sequenced and the result compared with available international databases for identification.

Viable counts of SRB were performed twice. First, counts were made on groundwater from boreholes on three different occasions and second, counts were made on sediments collected from the sea bed above the Äspö tunnel eg, at Stations I and II (Figure 2-1). Serial dilutions in anoxic agar medium called agar roller tubes, were used according to Widdel and Bak (1991). This technique was used throughout except on one occasion (1995-05-19) when the MPN technique according to Pedersen and Ekendahl (1990) was used. The tubes were incubated at room temperature and counted after 40 days. The sediment cores from Stations I and II had a diameter of 5cm. When brought to land they were sub-cored with Plexiglas tubes, 2.6 * 20cm, and transported to the mobile field laboratory standing at the entrance to the HRL tunnel. A core injection technique was used for the application of the radioisotope into the sediments. A volume of 10μl carrier-free $^{35}SO_4^{2-}$ was injected at 1cm intervals into intact cores from each station. The isotope was $Na_2{}^{35}SO_4$ with a specific activity of $3.7*10^7$ MBq $mmol^{-1}$ giving a final concentration of $1.9*10^{-8}$ mmol $^{35}SO_4^{2-}$ cm^{-3} in the sediments. The sediments were incubated for 26.5 hours at an ambient temperature of 18°C, and then deep-frozen until analysis. Sections of 2cm were taken from 8 and 24cm depths and transferred to sterile anoxic tubes with 15ml of 30% zinc acetate precipitating H_2S (Fossing and Jörgensen, 1989). Then acidification to a pH of below 2 was made with 5M HCl and the sulphide was stripped with nitrogen gas to a series of traps containing 10ml of 5% zinc acetate +0.1% acetic acid (Fossing and Jörgensen, 1989). Nitrogen was bubbled through for 30 minutes. From each of two traps 3ml subsamples were mixed with 15ml Hionic Flour and measured using a liquid scintillation technique. Testing the method with *Desulfomicrobium baculatum* previously isolated from another groundwater (Pedersen and Ekendahl, 1990) and sub-culture in the laboratory showed that the method worked well. Samples from the sediments were centrifuged and the sulphate concentration measured in the pore water with a turbidometric method according to Ekendahl and Pedersen (1994). Sediments were also dried in an oven at 80°C until no further loss of weight was registered.

A new technique used for identification of SRB present was by analysis of 16S-rRNA gene sequences. A basic requirement in the work with bacteria is good methods for the identification of different bacterial species and coherent bacterial groups. The use of methods such as numerical and chemical taxonomy has greatly influenced our views on how bacteria should be characterised, classified and identified. However, the development and deployment of nucleic acid based techniques is changing the current approach. It has been found that the DNA-sequence for the bacterial ribosomal 16S-unit is very suitable for identification. This sequence has conservative regions that are the same for all or large groups of bacteria as well as variable regions that are unique for single species. Through synthesis of short PCR-primers for the conservative regions, 16S-rRNA genes from very small samples can be amplified to amounts suitable for cloning and sequencing (Stackebrandt and Goodfellow, 1991). The sequences obtained can subsequently be compared with sequences available in databases (Olsen et al., 1991). There are also methods to synthesise species-specific sequences with fluorescent or radioisotopic labels. Such RNA-probes can be used for the in situ identification of single bacteria. This technique has provided new information. A common problem in the study of bacteria in nature is that only a minor part of all the bacteria observed can usually be successfully enriched and cultured. The 16S-technique now makes it possible to achieve information without culturing. The DNA from all present organisms can be extracted, amplified with PCR, cloned, sequenced and analysed. This technique was applied on samples from Äspö and the results are currently being analysed and will be reported separately. This report covers only the results on SRB. For a more extensive reporting of the results, see Pedersen and Karlsson (1995). Details about the technique can be obtained from Ekendahl et al. (1994).

7.3 Results of the microbe analysis

The total number of unattached and attached bacteria decreased from approximately 10^6 cells ml^{-1} in surface waters to between 10^4 to 10^5 cells ml^{-1} in deep groundwater as shown in Figure 7-1.

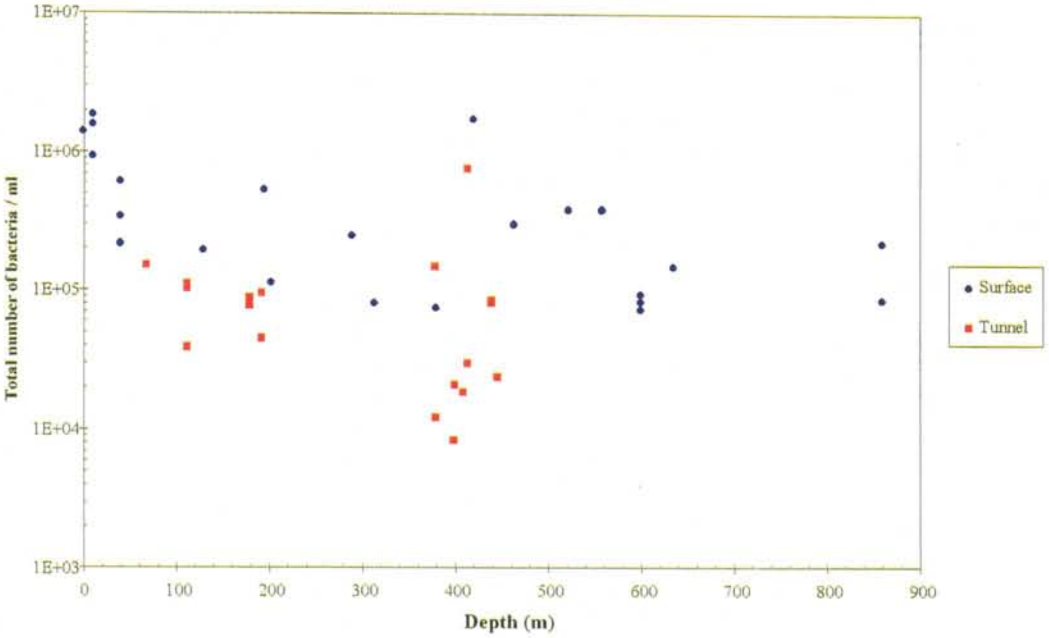


Figure 7-1. *Total number of bacterial cells from different depths and groundwaters in the Äspö area, sampled in surface boreholes and tunnel boreholes.*

The results from the enrichment and identification of SRB in groundwater from boreholes showed that SRB could not be detected in HBH01 and 02, and KR0012-15. Significant numbers of SRB could be obtained from SA813B, SA1327A and occasionally from SA1420A (Table 7-2).

Table 7-2. Results from SRB enrichment and number of SRB, determined with viable counts in agar roller tubes, in water samples from different boreholes in the Äspö HRL tunnel area. Enrichment cultures show presence/absence of sulphate-reducing bacteria. SRB ml^{-1} shows the number of SRB that grew in a multipurpose medium for sulphate reducers.

Borehole ID	Sulphate-reducing bacteria enrichment 921202	Sulphate-reducing bacteria ml^{-1} 930207	Sulphate-reducing bacteria ml^{-1} 950519
HBH02	no	b.d.[b]	-[a]
HBH01	no	b.d.	-
KR0012	-	b.d.	1
KR0013	no	b.d.	2
KR0015	-	b.d.	0.8
SA813	yes	2,420	42
SA923	-	b.d.	-
SA1062	yes	-	-
SA1327	yes	130	22
SA1420	no	b.d.	20
KAS03	yes	1390	-

[a] no data

[b] below the detection limit

A compilation of all results obtained on the viable numbers of SRB in the Äspö area are shown in Figure 7-2. Recent analyses of deeper boreholes have revealed a high number of SRB at a depth of 400m in boreholes KA3105A and KA3110A (Figure 7-2). This data has not been evaluated in this report.

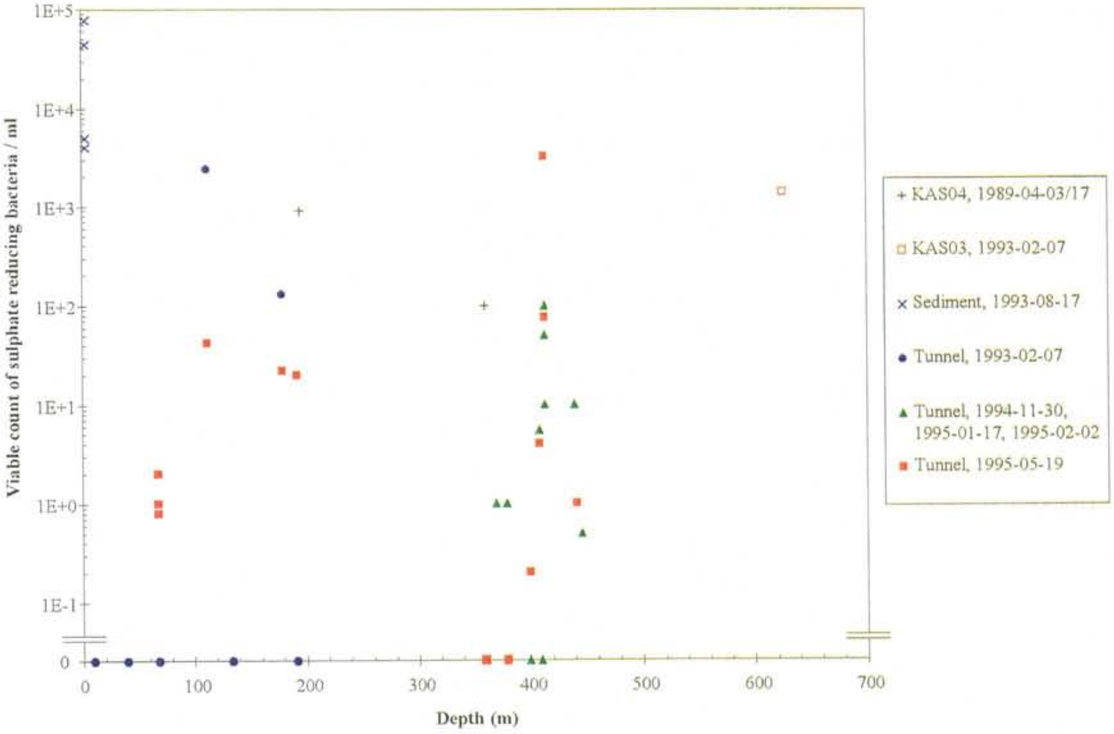


***Figure** 7-2. A compilation of all results obtained on the viable numbers of SRB in the Äspö area. The KAS03-04 data are from Pedersen and Ekendahl (1990). Data from 1995 has not been evaluated in this report.*

The viable count of SRB from February 7, 1993 on groundwater from boreholes confirms the enrichment analysis made in December 1992 (Table 7-2). There were from 100 up to 2420 viable, cultivable SRB ml^{-1} in the sampled groundwaters. One sample from SA0813 was turbid and therefore sampled once again when the groundwater was clear. However, when the turbid sample was analysed, it showed that large amounts (830000 cells ml^{-1}) of SRB related to sediments and precipitates were present in this borehole.

