

R-09-02

Evaluating hydrochemical data from shallow groundwater in Laxemar from a microbiological perspective

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Januari 2009

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This report concerns a study which was conducted for SKB. The conclusions and viewpoints presented in the report are those of the author and do not necessarily coincide with those of the client.

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Abstract

Oxygen is one of the chemical species that can corrode a copper canister in a KBS-3 repository. It is therefore important to determine whether oxygen dissolved in precipitation or groundwater could reach repository depth by means of groundwater transport. This can be determined by gaining an understanding of the oxygen-consuming microbial processes that take place in shallow groundwater in the area of interest.

This report evaluates hydrogeochemical data from shallow groundwater in the Laxemar area from a microbiological perspective.

Hydrogeochemical data were gathered from soil pipes at depths from 1.6 to 16.5 m and from percussion-drilled boreholes having mid-point depths of between 28.5 and 131 m. Only a few of the percussion-drilled boreholes had packers installed; the sampled sections were therefore very extended, allowing groundwaters from many different depths to mix. Oxygen and oxygen-reduction potential (ORP) were not measured in groundwater from soil pipes or percussion-drilled boreholes. The report therefore focuses on parameters that indicated ongoing anaerobic microbial processes, such as nitrite, ferrous iron, dissolved manganese, and sulphide, that were found in many soil pipes.

Even though many of the soil pipes were located in similar environments and at relatively similar depths, ranging from 3.5 to 6 m, they displayed individual chemical profiles in terms of chemical species related to microbial activity. The microbial activity could not be linked to the classes of soil pipe, i.e. recharge, discharge, or intermittent.

Existing soil pipes and percussion-drilled boreholes could be used for additional sampling to measure microbial parameters. Such sampling would benefit from the careful hypothesis-driven description of the sampling parameters and experience-guided choice of sampling methods.

Sammanfattning

Syre är ett av de kemiska ämnen som kan korrodera en kopparkapsel i ett KBS-3-förvar. Därför är det viktigt att bestämma om syre som är löst i nederbörd eller i grundvatten kan transporteras med grundvatten ner till förvarsdjup. För att förstå detta bör syrekonsumerande mikrobiella processerna som pågår i de grunda grundvattnen i området av intresse studeras.

Syftet med den här rapporten var att från ett mikrobiologiskt perspektiv utvärdera hydrogeokemiska data från grunda grundvatten i Laxemarområdet.

Hydrogeokemiska data har samlats in från jordrör med djup från 1,6 till 16,5 meter och från hammarborrade borrhål med ett medeldjup av 28,5 till 131 m. Få hammarborrade hål hade packers installerade och därför är många av de provtagna sektionerna från dessa hål väldigt långa och innehöll förmodligen en blandning av grundvatten från olika djup. Syre och oxidation-reduction potentialen (ORP) har inte mätts i jordrör och inte heller i hammarborrade hål. Därför kan man inte avgöra vid vilket djup syre är konsumerat. Mätbara halter av kemiska parametrar som indikerade att anaeroba mikrobiella processer pågick, t ex nitrit, tvåvärt järn, löst mangan och sulfid fanns i flera av jordrören.

Trots att många av jordrören var placerade i liknande biotoper och med samma provtagningsdjup, 3,5 m till 6 m, uppvisade de individuella kemiska karaktärer med avseende på kemiska specier som är relaterade till mikrobiell aktivitet. Den mikrobiella aktiviteten kunde inte kopplas till utflödes- eller inflödeszoner.

De befintliga jordrören och borrhålen kan användas för ytterligare provtagningar av mikrobiella parametrar. En sådan provtagning bör vara hypotesdriven och provtagningarna och valet av parametrar och provtagningsmetoder väljas utifrån erfarenhet från liknade uppdrag.

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1 Introduction

One of the most important conditions for a KBS-3 type repository /Anonymous 1983/ for radioactive waste in the bedrock in Sweden is the maintenance of anoxic and reduced conditions. The source of oxygen that could reach the repository is air that dissolves in precipitation or directly in groundwater. The oxygen could then theoretically reach the repository depth of 500 m by means of groundwater transport.

Oxygen in shallow groundwater is effectively consumed by microorganisms during the degradation of organic matter, as oxygen functions as an electron acceptor in microbial metabolism. Other compounds, such as methane, sulphide, ammonium, iron, and manganese, can also be used by microorganisms as energy sources, when coupled with oxygen reduction. All such microbially catalysed reactions will rapidly consume the available oxygen; when the oxygen is depleted, the degradation of organic matter will be continued by anaerobic microorganisms. These organisms use other compounds as electron acceptors. They follow a thermodynamic hierarchy, depending on the energy exchange with the available electron donors and acceptors (see Figure 1-1 and 1-2). As can be seen, the manganese- and iron-reducing bacteria (MRB and IRB, respectively) will come into play when the oxygen is gone. Their sources of electron acceptors are oxidised iron and manganese, which are solid phases found on fracture surfaces; these bacteria are active only if such mineral phases are present. One group of iron and manganese reducers (including the genus *Shewanella*) is very versatile and can use an array of different electron acceptors ranging from oxygen and nitrate to highly crystalline iron oxides; these microbes are facultatively anaerobic, meaning that they can use both oxygen and nitrate as electron acceptors. The manganese and iron reducers are followed by nitrate-reducing bacteria (NRB), which are also often facultatively anaerobic. The next organism group is the sulphate-reducing bacteria (SRB), which use sulphate as an electron acceptor and reduce the sulphur atom in sulphate to sulphide, S^{2-} . Sulphate reducers are present in almost all waters, though in different numbers.

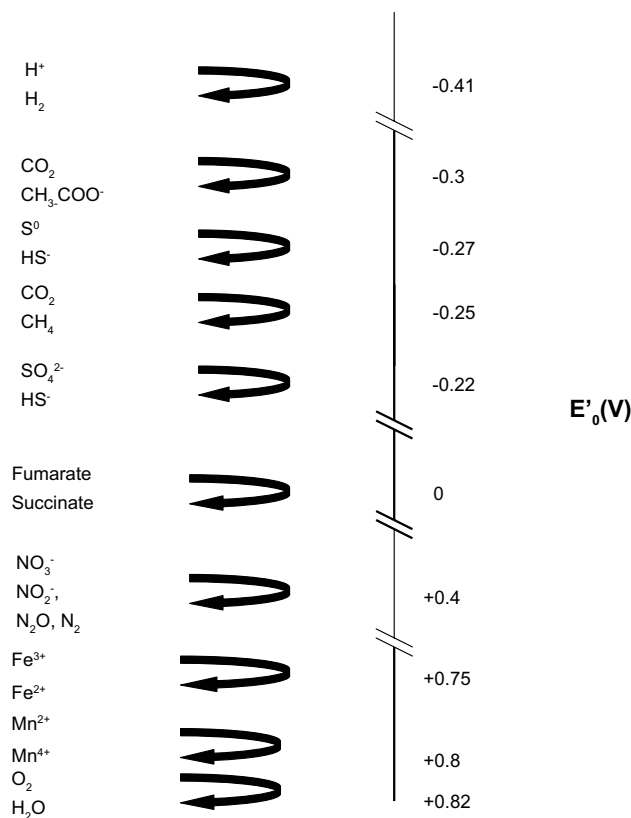


Figure 1-1. Redox couples found in deep groundwater:

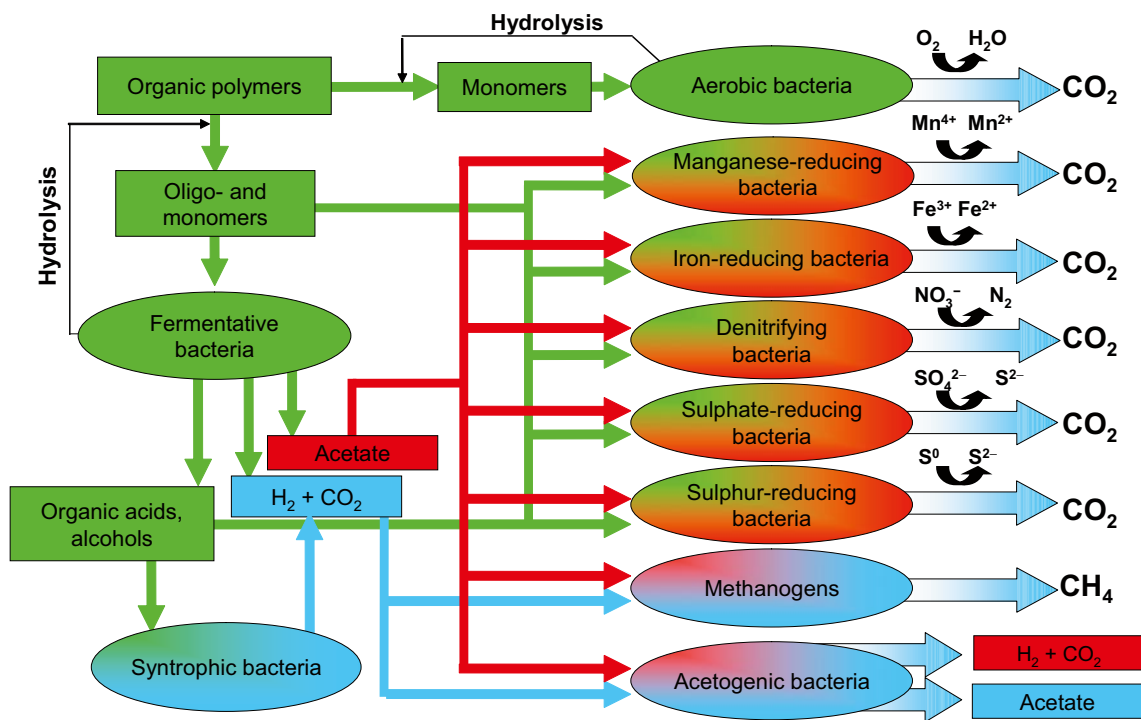


Figure 1-2. Possible pathways for the flow of carbon in the subterranean environment. Organic carbon is respired with oxygen, if present, or else fermentation and anaerobic respiration occur using an array of different electron acceptors.

Along with the anaerobic microbes described above, fermenting microorganisms are also active. Fermentation consists of degradation pathways that do not require external electron acceptors; instead, electrons are shuffled around within the degraded molecule that has been split into two or more compounds. The degradation products consist of both more reduced and more oxidised organic molecules, such as organic acids, ketones, alcohols, and hydrogen gas (hydrogen is an important energy source for the autotrophic microorganisms that thrive in groundwater). Apart from fermentation, hydrogen also comes from sources deeper in the bedrock /Apps and Van de Kamp 1993/. Two important groups of autotrophic organisms that can use the hydrogen in groundwater are methanogens and acetogens, the end products of which are methane and acetate. Both groups fix carbon dioxide using the energy in hydrogen (see Eqs. 1 and 2).



There are also microorganisms that can use organic compounds with one or two carbons and produce acetate and methane. Table 3-1 presents a summary of the different organism groups and their effects on the environment.

The site investigation in Laxemar includes studies of microbial populations in groundwater from core-drilled boreholes from depths of 150 m to 922 m. Unfortunately, no microbial data are available for the interval from the ground surface to a depth of 150 m, though *chemical* data are available from soil pipes and percussion-drilled boreholes for the 1.5–139 m depth interval. Some of the soil pipes yield data that can give clues as to the redox conditions, and thus information about the active microbial populations in the groundwater. The parameters influenced by microbial activity and therefore of interest are oxygen, redox potential or oxygen-reduction potential (ORP), nitrogen species, ferrous iron and manganese, sulphate and sulphide, dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), and bicarbonate. In addition, stable isotope data for inorganic carbon and sulphate can give information about microbial processes. Some of these parameters were measured in water samples from the soil pipes, but unfortunately not oxygen, ORP, or DIC. Nitrate, nitrite, ferrous iron, manganese, sulphide, sulphate, and DOC could be measured in samples from the soil pipes, but not all parameters on all occasions and not for all

soil pipes. For some soil pipes, there are measurements covering quite long periods and thus also covering seasonal variations over the year. Chemical data for the percussion-drilled boreholes are much fewer than those for the soil pipes: oxygen and ORP were not measured in any percussion-drilled boreholes; manganese, ferrous iron, DOC, sulphate, and sulphide were measured in some of them, but on few occasions.

This study aims to answer the following questions with the help of available data:

- Is it possible to determine from area-specific chemistry and the results of the microbe investigation in Olkiluoto, Finland, what microbial processes are active in different parts of the Laxemar-Simpevarp area?
- Which process or processes dominates/dominate the degradation of organic matter? Do different processes dominate in different areas and/or depths? Could the area be divided into sub-areas based on depth, recharge/discharge areas or other factors?
- Is it possible to establish a depth limit for the presence of oxic groundwater? Does this limit vary over the course of the year or between years? Where is the oxygen consumed, if it is not transported down to the bedrock?
- Are we still missing some chemical parameters necessary for a full understanding of the microbiology in the oxic/anoxic zone in Laxemar?

This report will evaluate the available data with respect to microbial activity. It is hoped that the evaluation will reveal seasonal variations, environmental variations, and depth variations present in shallow groundwater in Laxemar.

Table 1-1. Activities and effects of the different physiological groups of microorganisms found in deep groundwater.