The marine sediments (Stations I and II) had SRB ranging from approximately 5000 up to 10000 cells per cm^3 depending on sample location and depth as shown in Table 7-3. Sulphate assimilation and reduction showed that there was a significant sulphate reduction in all samples which was larger in the upper samples at 8cm depth than at 24cm depth. The activity was also approximately 10 times greater at Station II than at Station I.

Table 7-3. Results from measurements of viable counts of SRB and the rate of sulphate-reducing activity in sea bed sediments sampled above the Äspö HRL tunnel in August 1993 (see Figure 2-2e for location).

Station	Depth	Pore water	Dry content	SO_4^{2-} reduced	Viable counts of sulphate-reducing bacteria cells
	cm	%	%	cm^{-3} day^{-1} $mole^{-1}$ * 10^{-9}	cm^{-3} x 10^{4}
I	8	62	8.7	16	0.5
I	24	40	16	0.9	4.2
II	8	56	7	140	7.8
II	24	46	12	13	0.4

Analysis of 16S-rRNA gene sequences from the sampled groundwaters showed that SA0813 was dominated by SRB undetected by the other methods (compare Table 7-2). In all the other boreholes that were analysed the number of sulphate-reducing sequences was below the detection limit of this DNA technique.

Identification with 16S-rRNA gene sequencing revealed 3 different species of SRB. One of them was identified as *Desulfomicrobiumbaculatum* while the other two could not be identified at species level - shown in Table 7-4 below. The closest related bacterium was *Desulfovibrio salexigens* (Figure 7-3). Interestingly, two non-sulphate-reducing bacteria that can grow in the multipurpose medium for sulphate reducers from SA0813 were isolated. One of them, group IV, was the other dominating bacteria in this borehole as determined with the 16S-rRNA technique in addition to sulphate reducers.

Table 7-4. Characterisation of isolates from the Äspö HRL tunnel area with the 16S-rRNA gene sequencing technique. Choice of isolates was based on different colony characters. Despite this, many of the isolates turned out to have the same 16S-rRNA genotype indicated by similar grouping. Isolate 1 and 6-21 are all SRB. Isolates 2-5 do not reduce sulphate.

Isolate	Borehole	Group	Species	Similarity
1	KR0013	II	*Desulfovibrio salexigens*	94%
2	SA0813	IV	*Eubacterium* sp	92%
3	SA0813	IV	*Eubacterium* sp	92%
4	SA0813	V	*Clostridium* sp	92%
5	SA0813	V	*Clostridium* sp	92%
6	SA1062	III	*Desulfovibrio salexigens*	92%
7	SA1062	III	*Desulfovibrio salexigens*	92%
8	HA1327	I	*Desulfomicrobium baculatum*	100%
9	HA1327	I	*Desulfomicrobium baculatum*	100%
10	HA1327	II	*Desulfovibrio salexigens*	94%
11	HA1327	II	*Desulfovibrio salexigens*	94%
12	KAS03	I	*Desulfomicrobium baculatum*	100%
13	KAS03	I	*Desulfomicrobium baculatum*	100%
14	KAS03	I	*Desulfomicrobium baculatum*	100%
15	KAS03	I	*Desulfomicrobium baculatum*	100%
16	KAS03	I	*Desulfomicrobium baculatum*	100%
17	KAS03	II	*Desulfovibrio salexigens*	94%
18	KAS03	II	*Desulfovibrio salexigens*	94%
19	KAS03	II	*Desulfovibrio salexigens*	94%
21	KAS03	II	*Desulfovibrio salexigens*	94%

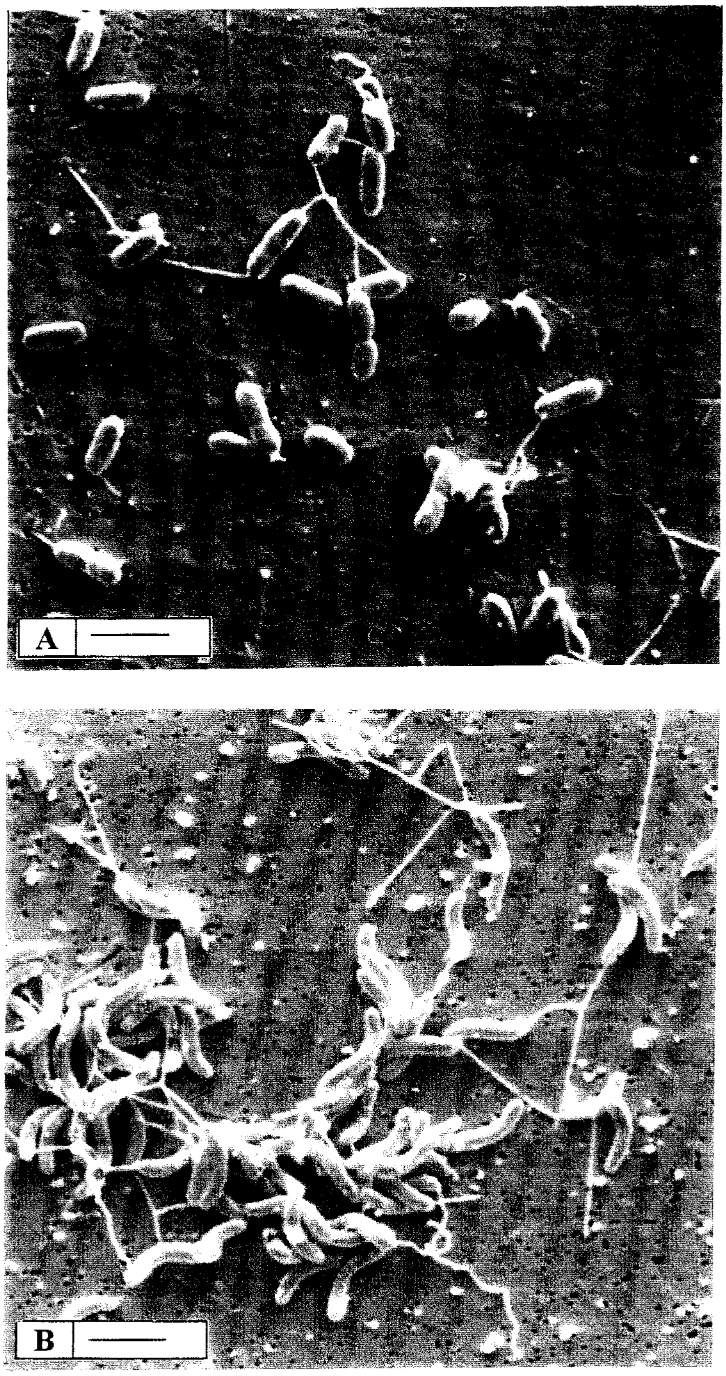


Figure 7-3a,b. *Scanning electron microscopy image of sulphate reducing bacteria that have been enriched and isolated from the Äspö HRL environment. DNA sequences have demonstrated the bacterium in Figure 7-3A to be Desulfomicrobium baculatum (group I in Table 7-4), while the bacterium in Figure 7-3B was found to be related to the genus Desulfovibrio (group III in Table 7-4). The bar in the figures denote 2 μm.*

7.4 Discussion of the results from the microbial investigation

Different techniques were used for the detection of sulphate reducers and their activity in groundwater and in sediments: enrichment cultures, viable counts in agar roller tubes, most probable number assays, sulphate reduction activity and 16S-rRNA gene sequencing. All the techniques indicated the presence in some cases of dense populations of sulphate reducers. SA813B and HA1327A in particular, and occasionally 1420A, harboured SRB in significant numbers (Table 7-2) in relation to the total numbers measured (Figure 7-1). Figure 5-9 shows that sulphate reduction may occur between 900-1300m tunnel length which is close to boreholes SA813B and HA1327A where sulphate reducers appeared. Much later, during autumn 1994 and spring 1995, white mats of bacteria were observed at some places along the tunnel within this section. Pedersen and Karlsson (1995) show that these mats are composed of various types of sulphide oxidising bacteria, oxidising sulphide in the groundwater as the sole energy source. The observation suggests that sulphide production is present in the groundwater percolating over the observed mats. A possible source of this sulphide is ongoing bacterial sulphate reduction behind the tunnel rock wall.

Recent findings during the SELECT investigations have revealed very high numbers of SRB in a recently drilled borehole called KA3105A with a groundwater Cl^- content of 3960 mg/l (not included in this report). According to drilling personnel, a gas pocket with a strong smell of sulphide was penetrated when drilling this borehole. The presence of sulphate reduction at depth (400m) was indicated during the pre-investigation phase through positive culturing results of SRB from KAS03 (Figure 7-2, Table 7-2) and KAS04 (Figure 7-2).

Groundwater data in this study indicated obvious sulphate reduction in groundwater with a salinity (indicated by Cl^-) of 4000-6000 mg/l. Data based on culturing with the isolates, as depicted in Table 7-4, has shown that their optimum salinity for growth, expressed as NaCl, is 7-10 g/l (Cl^- = 4200-6000 mg/l), which agrees with the indication reported here.

There seems to be a need for a high DOC content, above 10 mg/l, in the reported data for obvious sulphate reduction to occur. This limit may however be lower, as recent data from the SELECT investigations revealed an obvious occurrence of sulphate reducers in one borehole (KA3105A, see above) with a DOC content of 2.9 mg/l. Some sulphate reducers can use hydrogen as an electron donor instead of organic carbon and will produce hydrogen sulphide regardless of the presence of organic carbon. However, laboratory experience indicates this to result in a less obvious sulphide production than with an organic electron donor (see section 7.1).