Metabolic groups of microorganisms	Activity	Effect on the environment
Aerobic respiration	Oxidation of organic material by means of oxygen reduction	Depletion of oxygen and organic material Increased alkalinity Lowered redox potential
Anaerobic respiration	Oxidation of organic material and reduction of compounds other than oxygen	See below for each specific group of bacteria
Iron-reducing bacteria	Oxidation of organic material and reduction of ferric iron	Depletion of organic material and ferric iron Increased ferrous iron concentration and alkalinity Lowered redox potential
Manganese-reducing bacteria	Oxidation of organic material and reduction of manganese(IV) ions	Depletion of organic material and manganese(IV) Increased manganese(II) concentration and alkalinity Lowered redox potential
Sulphate-reducing bacteria	Oxidation of organic material and reduction of sulphate	Depletion of organic material and sulphate Increased sulphide concentration and alkalinity Lowered redox potential
Methanogenesis		
Heterotrophic methanogens	Convert short-chained organic material to methane and carbon dioxide	Depletion of organic material Increased methane gas and carbon dioxide (alkalinity) concentrations Redox not influenced
Autotrophic methanogens	Oxidation of hydrogen gas and reduction of carbon dioxide to methane gas	Depletion of hydrogen gas and alkalinity Increased methane gas concentration Redox lowered
Acetogenesis		
Heterotrophic acetogens	Convert organic material to acetate	Depletion of organic material other than acetate Increased acetate concentration Redox not influenced
Autotrophic acetogens	Oxidation of hydrogen gas and reduction of carbon dioxide to acetate	Depletion of hydrogen gas and alkalinity Increased acetate concentration Redox lowered

2 Data evaluation

The data used here are from measurements made in soil pipes and percussion-drilled boreholes in the Laxemar sub-area. The data were compiled by Mats Tröjbom and are the same as used in SKB report R-08-46 /Tröjbom et al. 2008/.

2.1 Depths

The parameter “mean screen depth” (MSD) is used as the depth of soil pipes in this report. Thus, the sampling depth of the shallowest soil pipe was 1.5 m and of the deepest soil pipe that provided a sufficient amount of data was 16.5 m. Most of the soil pipes were 3–6 m deep. For the percussion-drilled boreholes, the mid-point depths of the sections (“sec mid” depths) are used as the depth. This makes the data uncertain, since some of these boreholes had no packers installed, making some sampled sections as long as 200 m; water samples from such boreholes may therefore be a mixture of shallow and deep groundwater. The important parameters oxygen and oxygen-reduction potential (ORP) were not measured in either the soil pipes or percussion-drilled boreholes. Using the sec mid value, the shallowest percussion-drilled borehole had a depth of 28.5 m and the deepest 131 m. This means that no data at all are available from depths between 16.5 and 28.5 m. This is unfortunate, since this depth interval is where the greatest decrease of oxygen in has been observed to occur in Olkiluoto groundwater, as can be seen in Figure 2-1 /Pedersen 2006/. This figure also shows the difference in oxygen concentration between spring and autumn in samples from soil pipes and boreholes in Olkiluoto, Finland.

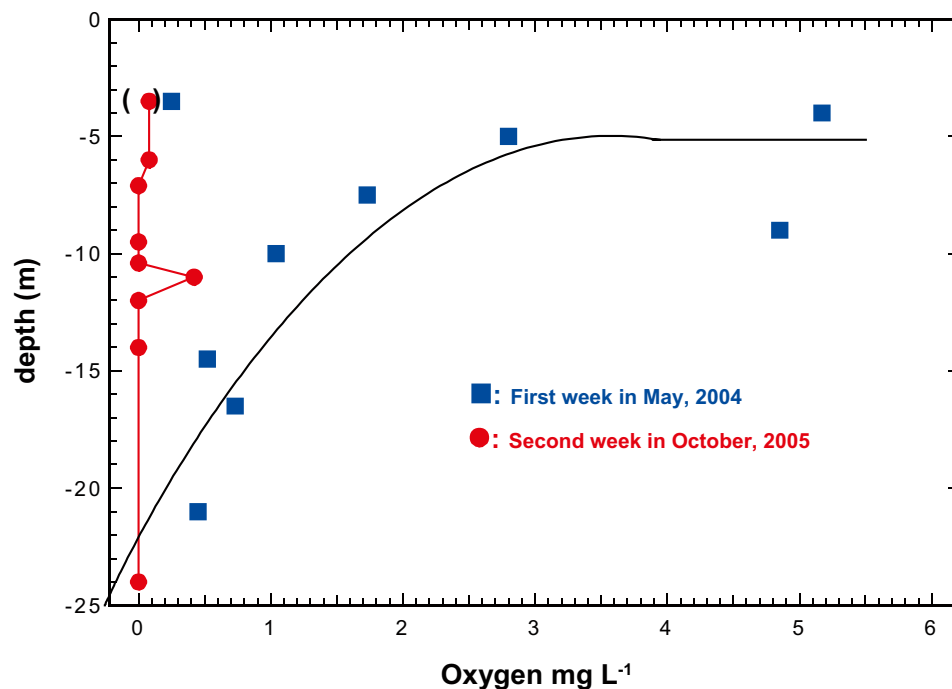


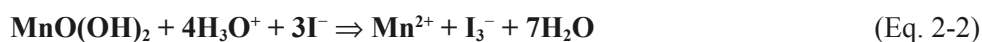
Figure 2-1. The concentration of dissolved oxygen in shallow groundwater in Olkiluoto, Finland, analysed using Winkler titration in the laboratory /Pedersen 2006/.

2.2 Oxygen

Oxygen was not measured in any of the soil pipes or percussion-drilled boreholes in Laxemar, mainly because it is difficult to get reliable data from these measurements. Measuring oxygen concentrations is complicated and is done in two main ways: in the field using an oxygen electrode or in the laboratory using a modified Winkler method, as described in detail in /Carritt and Carpenter 1966/. In the Winkler method, groundwater is carefully sampled in 115-mL, glass-stoppered Winkler bottles; the dissolved oxygen is immediately precipitated as manganese oxyhydroxide ($\text{MnO}(\text{OH})_2$) by adding manganese ions to an alkaline medium. With the oxygen bound in the precipitate, the samples can be stored and the actual analysis is done in the laboratory; the following are the reactions that take place during sampling and analysis:



In the laboratory, the manganese hydroxide is dissolved in acid and reduced by iodine ions, as follows:



Finally, the I_3^- ions produced are determined by titration, using thiosulphate ions and soluble starch as the titration indicator, as follows:



The field measurements in Forsmark site investigation were made using two multiparameter sondes, YSI 600 QS and YSI 6600 EDS (YSI Inc. Yellow Springs, OH, USA), that measured pH, water temperature, oxygen, ORP, and electrical conductivity. The measurements were made in a simple flow-through cell, and the probe used for measuring oxygen was a membrane electrode. This method for measuring oxygen is somewhat less reliable than the Winkler titration method. /Nilsson and Borgiel 2005/ concluded that if an electrode measurement indicated a value of 4 mg L^{-1} , the true value might well be below the detection limit or even zero.

The membrane electrode used in the monitoring program in Forsmark, the YSI Rapid Pulse Dissolved Oxygen electrode (YSI Inc. Yellow Springs, OH, USA), has a measurement range of $0\text{--}50 \text{ mg L}^{-1}$ with a resolution of 0.01 mg L^{-1} ; the accuracy of the electrode is $\pm 0.2 \text{ mg L}^{-1}$ over the $0\text{--}20 \text{ mg L}^{-1}$ range. This type of electrode needs continuous calibration and maintenance, for example, the membrane must be changed on a regular basis. Another type of electrode is available, which uses luminescence to detect oxygen and is easier to handle. In sampling shallow groundwater at Olkiluoto, Finland, both types of electrodes were used but on different occasions. The conclusion was that the Hach HQ10 Portable LDO Dissolved Oxygen Meter (cat. no. 51815-00; Hach, Stockholm, Sweden) gave lower and more reliable results and was easier to handle than the pIONeer 20 portable oxygen meter equipped with a DOX20T-T oxygen probe /Pedersen 2008/. For all oxygen measurements in water containing low or zero amounts of oxygen, it must be established that a zero value can indeed be measured by making a reference measurement in completely oxygen-free water.