The multivariate mixing and mass balance calculations (section 5.2) have demonstrated that an understanding of the fluxes of compounds, rather than measurements of concentrations only, is necessary for modelling sulphate consumption (the electron acceptor) and carbon dioxide production by SRB. The same argument is valid for the energy sources that SRB can utilise ie, low weight organic carbon compounds and hydrogen. Prediction of SRB activity over time must also include fluxes of these compounds since they limit the rate with which SRB can grow and produce hydrogen sulphide. These fluxes have not yet been determined for groundwater in deep crystalline bed-rock. Other components that bacteria need for growth such as nitrogen and phosphorus can be obtained at depths. Nitrogen is supplied through nitrogen fixation of the plentiful amount of nitrogen gas that is found in all deep groundwater. Phosphorus comes from phosphorus-bearing minerals such as apatite, also present in most crystalline bedrock environments (Pedersen and Karlsson, 1995).

8. CONCLUSION OF THE INTEGRATED STUDY OF SULPHATE REDUCTION

Evidence and indications of sulphate reduction based on geological, hydrogeological, groundwater, isotope and microbial data in and around the Äspö Hard Rock Laboratory (HRL) tunnel have been evaluated.

Geological data were evaluated to find the amount of sulphide which could be calculated to result from the sulphate reduction. The conclusion is that the amount of pyrite normally occurring in the fracture coatings could explain the amount produced. However, there are other processes in the geological time span which have also produced pyrite. Therefore the existence of pyrite is not the only evidence for sulphate reduction. The role of colloidal sulphur has not yet been investigated in boreholes with a high concentration of SRB, but samples from deep boreholes containing HS^- show that the S on the colloidal phase is several orders of magnitude lower than that expected from a sulphate reduction process.

The hydrogeological conditions were evaluated in order to describe the possible transport phenomena related to the sulphate reduction. The questions to be answered were: Can sulphate reduction take place in the bottom sediments and the modified water be transported to the tunnel? Could the groundwater flow conditions in the tunnel either increase or decrease the effect of biological sulphate reduction? The answer to the first of the questions is yes, the process can occur in the sea bed sediments and the effect in hydrochemistry can be observed in the water inflow in the tunnel. Hydrogeological calculations imply a transport time of approximately 100-400 days for the water passing through the sediments to reach the tunnel in a proportion of 25%. This water might be Baltic sea water which has recently been modified by sulphate reduction on its way through the bottom sediments. The second answer is that the relatively simple groundwater flow conditions around the tunnel would not affect the biological process directly. However, if the sulphate reduction had been an ancient process, then the effects would soon be washed out, which has not been the case. In addition, the existence of high bicarbonate and low sulphate concentrations in the probing holes on the very first sampling occasion strongly imply that the process has been ongoing.

The groundwater chemistry was evaluated by multivariate mixing and mass balance calculations. The calculations demonstrated that an understanding of the fluxes of compounds, rather than measurements of concentrations only, is necessary for modelling sulphate consumption and bicarbonate production by SRB. These calculations defined the specific conditions where the process could be ongoing. The results show that the salinity range of 4000-6000 mg/l of chloride is the optimal one. This is also supported by laboratory experiments. Dissolved organic content above 10 mg/l is also strongly correlated to the high number of bacteria. Sulphate reduction seems to occur in anaerobic brackish groundwater with access to dissolved sulphate and organic carbon or hydrogen. These conditions are mainly found in the sea bed sediments, in the tunnel section under the Baltic Sea and in some deep groundwaters.

Isotope data were expected to give a definite answer to where the sulphate reduction takes place, since the bacterial processes always result in an enrichment of the lighter isotopes. Concerning both the $\delta^{13}C$ and the $\delta^{34}S$ isotopes the results generally point towards the existence of bacterial sulphate reduction. However, there are several processes in the geological evolution which could have given the same isotopic signatures as well. Therefore the isotopic data provide indications of biological sulphate reduction but no evidence.

Microbiological data were collected in the selected boreholes where the hydrochemistry had indicated an ongoing or previously ongoing sulphate reduction. The results show that sulphate-reducing bacteria are present, sometimes in large quantities, and that they can be correlated to the modified groundwater composition with high bicarbonate and low sulphate concentrations. The amount of bacteria vary from time to time in the sampling points. Generally there are more bacteria that occur more frequently in the boreholes investigated below the sea bed than there are in the tunnel spiral. The conclusion of this is that sulphate reduction is continuously ongoing in the sea bed sediments and probably also in the bedrock below the sea bed.

Indications and evidence of sulphate reduction based on the different methods are shown in Figures 8-1 to 8-7. The extent of the sampling programme and the sampling occasions varied between the methods. Modified Baltic Sea water is believed to be connected with obvious sulphate reduction. The influx of this water type has been calculated using multivariate mixing calculations. The observations have then been interpolated using Kriging (Henley, 1981; Fortner, 1992).

Influx of Modified Baltic Sea water, experimental day 150 (1991-03-13)

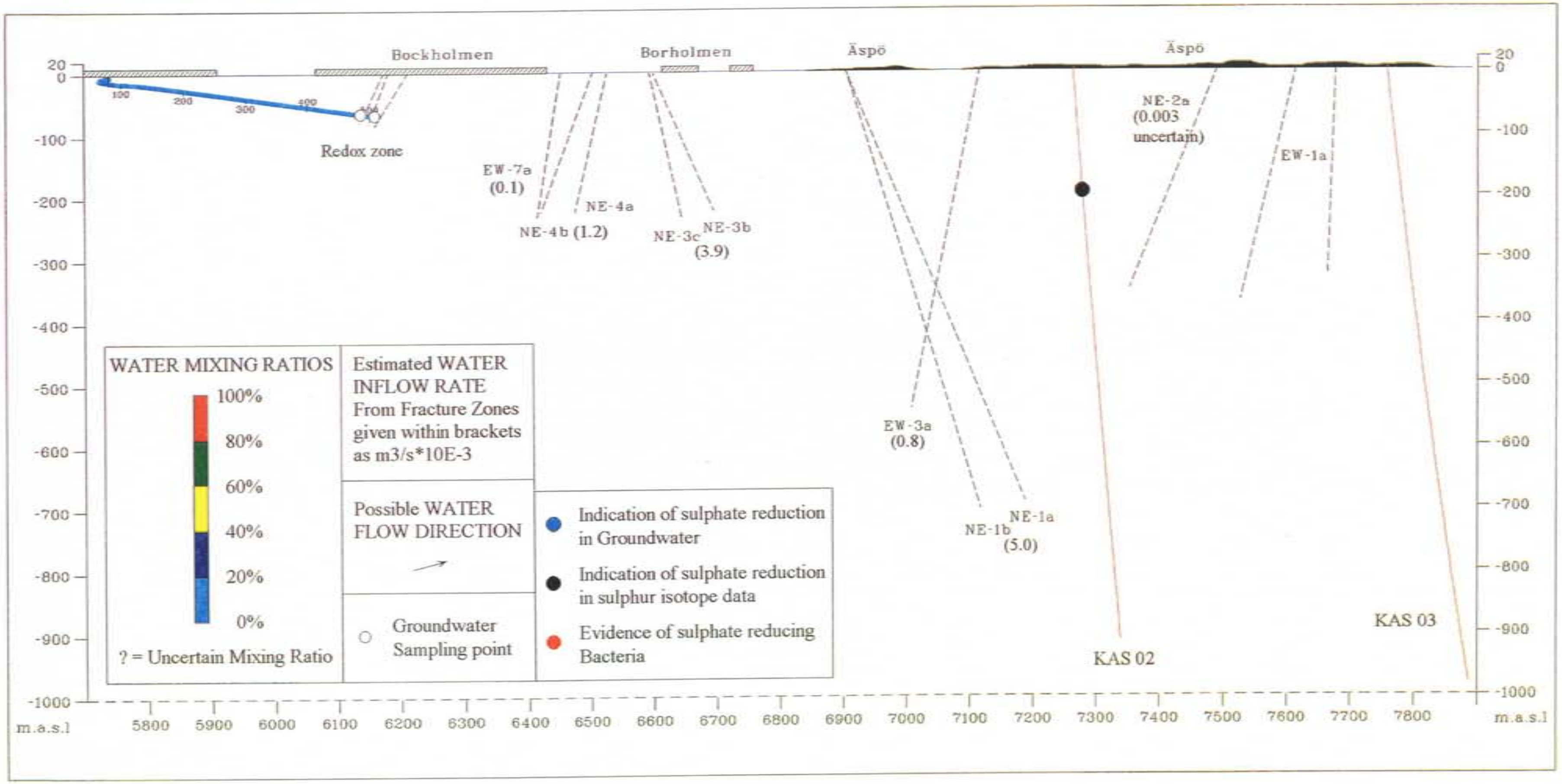


Figure 8-1. *Evidence/indications of sulphate reduction based on groundwater, isotopical and microbial data on experimental day 150 (0.4 year) (1991-03-13) of the HRL tunnel construction. Based on the multivariate mixing calculations the influx of Modified Baltic Sea water, which is believed to be associated with obvious sulphate reduction is shown in %. The estimated flow rate from the fractures is given within brackets.*

Influx of Modified Baltic Sea water, experimental day 350 (1991-09-29)

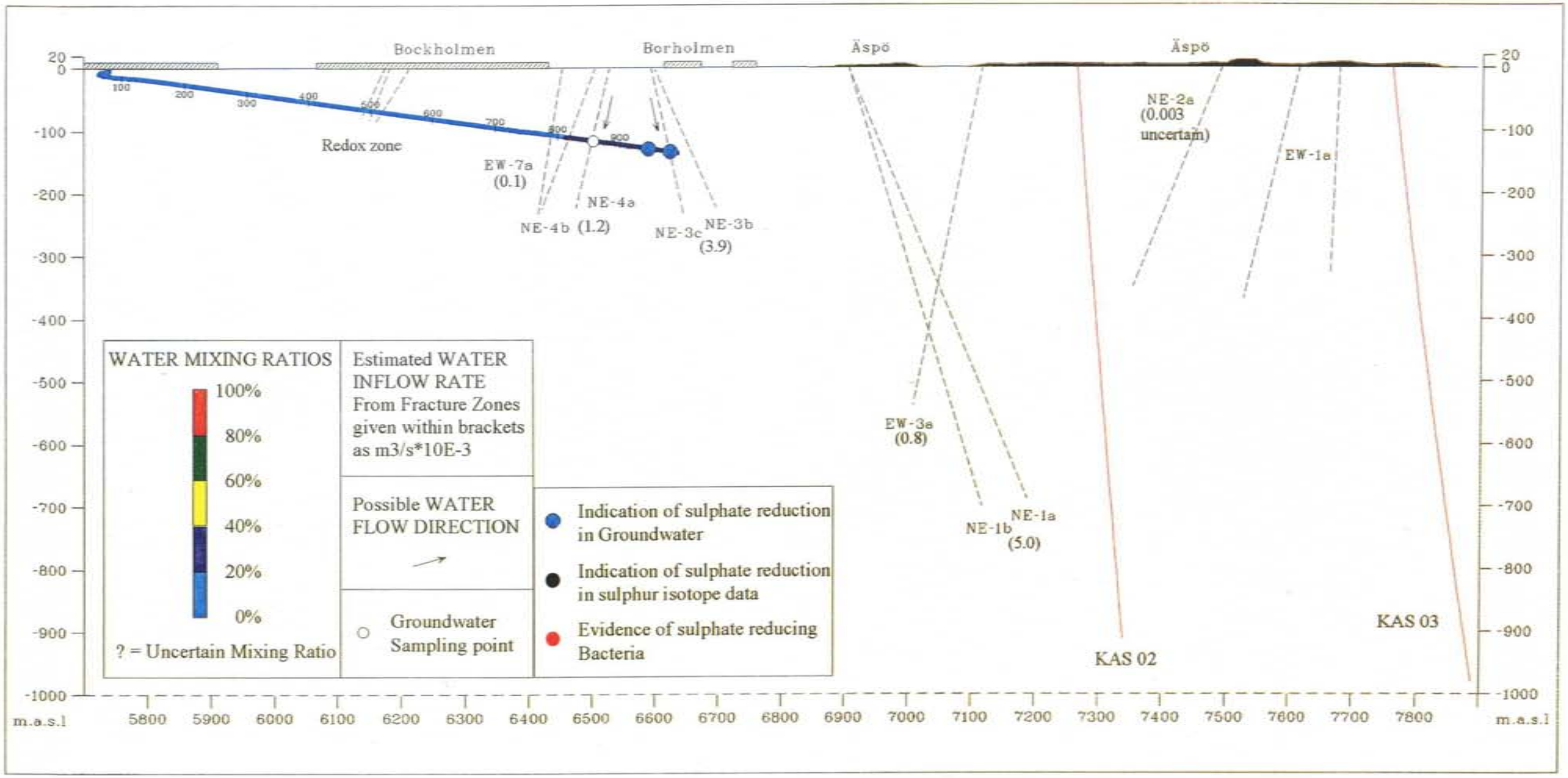
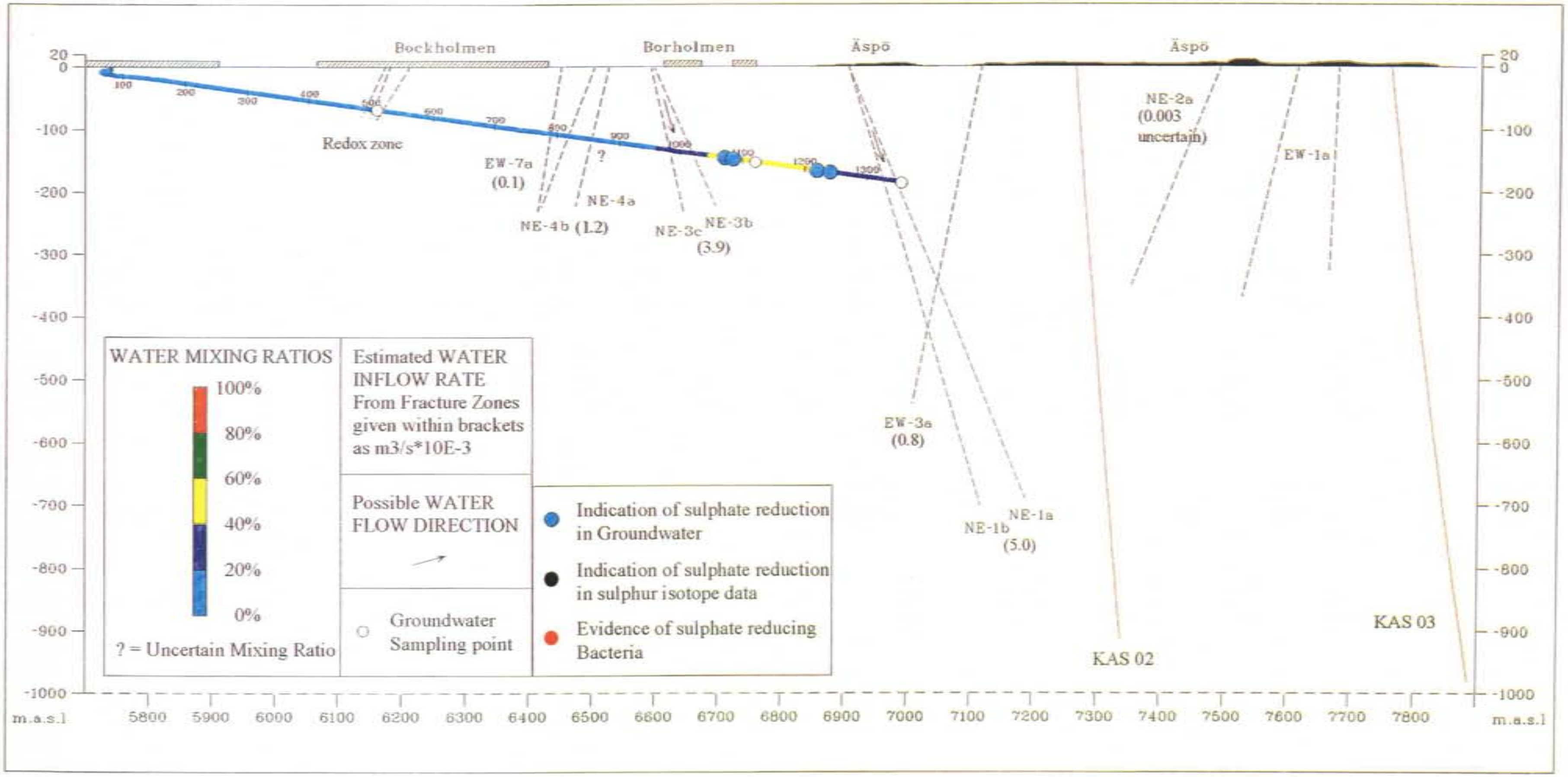


Figure 8-2. *Evidence/indications of sulphate reduction based on groundwater, isotopical and microbial data on experimental day 350 (1 year) (1991-09-29) of the HRL tunnel construction. Based on the multivariate mixing calculations the influx of Modified Baltic Sea water, which is believed to be associated with obvious sulphate reduction is shown in %. The estimated flow rate from the fractures is given within brackets.*



Influx of Modified Baltic Sea water, experimental day 550 (1992-04-16)

__Figure 8-3.__ Evidence/indications of sulphate reduction based on groundwater, isotopical and microbial data on experimental day 550 (1.5 year) (1992-04-16) of the HRL tunnel construction. Based on the multivariate mixing calculations the influx of Modified Baltic Sea water, which is believed to be associated with obvious sulphate reduction is shown in %. The estimated flow rate from the fractures is given within brackets.

Influx of Modified Baltic Sea water, experimental day 750 (1992-11-02)

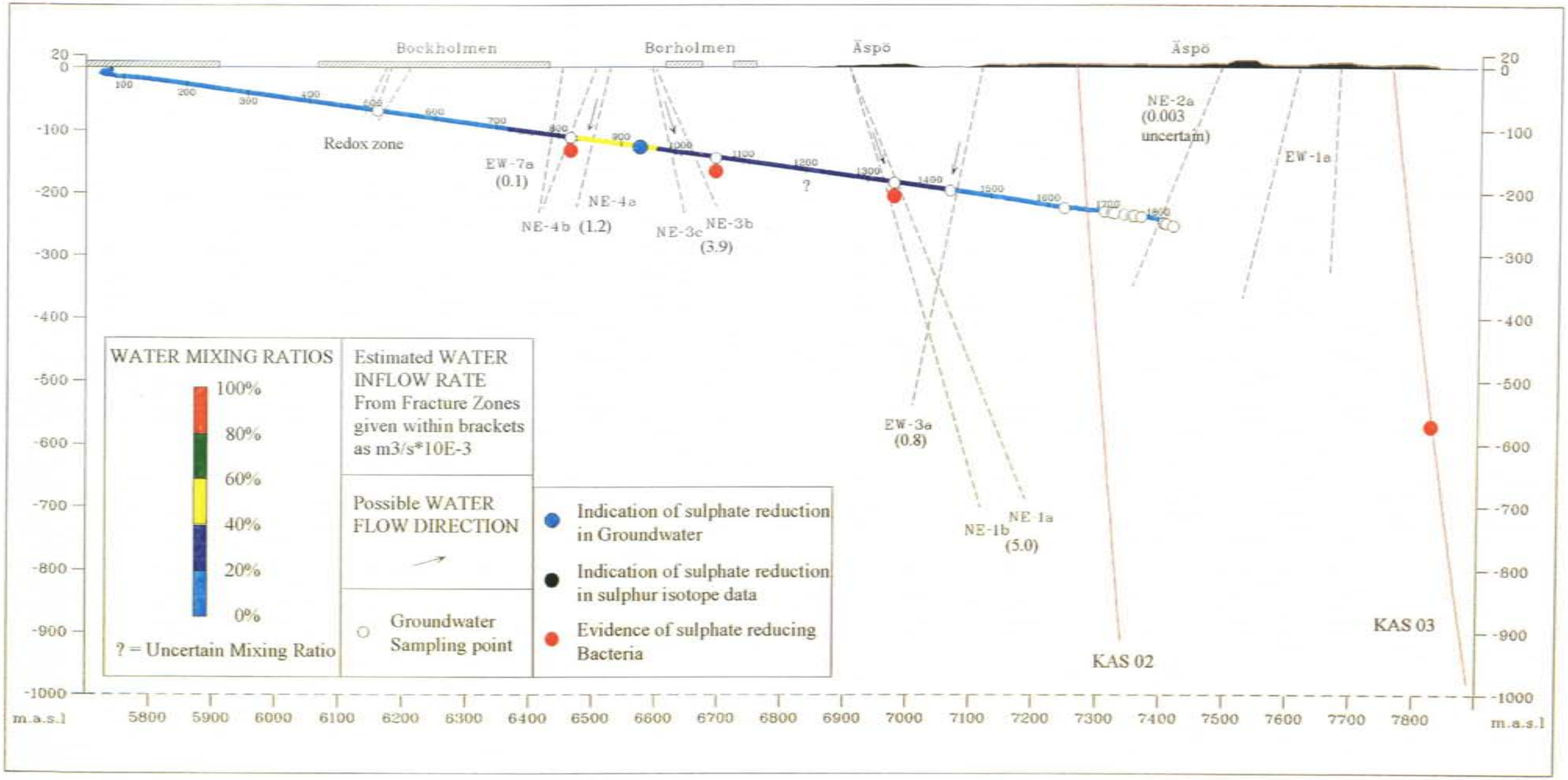


__Figure 8-4.__ Evidence/indications of sulphate reduction based on groundwater, isotopical and microbial data on experimental day 750 (2 years) (1992-11-02) of the HRL tunnel construction. Based on the multivariate mixing calculations the influx of Modified Baltic Sea water, which is believed to be associated with obvious sulphate reduction is shown in %. The estimated flow rate from the fractures is given within brackets.