2.3 Oxygen-reduction potential

Oxygen-reduction potential (ORP) is measured using one electrode, often made of a precious metal such as gold, silver, or platinum, with a silver–silver chloride electrode used as the reference. ORP was measured in the surface program in Forsmark but not in Laxemar. When sampling deep groundwater, several electrodes are used instead of only one as in the ORP measurements.

The data for the deep groundwater samples are reported as the redox potential (Eh), which is the recalculated potential versus that of the hydrogen reference electrode. In this report, the ORP values are used. The E_h value can be calculated using the following equation:

$$Eh = E_{meas} + E_{ref} \quad (\text{Eq. 2-4})$$

where Eh is the redox potential, E_{meas} is the ORP value, and E_{ref} is the potential of the electrode versus that of the hydrogen electrode. The potential of the used electrode at 15°C is +214 mV.

Redox potential or oxygen-reduction potential is a measure of a system's tendency to donate electrons. Negative values indicate a high tendency to donate electrons and positive values a low tendency and that the system more likely accepts electrons.

2.3.1 Reduced species in shallow groundwater

Even though no oxygen data are available for shallow groundwater in Laxemar, it is possible to infer the presence of oxygen by studying the reduced chemical species ferrous iron (Fe^{2+}) and sulphide (S^{2-}). These species oxidise rapidly in the presence of oxygen. If ferrous iron and sulphide are present in measurable amounts, the oxygen concentrations are very low. Data for these species are available for the soil pipes, and Figures 2-2 and 2-3 show ferrous iron and sulphide versus depth in soil pipes in Laxemar. Both species were present but in various concentrations in the different soil pipes. In some soil pipes, the ferrous iron concentration was as high as 11 mg L^{-1} , higher than measured in any soil pipes in Forsmark. The sulphide concentrations were near or below 0.1 mg L^{-1} (this parameter is difficult to measure; see also section 3.2.3 where this issue is discussed further). Ferrous iron and sulphide were not measured in groundwater from percussion-drilled boreholes.

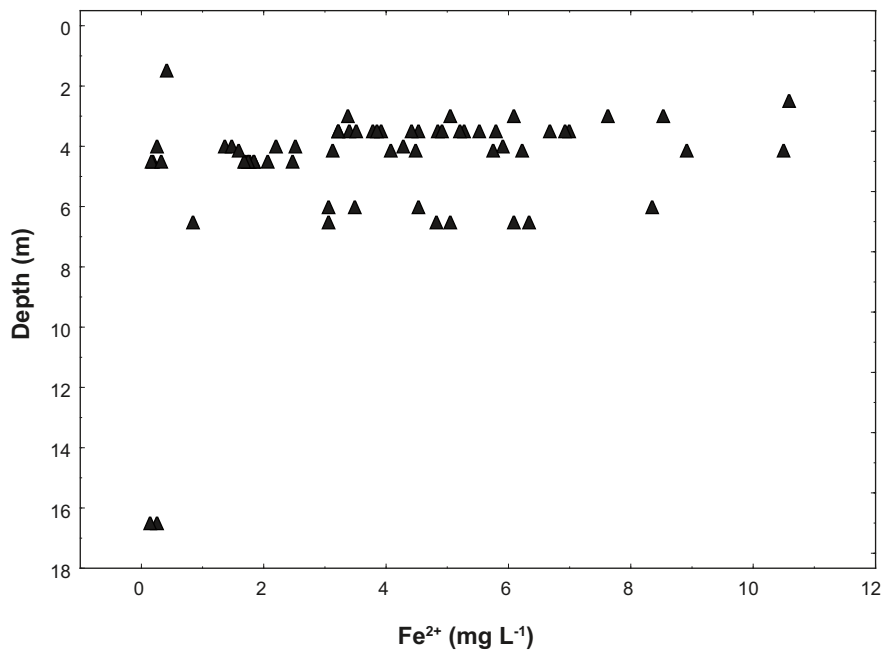


Figure 2-2. Ferrous iron versus depth in shallow groundwater in Laxemar.

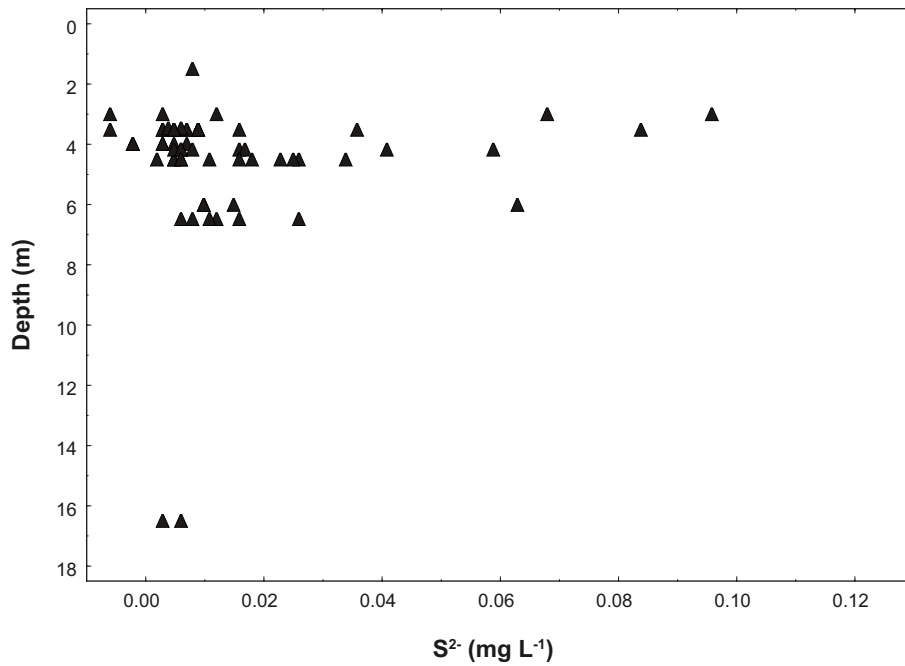


Figure 2-3. Sulphide versus depth in shallow groundwater in Laxemar.

2.4 Conclusions

- Oxygen and ORP data were missing from the dataset for both the soil pipes and percussion-drilled boreholes. The oxygen depletion in Olkiluoto, Finland, occurred at depths of 10–25 m. The oxygen and ORP parameters could not be evaluated using the dataset from Laxemar.
- The presence of reduced species of iron and sulphur, ferrous iron and sulphide, indicate that anoxic conditions were present at some point in the sampled soil pipes.

3 Characterisation of soil pipes and percussion-drilled boreholes

Since one question to be answered by this study was whether there were any differences between soil pipes containing discharging or recharging groundwater, initial analyses focused on these two types of soil pipes. Only two soil pipes in the Laxemar area are classified as recharge groundwater soil pipes, SSM000039 and SSM000230. Neither of these displayed any special characteristics when the dataset was analysed.