Influx of Modified Baltic Sea water, experimental day 950 (1993-05-21)

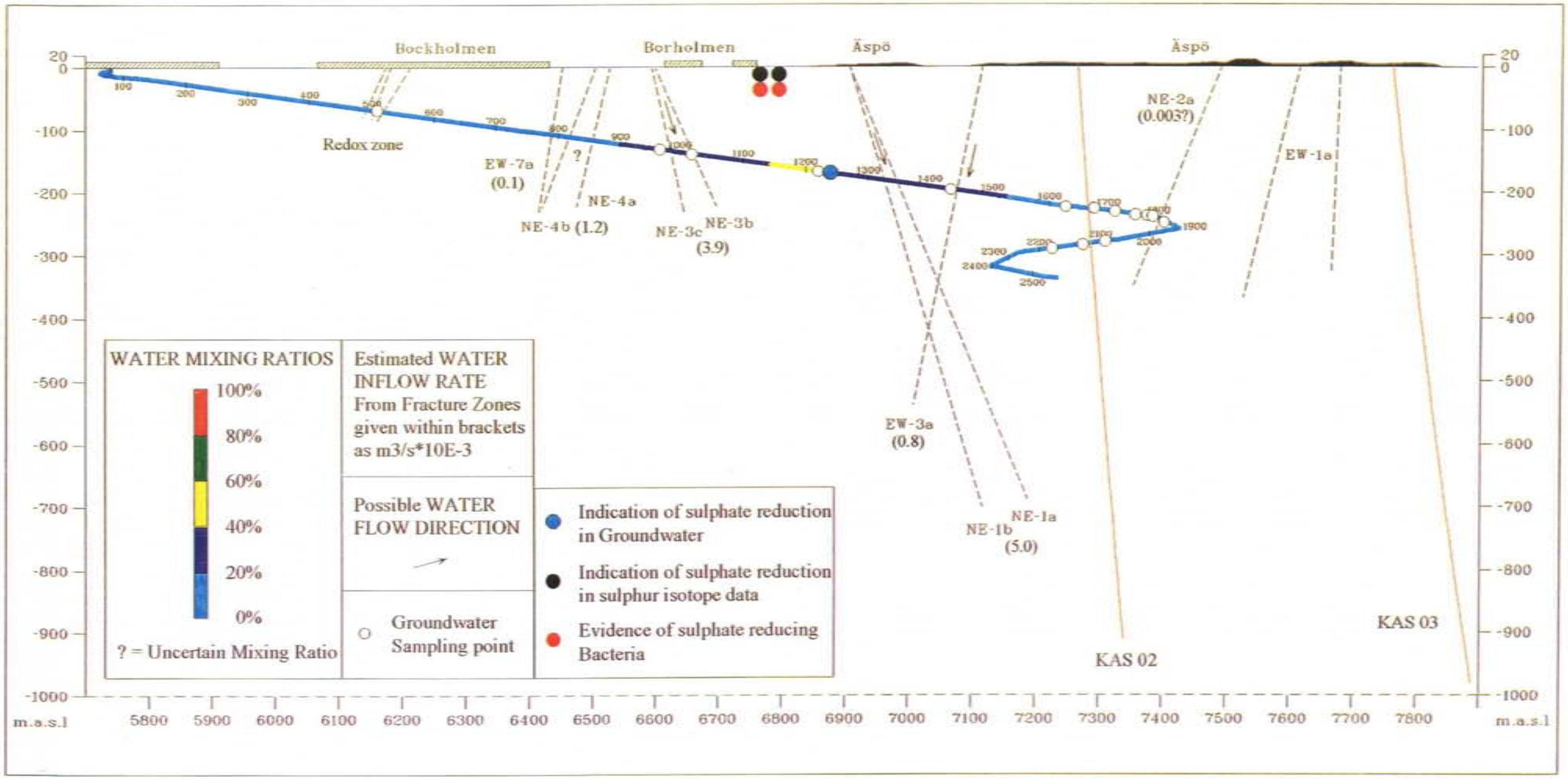


Figure 8-5. Evidence/indications of sulphate reduction based on groundwater, isotopical and microbial data on experimental day 950 (2.6 years) (1993-05-21) of the HRL tunnel construction. Based on the multivariate mixing calculations the influx of Modified Baltic Sea water, which is believed to be associated with obvious sulphate reduction is shown in %. The estimated flow rate from the fractures is given within brackets.

Influx of Modified Baltic Sea water, experimental day 1150 (1993-12-07)

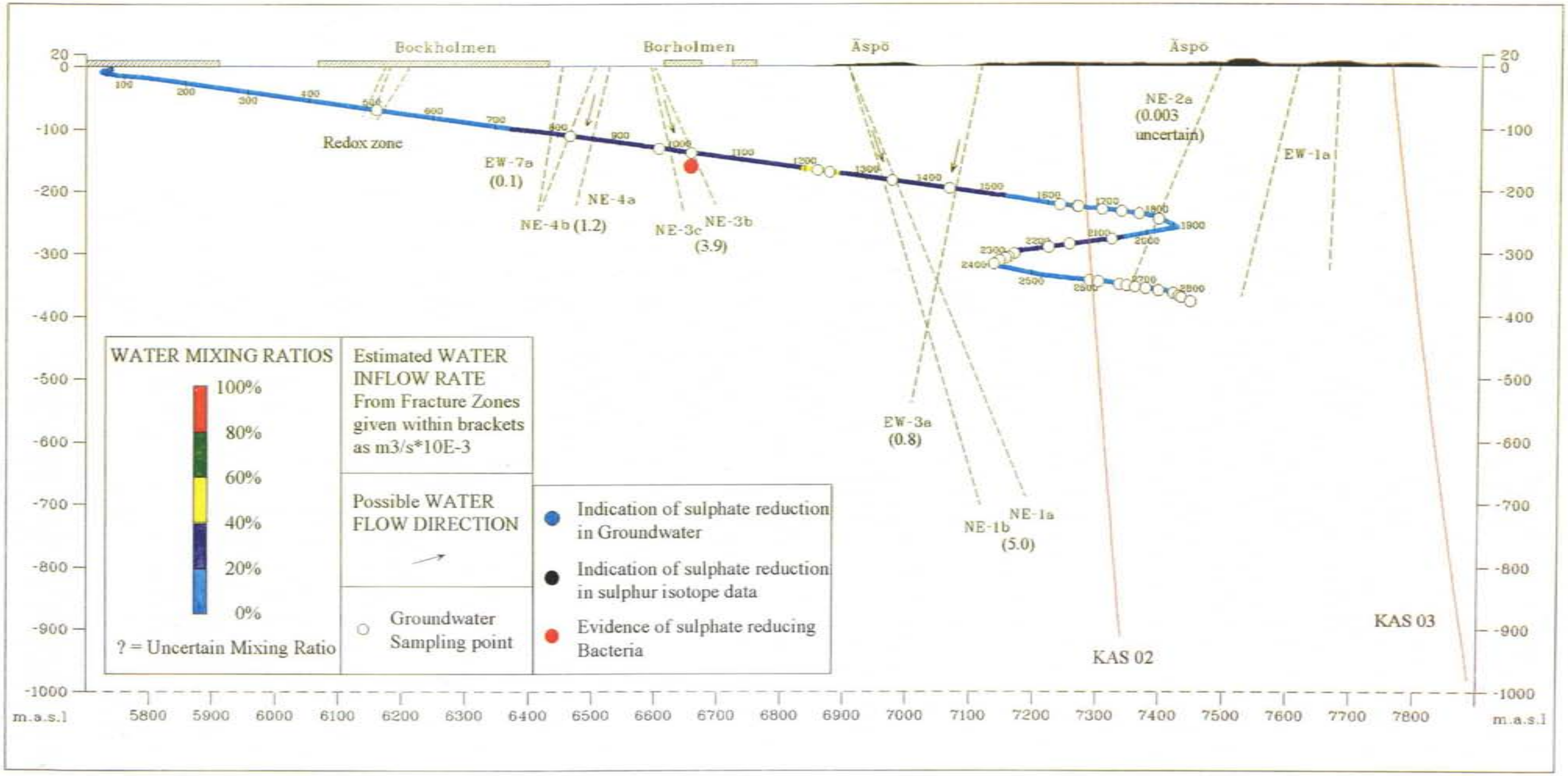


Figure 8-6. *Evidence/indications of sulphate reduction based on groundwater, isotopical and microbial data on experimental day 1150 (3.2 years) (1993-12-07) of the HRL tunnel construction. Based on the multivariate mixing calculations the influx of Modified Baltic Sea water, which is believed to be associated with obvious sulphate reduction is shown in %. The estimated flow rate from the fractures is given within brackets.*

Influx of Modified Baltic Sea water, experimental day 1350 (1994-06-25)