A study of shallow groundwater in Olkiluoto, Finland /Pedersen 2008, Pedersen et al. 2008/ found that most of the soil pipes sampled had chemical characteristics that remained stable from season to season and from one year to another.

When some of the parameters measured in Laxemar were plotted against each other, a borehole specific chemical composition pattern became evident. Data from the following soil pipes were included in this evaluation, (the prefix SSM0000 is omitted from the following soil pipe names): 27, 29, 30, 31, 34, 35, 37, 39, 40, 41, 42, 224, 226, 227, 228, 230, and 240; soil pipes 27, 30, 31, 34, 37, 42, and 240 were analysed more closely. Table 3-1 presents the biotopes and characteristics of the identified soil pipes.

Data for percussion-drilled boreholes were even scarcer than for the soil pipes, with no oxygen, ferrous iron, ORP, or DIC data being available for them. DOC was measured in a few boreholes, while manganese and nitrogen species were measured only in some boreholes on a few occasions. Data for chloride, hydrogen carbonate, $\delta^{13}\text{C}$, sulphate, and $\delta^{34}\text{S}$ were available for all boreholes and measured over longer periods. As described in section 2.1, many of the percussion-drilled boreholes had no packers, so the water sampled was a mixture of water from many different depths. The size of the sections often varied between samplings. The sampled percussion-drilled boreholes and the SECUP, SECLW, and SECMID depths of the sections are shown in Table 3-2.

Table 3-1. Depths, main biotopes, and symbols of well-characterised soil pipes in Laxemar.

Soil pipe	Depth (m)	Main biotope	Symbol in figures
SSM000027	4.0	missing	▲
SSM000030	4.5	Open landscape	▲
SSM000031	3.5	Open landscape	▲
SSM000034	3.5	Open landscape	▲
SSM000037	3.5	Open landscape	●
SSM000042	4.2	Open landscape	■
SSM000240	4.9	Sea bay	◆

Table 3-2. Percussion-drilled boreholes and sections sampled for hydrogeochemistry in the Laxemar area.

IDCODE	SECUP (m)	SECLow (m)	SECMID (m)
HLX01	50	100	75
HLX03	25	100	62.5
HLX06	45	100	72.5
HLX07	20	100	60
HLX10	0	85	42.5
HLX10	3	85	44
HLX14	0	95	47.5
HLX14	11.9	115.9	63.9
HLX14	96	115.9	105.95
HLX18	15.1	181.2	98.15
HLX20	0	69	34.5
HLX20	9.03	202.2	105.61
HLX20	9.1	202.2	105.65
HLX20	70	80	75
HLX20	81	202	141.5
HLX21	0	72	36
HLX21	0	150.3	75.15
HLX21	73	150.3	111.65
HLX22	0	163.2	81.6
HLX23	0	160.2	80.1
HLX24	0	175.2	87.6
HLX27	0	95.5	47.75
HLX27	6.01	164.7	85.35
HLX27	96.5	148.5	122.5
HLX27	149.5	164.7	157.1
HLX28	0	6.5	3.25
HLX28	6.03	154.2	80.11
HLX28	7.5	69	38.25
HLX28	70	90	80
HLX28	91	154	122.5
HLX30	0	163.4	81.7
HLX32	0	19	9.5
HLX32	12.3	162.6	87.45
HLX32	20	30	25
HLX32	31	162.6	96.8
HLX33	0	49	24.5
HLX33	9.03	202.1	105.56
HLX33	50	202	126
HLX34	0	151.8	75.9
HLX35	0	119	59.5
HLX35	6.03	151.8	78.92
HLX35	120	135	127.5
HLX35	136	151.8	143.9
HLX37	0	93	46.5
HLX37	12.03	199.8	105.92
HLX37	12.1	199.8	105.95
HLX37	94	110	102

HLX37	111	149	130
HLX37	150	199.8	174.9
HLX38	15.02	199.5	107.26
HLX39	0	50	25
HLX39	6.1	199.3	102.7
HLX39	51	186	118.5
HLX39	187	199	193
HLX42	9.1	152.6	80.85
HLX43	0	29	14.5
HLX43	6	170.6	88.3
HLX43	30	74	52
HLX43	75	134	104.5
HLX43	135	147	141

3.1 Dissolved organic carbon, dissolved inorganic carbon and hydrogen carbonate

In Figure 3-1, the dissolved organic carbon (DOC) and the bicarbonate (HCO_3^-) values for all soil pipes in Laxemar are plotted against each other. From this it can be seen that most of the data cluster, DOC values being between 6 and 14 mg L^{-1} and HCO_3^- between 50 and 300 mg L^{-1} . The DOC and HCO_3^- data were typical of each soil pipe, and soil pipes located in similar environments displayed data coherence from one sampling occasion to another. In Figure 3-2, seven soil pipes were chosen and the DOC and HCO_3^- data were plotted using symbols to identify each soil pipe. The typical DOC and HCO_3^- profiles seem to be linked to the biotope where the soil pipes were located. Groundwater from soil pipe SSM000034 has DOC values in the same order of magnitude as those of most of the sampled groundwater, approximately 7 mg L^{-1} , but its HCO_3^- content is over twice as high at approximately 550 mg L^{-1} . This soil pipe is located in a fen, in an area mostly covered with water. In such areas, there are large amounts of organic material that degrade slowly due to the limited availability of oxygen. The produced carbon dioxide

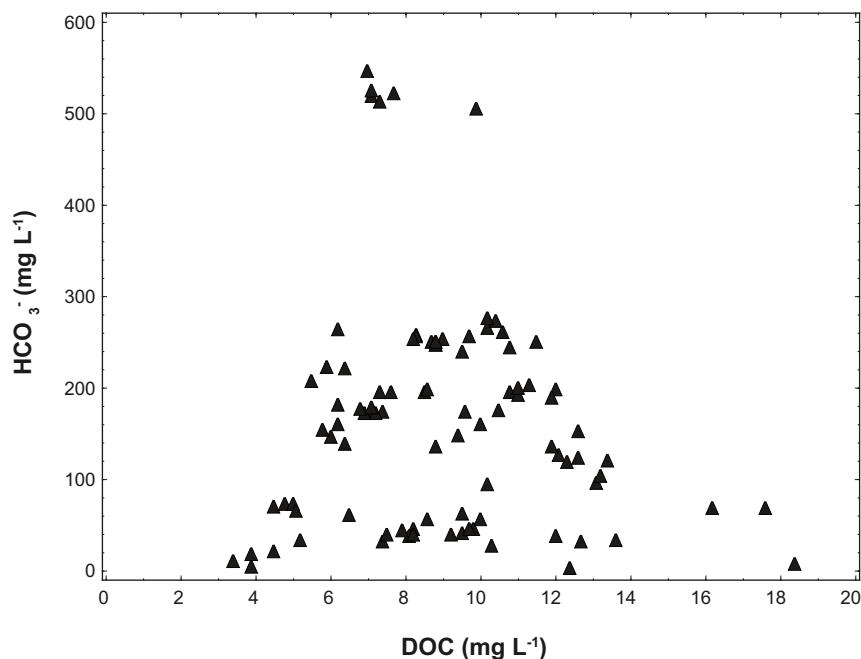


Figure 3-1. DOC versus bicarbonate (HCO_3^-) in groundwater from soil pipes in Laxemar.