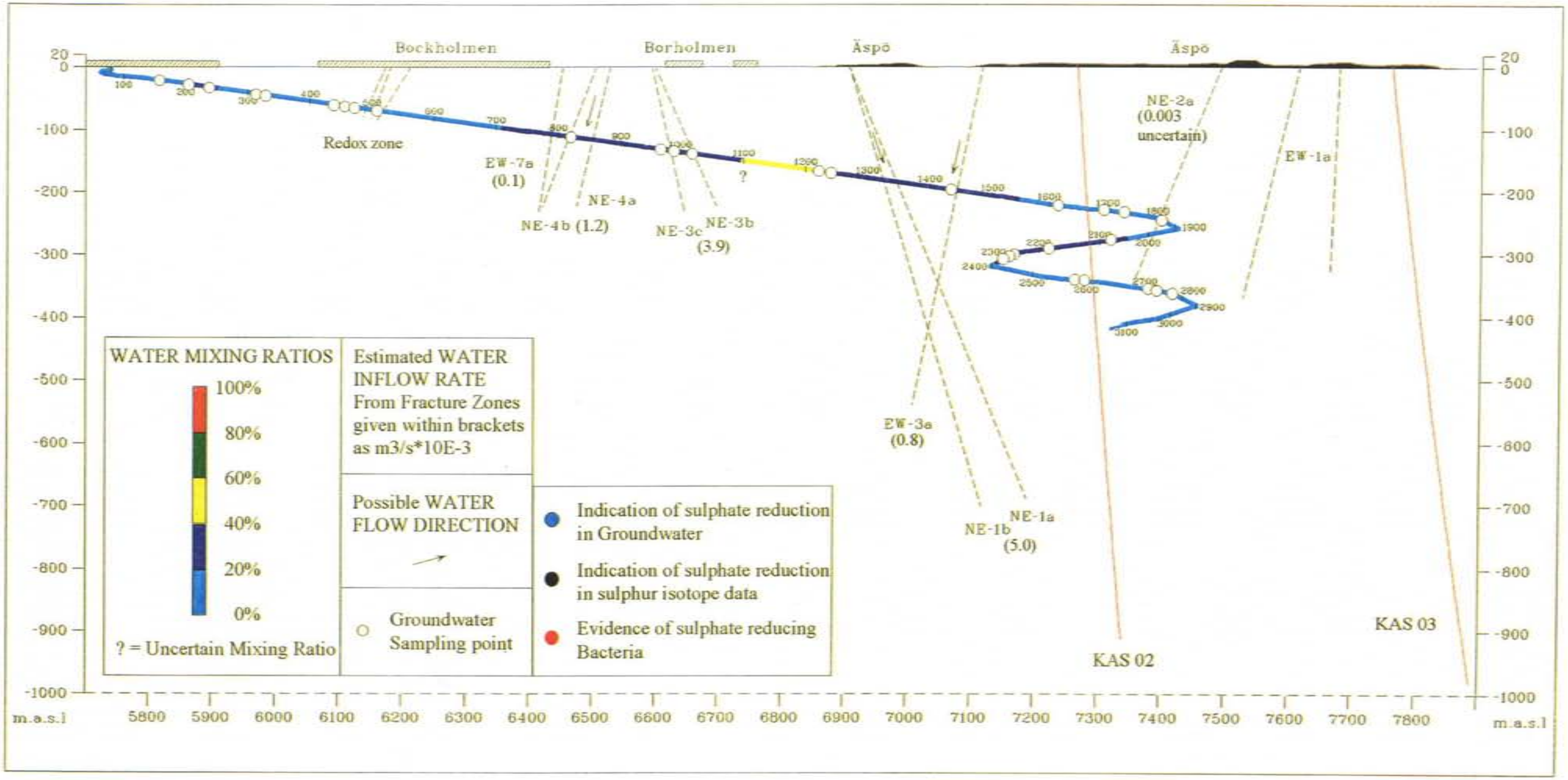


Figure 8-7. *Evidence/indications of sulphate reduction based on groundwater, isotopical and microbial data on experimental day 1350 (3.8 years) (1994-06-25) of the HRL tunnel construction. Based on the multivariate mixing calculations the influx of Modified Baltic Sea water, which is believed to be associated with obvious sulphate reduction is shown in %. The estimated flow rate from the fractures is given within brackets.*

The fracture zones with detected sulphate reduction in the tunnel were NE-3c on day 350 of the tunnel construction, NE-4b and NE-1a on day 750 of the tunnel construction and NE-3b on day 1150 of the tunnel construction. The Principal Component Analysis plot (Figure 8-8) was used to indicate areas with obvious sulphate reduction in the Äspö groundwaters. The results from groundwater modelling and bacterial investigations are included in this report and seem to correlate. The area where obvious sulphate reduction occurs is in waters with a relative young age (see Figure 5-6). The influx of the water with obvious sulphate reduction (Modified Baltic sea water) is shown as mixing iso-lines. The mixing calculations show that the effect of sulphate reduction can affect many of the samples from the HRL tunnel especially in the tunnel sections under the Baltic Sea or in the near vicinity.

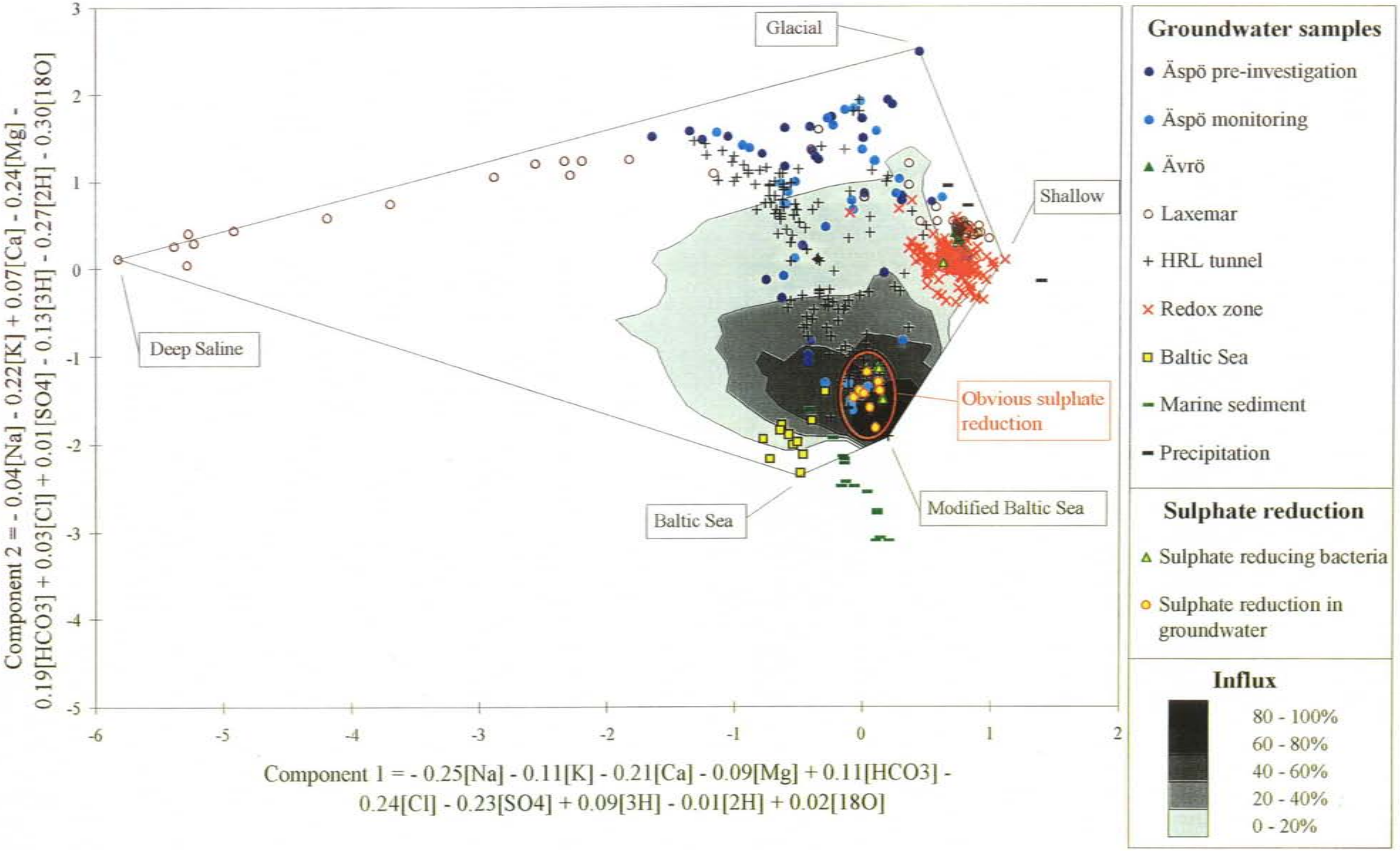


Figure 8-8. *Observations with obvious sulphate reduction (circled area) and the effect of the influx of sulphate reduced water (Modified Baltic Sea water) in the HRL tunnel. The weight for the different elements is shown in the equations for the first and second Principal Component respectively.*

The conclusions are:

- Sulphate reduction has occurred in the past. This is supported by geological and isotopical indications.
- Presence of sulphate reduction is supported by bacterial evidence, groundwater and isotopical indications.
- Hydrogeological modelling indicated that sulphate reduction can take place in the sediments and the products can be transported to the tunnel by the water flow.
- Sulphate reduction takes place in the sediments but also around the HRL tunnel sections under the sea and in the deep groundwaters.
- The anaerobic SRB live in locations such as sea bed sediments, tunnel sections passing under the sea and at large depths.
- Obvious sulphate reduction in groundwater, calculated after compensating for influx of different water types (expressed as mole fraction: $[HCO_3^-]/[SO_4^{2-}] > 1.27$), seems to require a limited salinity interval ($Cl^- = 4000\text{-}6000$mg/l).
- Most sulphate reducers use organic matter as an electron donor. A more vigorous sulphate reduction is possible when the organic content in the groundwater is high (>10mg/l) which is the case in the sediments and in the groundwaters under the sea. In this case a clear response of high HCO_3^- and low SO_4^{2-} content is found in the groundwater. Mass balance calculations and bacterial investigation showed that obvious sulphate reduction may still occur when the lower boundary condition of 0.6 mg/l is used. It is therefore premature to establish a fixed lower limit for the organic matter.
- Sulphate-reducing bacteria can use hydrogen (geogas) as an electron donor instead of organic carbon in a less vigorous reaction than when organic carbon is consumed. These bacteria can live at larger depths were the access to organic material is limited.
- The tunnel construction seemed initially to lower the potential for sulphate reduction due to increased flow but new bacterial measurements indicate the presence of a sulphate reduction process in many samples in the tunnel some years after the start of the tunnel construction. This may indicate that the sulphate reducers have adapted to the prevailing circumstances.
- The complete process of sulphate reduction is not yet known - eg, the full role of inhibitors, accelerators, distribution, alternative electron donators such as HS^- - and need further investigation.