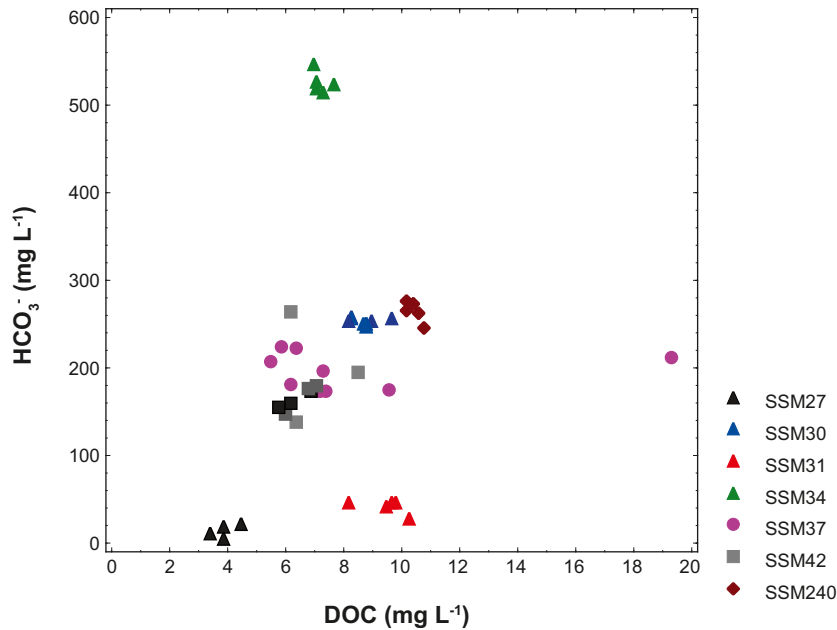


Figure 3-2. Dissolved organic carbon (DOC) versus bicarbonate (HCO_3^-) in groundwater from soil pipes in Laxemar.

dissolves in water giving a high concentration of HCO_3^- . The soil layer here is two metres of peat and gyttja. Groundwater from soil pipes SSM000027 and SSM000031, on the other hand, have the lowest HCO_3^- values, below 50 mg L^{-1} . The soil layers here were more porous, and some of the produced carbon dioxide probably escaped as gas and did not dissolve in water to form bicarbonate. Groundwaters from the other soil pipes in Figure 3-2, SSM000037 and SSM000042, are grouped in the middle between the two extremes. The soils in these two soil pipes are sandy with gravel. The calcium concentration in groundwater from SSM000034 was approximately 100 mg L^{-1} , versus the concentration in SSM000027 only approximately 5 mg L^{-1} . The molar ratio of HCO_3^- and calcium is 9 in SSM000037, indicating that there has been calcite dissolution due to carbon dioxide production from the degradation of organic material. When carbon dioxide from the degradation dissolves in water, carbonic acid is produced; calcite will then dissolve in the water because of its lowered pH.

There is no indication of seasonal variations in the DOC or HCO_3^- levels in the groundwater from soil pipes in Laxemar. The identified soil pipes were not located in forested areas, but rather in meadows, so the variation may not be that great in open areas without leaf fall in autumn.

DOC was measured in some of the percussion-drilled boreholes on a few occasions. Figure 3-3 shows the distribution of DOC versus depth for percussion-drilled boreholes. All samples were taken at depths of 60–80 m. Here, the DOC concentrations were below 10 mg L^{-1} , except in two samples from HLX01 and HLX07 that had DOC concentrations of 24 and 30 mg L^{-1} , respectively. Groundwaters from both boreholes were sampled on other occasions, when they had values below 10 mg L^{-1} . Figure 3-4 shows DOC versus HCO_3^- in groundwater from the percussion-drilled boreholes. Here it can be also seen that the HCO_3^- concentrations in the samples from HLX01 and HLX07 differ from those in the other samples by being much lower, at 150 and 120 mg L^{-1} , respectively.

Biological processes discriminate against heavy isotopes, as can be seen in the isotopic fractionation, for example, of DOC or bicarbonate in groundwater. Photosynthesis and produce organic carbon in plants that is lighter than the carbon found in atmospheric carbon dioxide. This light composition is also seen in the bicarbonate in shallow groundwater that originates from the degradation of organic matter from the ground surface. The isotopic carbon compositions of different carbon

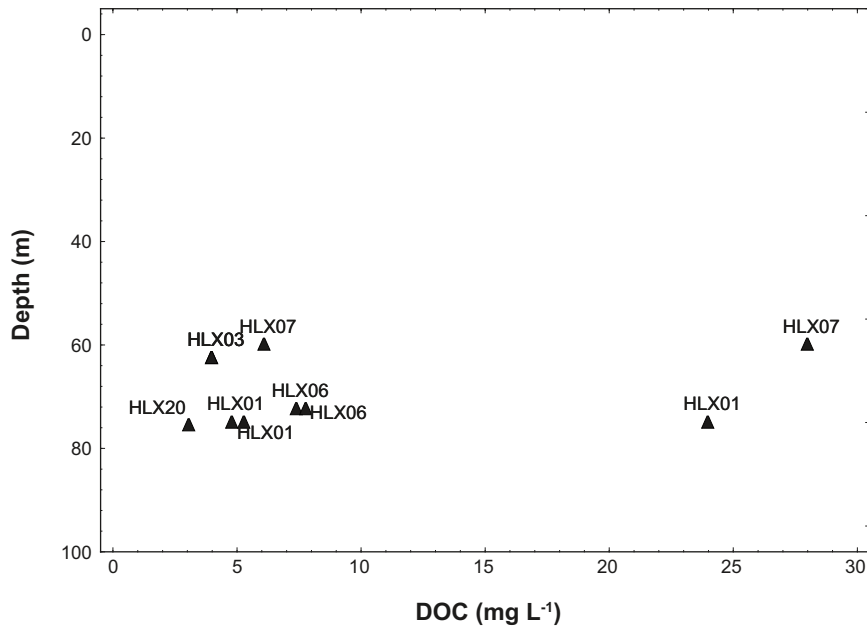


Figure 3-3. Dissolved organic carbon (DOC) versus depth in percussion-drilled boreholes in Laxemar.

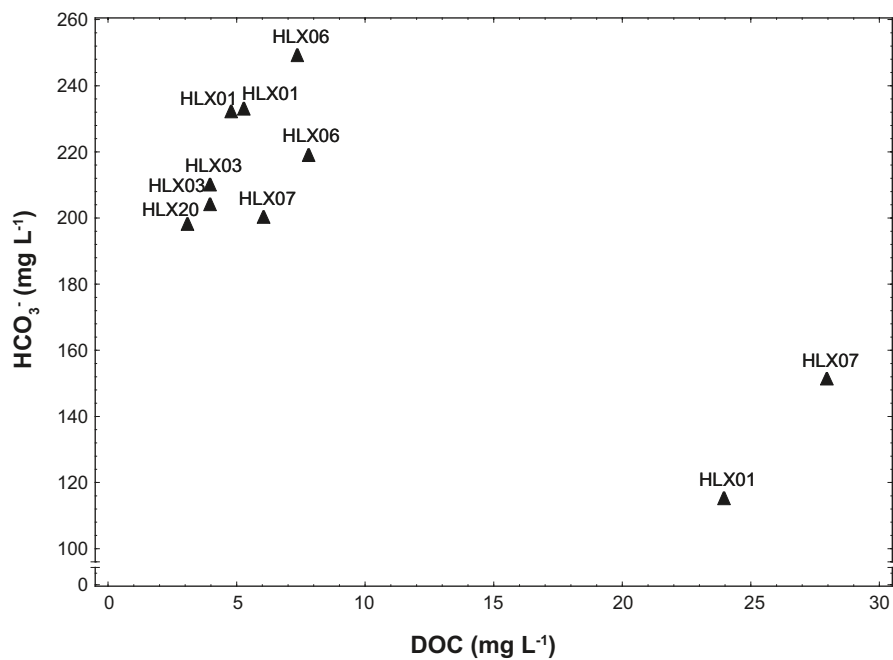


Figure 3-4. Dissolved organic carbon (DOC) versus bicarbonate (HCO_3^-) in percussion-drilled boreholes in Laxemar.

reservoirs on Earth are depicted in Figure 3-5. Notably, the isotopic composition tells us nothing about when the fractionation took place. As well, mixtures of waters having different isotopic signatures can obscure the picture. To be certain of what processes are currently ongoing, the microbial communities must be investigated.

Figure 3-6 shows $\delta^{13}\text{C}$ versus HCO_3^- in groundwater from soil pipes in Laxemar. The ^{13}C is compared with a standard, the Vienna Pee Dee Belemnite (PDB) carbonate, having a specific isotopic composition. The soil pipe containing the heaviest inorganic carbon was SSM000034, having $\delta^{13}\text{C}$ values from -10 . This soil pipe was located in a fen and is covered with water. The isotopic composition supports the theory that there was ongoing methanogenic and/or

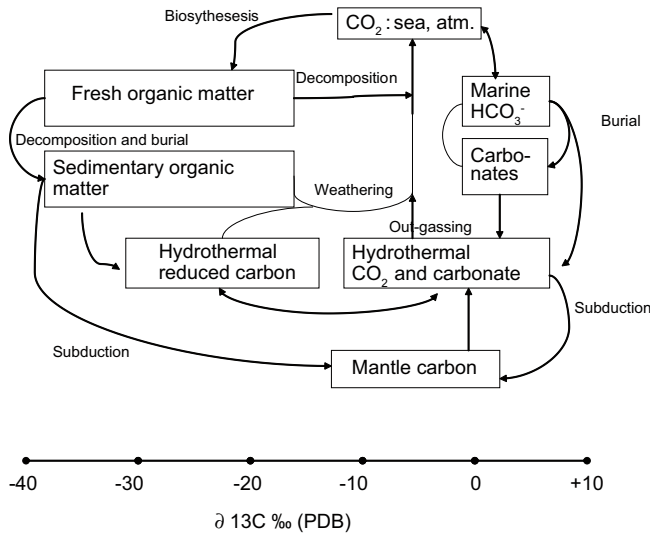


Figure 3-5. Carbon isotopic composition of carbon reservoirs (adapted from Des Marais 1996/).

acetogenic autotrophic activity. Both these processes discriminate against heavy carbon in carbon dioxide. The second explanation is that there could be ongoing calcite dissolution in the groundwater, as indicated by the higher calcium values mentioned earlier in this section. Since stable isotope measurements do not indicate when the process occurred, they must be complemented with microbial studies and analyses of groundwater gases.

The soil pipes located in forests and open landscapes contain inorganic carbon with light $\delta^{13}\text{C}$ signatures, which reveals the photosynthetic origin of the degraded organic matter.

Figure 3-7 shows the carbon isotopic composition of inorganic carbon versus bicarbonate measured in samples from percussion-drilled boreholes. No distinct pattern was evident for the different boreholes, but there was a trend towards lighter carbon in groundwater containing a

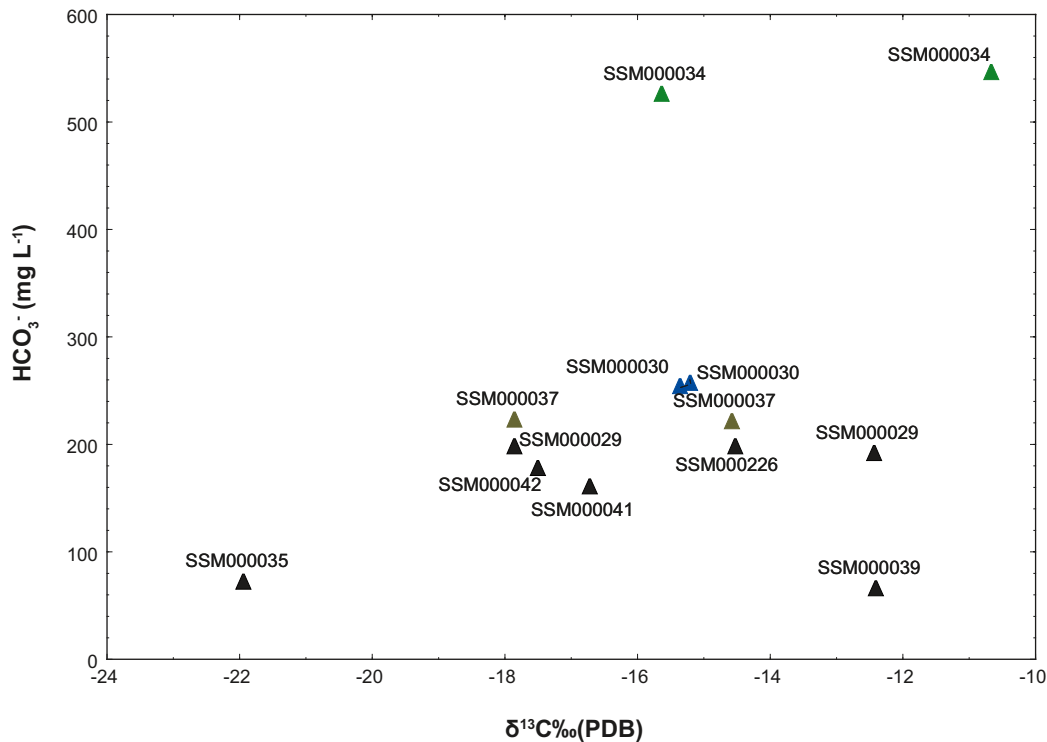


Figure 3-6. $\delta^{13}\text{C}$ versus DIC in groundwater from soil pipes in Laxemar.