Sulphate reduction seems to have occurred and will probably occur where the conditions are favourable for SRB such as anaerobic brackish groundwater with dissolved sulphate and organic carbon or hydrogen.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Fred Karlsson, SKB and Prof. Ingmar Grenthe, KTH who provided valuable help, comments and suggestions during the preparation of the manuscript. Izabella Hallberg, GeoPoint AB edited the language of the document.

REFERENCES

Alm P, 1994. Tre simuleringar av interferenstester gjorda i sprickzonen NE-1 vid Äspö. Sammanställning av resultaten. Geologiska Institutionen CTH, Göteburg. (Report in progress).

Back W, 1966. Hydrochemical facies and groundwater flow patterns in northern part of Atlantic Coastal Plain. U.S. Geol. Surv. Prof. Paper 498-A.

Banwart S, Gustavsson E, Laaksoharju M, Nilsson A-C, Tullborg E-L, Wallin B, 1994. Large scale intrusion of shallow water into a vertical fracture zone in crystalline bedrock: Initial hydrochemical pertubation during tunnel construction at the Äspö Hard Rock Laboratory, south-eastern Sweden. *Water Resources Research,* 30, No.6, 1747-1763.

Banwart S (ed), 1995. The Äspö redox experiment in block scale: results and conclusions from three years of field investigations. SKB Progress Report PR 25-95-06, Stockholm.

Beijerinck W M, 1885. Uber Spirillum desulfuricans als Ursache von Sulfatreduction. Zentralbl Bakteriol (2) 1-9.

Berner R A, 1972. Sulphate reduction, pyrite formation, and the ocean sulphur budget. The Changing Chemistry of the Oceans, D. Dryssen and D. Jagner, eds. Nobel Symposium 20. Wiley-Interscience, New York.

Berner R A, 1982. Burial of organic carbon and pyrite sulphur in the modern ocean: Its geochemical and environmental significance . Am. J. Sci., 282.

Berner R A, 1984. Sedimentary pirite formation: An update. Geochim. Cosmochim. Acta, 48.

Chatfield C, Collins A J, 1989. Introduction to multivariate analysis. Chapman and Hall.

Davis S N, De Wiest R J M, 1967. Hydrogeology, 2d Ed., John Wiley & Sons, New York.

Drever J I, 1988. The geochemsitry of natural waters. ISBN 0-13-351396-3. Prentice Hall, Inc., New Jersey.

Ekendahl S, Arlinger J, Ståhl F, Pedersen K, 1994. Characterization of attached bacterial populations in deep granitic groundwater from the Stripa research mine with 16S-rRNA gene sequencing technique and scanning electron microscopy. Microbiology 140.

Ekendahl S, Pedersen K, 1994. Carbon transformations by attached bacterial populations in granitic groundwater from deep crystalline bed-rock of the Stripa research mine. Microbiology 140.

Fortner B, 1992. The Data Handbook. Spyglass Inc. Champaign, Illinois, USA.

Fossing H, Jörgensen B B, 1989. Measurement of bacterial sulphate reduction in sediments: evaluation of a single step chromium reduction method. Biogeochemistry (8) 205-222.

Frape S K, Fritz P, 1987. Geochemical trends for groundwaters from the Canadian shield. In: Fritz P and Frape S K, Saline water and gases in crystalline rocks. Geol. Assoc. of Canada Spec. Pap., 33:19-38.

Fritz P, Fontes J-Ch, Frape S K, Luovat D, Michelot J-L, Balderer W, 1989. The isotope geochemistry of carbon in the groundwater at Stripa. Geochim. et Cosmochim. Acta, 53:1765-1775.

Gascoyne M, Davison C C, Ross J D, Pearson R, 1987. Saline groundwaters and brines in plutons in the Canadian shield. In: Fritz, P. and Frape, S. K., Saline water and gases in crystalline rocks. Geol. Assoc. of Canada Spec. Pap., 33:53-68.

Goldhaber M B, Kaplan I R, 1974. The sulphur cycle. The Sea, Vol. 5, E. D. Goldberg, ed. Wiley-Interscience, New York.

Hallberg R O, 1984. Computer simulation of sulphur isotope fractionation in a closed sulfuretium. Geomicrobial Journal 4.

Henley S, 1981. Nonparametric geostatistics. Elsevier Applied Science Publishers Ltd, Essex, England.

Kankainen T, 1986. Loviisa power station final disposal of reactor waste. On the age and origin of groundwater from the rapakivi granite on the island of Hästholmen. Nuclear Waste Commission of Finnish power Companies. Report YJT-86-29.

Laaksoharju M, 1990. Measured and predicted groundwater chemistry at Äspö. SKB Progress Report PR 25-90-13, Stockholm.

Laaksoharju M, Nilsson A C, 1989. Models of groundwater composition and of hydraulic conditions based on chemometrical and chemical analyses of deep groundwater at Äspö and Laxemar. SKB Progress Report PR 25 89 04, Stockholm.

Laaksoharju M, Skårman C, 1995. Groundwater sampling and chemical characterisation of the HRL tunnel at Äspö, Sweden. SKB Progress Report (report in progress).

Landström O, Tullborg E-L, 1993. Results from a geochemical study of zone NE-1, based on samples from the Äspö tunnel and drillcore KAS 16 (395 to 451 m). SKB Progress Report PR 25-93-01, Stockholm.

Landström O, Aggeryd I, Marthiasson L, Sundblad B, 1994. Chemical composition of sediments from the Äspö area and interaction between biosphere and geosphere. SKB Arbetsrapport AR 94-03, Stockholm.

Maddock R H, Hailwood E A, Rhodes E J, Muir Wood R, 1994. Direct fault dating trials at the Äspö hard rock laboratory. SKB Technical Report TR 93-24, Stockholm.

Morgan C O, Winner Jr M D, 1962. Hydrochemical facies in the 400 foot and 600 foot sands of the Baton Rouge Area, Louisiana. U. S. Geol. Surv. Prof. Paper 450-B.

Munier R, 1993. Segmentation, fragmentation and jostling of the Baltic shield with time. Thesis, Acta Universitatus Upsaliensis 37.

Nilsson A-C, 1995. Compilation of groundwater chemistry data from Äspö 1990-1994. SKB Progress Report PR 25-95-02, Stockholm.

Nurmi P A, Kukkonen I T, 1986. A new technique for sampling water and gas from deep drill holes. Canadian Journal of Earth Sciences. Volume 23, Number 9, 1986.

Olsen G J, Larsen N, Woese C R, 1991. The ribosomal RNA database project. Nucleic Acids Research (19) 2017-2021.

Pedersen K, Ekendahl S, 1990. Distribution and activity of bacteria in deep granitic groundwaters of south-eastern Sweden. Microb Ecol (20) 37-52.

Pedersen K, Karlsson F, 1995. Investigations of subterranean bacteria - Their influence on performance assessment of radioactive waste disposal. SKB Technical Report TR 95-10, Stockholm.

Piper A M, 1953. A graphic procedure in the geochemical interpretation of the water analyses, US Geological Survey, Water Res. Div. Ground Water Notes, Geochemistry. no. 12.

Rhén I, Danielsson P, Forsmark T, Gustafson G, Liedholm M, 1993. Äspö Hard Rock Laboratory. Geohydrological evaluation of the data from section 700-1475m. SKB Progress Report PR 25-93-06, Stockholm.

Rhén I, Danielsson P, Forsmark T, Gustafson G, Liedholm M, 1994. Äspö Hard Rock Laboratory. Geohydrological evaluation of the data from section 2265-2874m. SKB Progress Report PR 95-94-20, Stockholm.

Rhén I, Stanfors R, 1993. Passage through water-bearing fracture zones. Evaluation of investigations in fracture zones NE-1, EW-7 and NE-3. SKB Progress Report PR 25-92-18, Stockholm.

Rydström H, Gereben L, 1989. Seismic refraction survey on Äspö and Bockholmen. SKB Progress Report PR 25-89-18, Stockholm.

SKB 91, 1992. Final disposal of spent nuclear fuel. Importance of the bedrock for safety. SKB Technical report TR 92-20, Stockholm.

Smellie J A T, Laaksoharju M, 1992. The Äspö hard rock laboratory: final evaluation of the hydrogeochemical pre investigations in relation to existing geologic and hydraulic conditions. SKB Technical Report TR 92-31. Stockholm.

Stackebrandt E, Goodfellow M, 1991. Nucleic acid techniques in bacterial systematics.. John Wiley & Sons, Chichester.

Stanfors R, Gustafson G, Munier R, Olsson P, Stille H, Wikberg P, 1992. Evaluation of geological predictions in the access ramp 0-700m. SKB Progress Report PR 25-92-02, Stockholm.

Stanfors R, Liedholm M, Munier R, Olsson P, Stille H, 1993a. Geological-structural evaluation of data from section 700-1475m. SKB Progress Report PR 25-93-05, Stockholm.

Stanfors R, Liedholm M, Munier R, Olsson P, Stille H, 1993b. Äspö Hard Rock Laboratory. Geological-structural evaluation of data from tunnel section 1475-2265m. SKB Progress Report PR 25-93-10, Stockholm.

Stanfors R, Liedholm M, Munier R, Olsson P, Stille H, 1994. Äspö Hard Rock Laboratory. Geological-structural evaluation of data from tunnel section 2265-2874m. SKB Progress Report PR 25-94-19, Stockholm.

Trudinger P A, Chambers L A, 1973. Reversibility of bacterial sulphate reduction and it's relevance to isotope fractionation. Geochim. Cosmochim. Acta 37, 1775-1778.

Tullborg E-L, Landström O, Wallin B, 1995. Processes affecting low temperature, trace element mobility-indications from $\delta^{18}O$, $\delta^{13}C$ and $\delta^{34}S$ analyses of fracture calcite and pyrite. SKB (Report in progress).

Velde B, 1983. Diagenetic reactions in clays. (Eds. Parker & Sellwood). Sediments Diagenesis. NATO ASI series. D. Reidel Publishing Company, Dordrecht, Boston, Lancaster, 215-268.

Velde B, 1985. Clay minerals. A physico chemical explanation of their occurrence. Developments in sedimentology, 40. Elsevier, Amsterdam.

Wallin B, 1990. Carbon, oxygen and sulphur isotope signatures for groundwater classification at Laxemar, south-eastern Sweden. SKB Progress Report PR 25-90-12, Stockholm.

Wallin B, 1993. Organic carbon input in shallow groundwater at Äspö, south-eastern Sweden. Proc. ANS Publications Department, Chicago, Illinois, April 26-30, 1993. American Nuclear Society, La Grange Park, Illinois.

Wallin B, Peterman Z, 1994. Isotope systematics in ground water and hydrogenic deposits at Äspö, Sweden. Proceedings of the Fourth Annual International Conference, Las Vegas, Nevada, May 22-26, 1994. American Nuclear Society, 4:2692-2701.

Wallin B, 1995. Paleohydrological implications in the Baltic area and its relation to the groundwater at Äspö, south-eastern Sweden - A literature study. SKB Technical Report TR 95-06, Stockholm.

Widdel F, 1988. Microbiology and ecology of sulphate- and sulphur-reducing bacteria. Biology of anaerobic microorganisms Zender AJB. John Wiley & sons, New York.

Widdel F, Bak F, 1991. Gram-negative mesophilic sulphate-reducing bacteria. The prokaryotes. A handbook on the biology of bacteria: Ecophysiology, isolation, identification, applications. Balows A, Trüper HG, Dworkin M, Harder W, Schleifer K-H . Springer-Verlag, New York.

Winn K, Werner F, Erlenkeuser H, 1988. Hydrography of the Kiel Bay, Western Baltic during the Litorina Transgression. Meyniana, 40:31-46.

Wold S, 1987. Principal component analyses. Chemometric and Intelligent Laboratory Systems, 2, 35-37.

List of SKB reports

Annual Reports

1977-78
TR 121
KBS Technical Reports 1 – 120
Summaries
Stockholm, May 1979

1979
TR 79-28
The KBS Annual Report 1979
KBS Technical Reports 79-01 – 79-27
Summaries
Stockholm, March 1980

1980
TR 80-26
The KBS Annual Report 1980
KBS Technical Reports 80-01 – 80-25
Summaries
Stockholm, March 1981

1981
TR 81-17
The KBS Annual Report 1981
KBS Technical Reports 81-01 – 81-16
Summaries
Stockholm, April 1982

1982
TR 82-28
The KBS Annual Report 1982
KBS Technical Reports 82-01 – 82-27
Summaries
Stockholm, July 1983

1983
TR 83-77
The KBS Annual Report 1983
KBS Technical Reports 83-01 – 83-76
Summaries
Stockholm, June 1984

1984
TR 85-01
Annual Research and Development Report 1984
Including Summaries of Technical Reports Issued during 1984. (Technical Reports 84-01 – 84-19)
Stockholm, June 1985

1985
TR 85-20
Annual Research and Development Report 1985
Including Summaries of Technical Reports Issued during 1985. (Technical Reports 85-01 – 85-19)
Stockholm, May 1986

1986
TR 86-31
SKB Annual Report 1986
Including Summaries of Technical Reports Issued during 1986
Stockholm, May 1987

1987
TR 87-33
SKB Annual Report 1987
Including Summaries of Technical Reports Issued during 1987
Stockholm, May 1988

1988
TR 88-32
SKB Annual Report 1988
Including Summaries of Technical Reports Issued during 1988
Stockholm, May 1989

1989
TR 89-40
SKB Annual Report 1989
Including Summaries of Technical Reports Issued during 1989
Stockholm, May 1990

1990
TR 90-46
SKB Annual Report 1990
Including Summaries of Technical Reports Issued during 1990
Stockholm, May 1991

1991
TR 91-64
SKB Annual Report 1991
Including Summaries of Technical Reports Issued during 1991
Stockholm, April 1992

1992
TR 92-46
SKB Annual Report 1992
Including Summaries of Technical Reports Issued during 1992
Stockholm, May 1993

1993
TR 93-34
SKB Annual Report 1993
Including Summaries of Technical Reports Issued during 1993
Stockholm, May 1994

1994

TR 94-33

SKB Annual Report 1994

Including Summaries of Technical Reports Issued during 1994.

Stockholm, May 1995

List of SKB Technical Reports 1995

TR 95-01

Biotite and chlorite weathering at 25°C. The dependence of pH and (bi) carbonate on weathering kinetics, dissolution stoichiometry, and solubility; and the relation to redox conditions in granitic aquifers

Maria Malmström[1], Steven Banwart[1], Lara Duro[2], Paul Wersin[3], Jordi Bruno[3]

[1] Royal Institute of Technology, Department of Inorganic Chemistry, Stockholm, Sweden

[2] Universidad Politécnica de Cataluña, Departmento de Inginería Química, Barcelona, Spain

[3] MBT Tecnología Ambiental, Cerdanyola, Spain

January 1995

TR 95-02

Copper canister with cast inner component. Amendment to project on Alternative Systems Study (PASS), SKB TR 93-04

Lars Werme, Joachim Eriksson

Swedish Nuclear Fuel and Waste Management Co, Stockholm, Sweden

March 1995

TR 95-03

Prestudy of final disposal of long-lived low and intermediate level waste

Marie Wiborgh (ed.)

Kemakta Konsult AB, Stockholm, Sweden

January 1995

TR 95-04

Spent nuclear fuel corrosion: The application of ICP-MS to direct actinide analysis

R S Forsyth[1], U-B Eklund[2]

[1] Caledon-Consult AB, Nyköping, Sweden

[2] Studsvik Nuclear AB, Nyköping, Sweden

March 1995

TR 95-06

Palaeohydrological implications in the Baltic area and its relation to the groundwater at Äspö, south-eastern Sweden – A literature study

Bill Wallin

Geokema AB, Lidingö, Sweden

March, 1995

TR 95-07

Äspö Hard Rock Laboratory Annual Report 1994

SKB

April 1995

TR 95-08

Feasibility study for siting of a deep repository within the Storuman municipality

Swedish Nuclear Fuel and Waste Management Co., Stockholm

January 1995

TR 95-09

A thermodynamic data base for Tc to calculate equilibrium solubilities at temperatures up to 300°C

Ignasi Puigdomènech[1], Jordi Bruno[2]

[1] Studsvik AB, Nyköping, Sweden

[2] Intera Information Technologies SL, Cerdanyola, Spain

April 1995

TR 95-10

Investigations of subterranean microorganisms. Their importance for performance assessment of radioactive waste disposal

Karsten Pedersen[1], Fred Karlsson[2]

[1] Göteborg University, General and Marine Microbiology, The Lundberg Institute, Göteborg, Sweden

[2] Swedish Nuclear Fuel and Waste Management Co., Stockholm, Sweden

June 1995

TR 95-11

Solute transport in fractured media – The important mechanisms for performance assessment

Luis Moreno, Björn Gylling, Ivars Neretnieks

Department of Chemical Engineering and Technology, Royal Institute of Technology, Stockholm, Sweden

June 1995

TR 95-12
Literature survey of matrix diffusion theory and of experiments and data including natural analogues
Yvonne Ohlsson, Ivars Neretnieks
Department of Chemical Engineering and Technology, Royal Institute of Technology, Stockholm, Sweden
August 1995

TR 95-13
Interactions of trace elements with fracture filling minerals from the Äspö Hard Rock Laboratory
Ove Landström[1], Eva-Lena Tullborg[2]
[1] Studsvik Eco & Safety AB
[2] Terralogica AB
June 1995

TR 95-14
Consequences of using crushed crystalline rock as ballast in KBS-3 tunnels instead of rounded quartz particles
Roland Pusch
Clay Technology AB
February 1995

TR 95-15
Estimation of effective block conductivities based on discrete network analyses using data from the Äspö site
Paul R La Pointe[1], Peter Wallmann[1], Sven Follin[2]
[1] Golder Associates Inc., Seattle, WA, USA
[2] Golder Associates AB, Lund, Sweden
September 1995

TR 95-16
Temperature conditions in the SKB study sites
Kaj Ahlbom[1], Olle Olsson[1], Stefan Sehlstedt[2]
[1] Conterra AB
[2] MRM Konsult AB
June 1995

TR 95-17
Measurements of colloid concentrations in the fracture zone, Äspö Hard Rock Laboratory, Sweden
Anna Ledin, Anders Düker, Stefan Karlsson, Bert Allard
Department of Water and Environmental Studies,Linköping University, Linköping, Sweden
June 1995

TR 95-18
Thermal evidence of caledonide foreland, molasse sedimentation in Fennoscandia
Eva-Lena Tullborg[1], Sven Åke Larsson[1], Lennart Björklund[1], Lennart Samuelsson[2], Jimmy Stigh[1]
[1] Department of Geology, Earth Sciences Centre, Göteborg University, Göteborg, Sweden
[2] Geological Survey of Sweden, Earth Sciences Centre, Göteborg, Sweden
November 1995

TR 95-19
Compaction of bentonite blocks. Development of technique for industrial production of blocks which are manageable by man
Lars-Erik Johannesson, Lennart Börgesson, Torbjörn Sandén
Clay Technology AB, Lund, Sweden
April 1995

TR 95-20
Modelling of the physical behaviour of water saturated clay barriers. Laboratory tests, material models and finite element application
Lennart Börgesson[1], Lars-Erik Johannesson[1], Torbjörn Sandén[1], Jan Hernelind[2]
[1] Clay Technology AB, Lund, Sweden
[2] FEM-Tech AB, Västerås, Sweden
September 1995

TR 95-21
Conceptual model for concrete long time degradation in a deep nuclear waste repository
Björn Lagerblad, Jan Trägårdh
Swedish Cement and Concrete Research Institute
February 1994

TR 95-22
The use of interaction matrices for identification, structuring and ranking of FEPs in a repository system. Application on the far-field of a deep geological repository for spent fuel
Kristina Skagius[1], Anders Ström[2], Marie Wiborgh[1]
[1] Kemakta, Stockholm, Sweden
[2] Swedish Nuclear Fuel and Waste Management Co, Stockholm, Sweden
November 1995

TR 95-23
Spent nuclear fuel. A review of properties of possible relevance to corrosion processes
Roy Forsyth
Caledon Consult AB
April 1995

TR 95-24

Studies of colloids and their importance for repository performance assessment

Marcus Laaksoharju[1], Claude Degueldre[2],
Christina Skårman[1]
[1] GeoPoint AB, Sollentuna, Sweden
[2] University of Geneva, Switzerland

December 1995