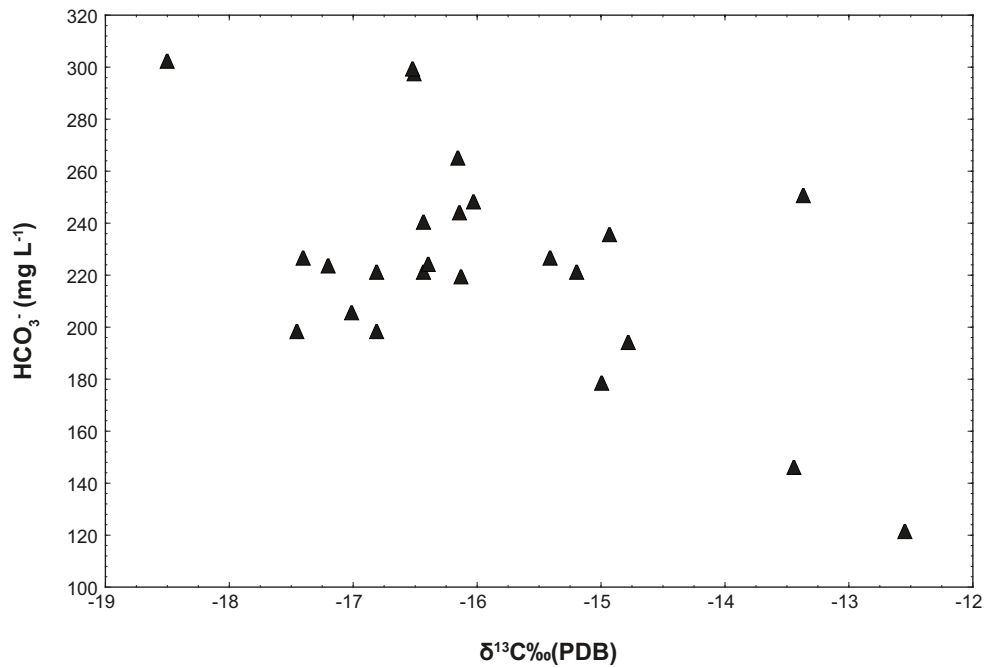


Figure 3-7. Carbon isotopic composition of inorganic carbon versus HCO_3^- in groundwater from percussion-drilled boreholes in Laxemar.

higher concentration of bicarbonate. It should be noted that in some boreholes, sampling was not consistently done at the same depths on different sampling occasions. Figure 3-8, on the other hand, presents $\delta^{13}\text{C}$ versus depth in the percussion-drilled boreholes. This indicates that the lighter carbon was found at shallower depths, together with higher bicarbonate concentrations than were found at greater depths.

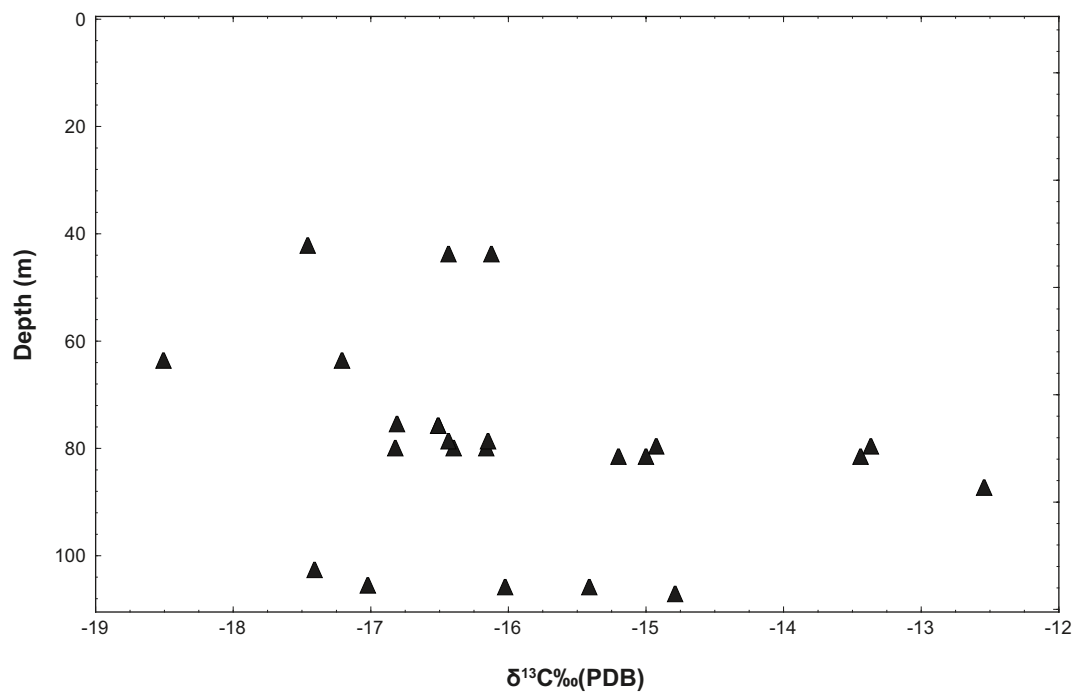


Figure 3-8. Carbon isotopic composition of inorganic carbon versus depth in groundwater from percussion-drilled boreholes in Laxemar.

3.2 Anaerobic microbial processes in groundwater

3.2.1 Nitrate reduction

It can now be concluded that there is variation in the amount of DOC and HCO_3^- in groundwater from the soil pipes. In samples from some pipes, the DOC content is higher and there is evidence of degradation of organic material. Since there are no oxygen data, they cannot be used to determine the depth at which the oxygen becomes depleted. Because of that, the alternate electron acceptor used when oxygen is consumed and anaerobic degradation takes place, namely, nitrate, will be used instead. Many aerobic microorganisms have the ability to use nitrate as an alternate electron acceptor when oxygen is depleted, and for this reason are called facultative anaerobes. Concentrations of nitrate are often low in groundwater, because nitrate is frequently used as an electron acceptor by microorganisms and as a nitrogen source by plants and microorganisms. The end product of nitrate reduction is often nitrogen gas, but this cannot be used to indicate ongoing nitrate reduction because of its high background concentration in air and groundwater. Some microorganisms that use nitrate oxidise it only partially to nitrite and not completely to nitrogen gas, so the presence of nitrite can indicate ongoing nitrate reduction. In Figure 3-9, nitrate is plotted versus nitrite for groundwater from soil pipes in Laxemar.

The detection limit for nitrite is 0.0003 mg L^{-1} and for nitrate approximately 0.001 mg L^{-1} (different values are found in the SKB database, Sicada). Figure 3-9 shows that high nitrite values coincide with low nitrate values, and vice versa. Soil pipes SSM000230, SSM000226, and SSM000227 display extremely high nitrate values, up to 25 mg L^{-1} on some sampling occasions (see Figure 3-10). There is no obvious explanation for this in available P reports. Usually high nitrate values can be found in winter or early spring, when degradation and mineralisation are complete but before new vegetation growth has started. The samples containing high nitrate values represented in this dataset display no obvious correlation with season, but these samples were taken only from late autumn to spring; there are no data from July or August in the dataset. Presence of nitrite indicates that the system is oxygen free.

Nitrate and nitrite were not measured in groundwater from percussion-drilled boreholes.

The investigations in Olkiluoto, Finland, revealed that the greatest variation in the number of nitrate-reducing bacteria was between groundwater samples from different soil pipes and not between samples from different seasons /Pedersen 2008, Pedersen et al. 2008/.

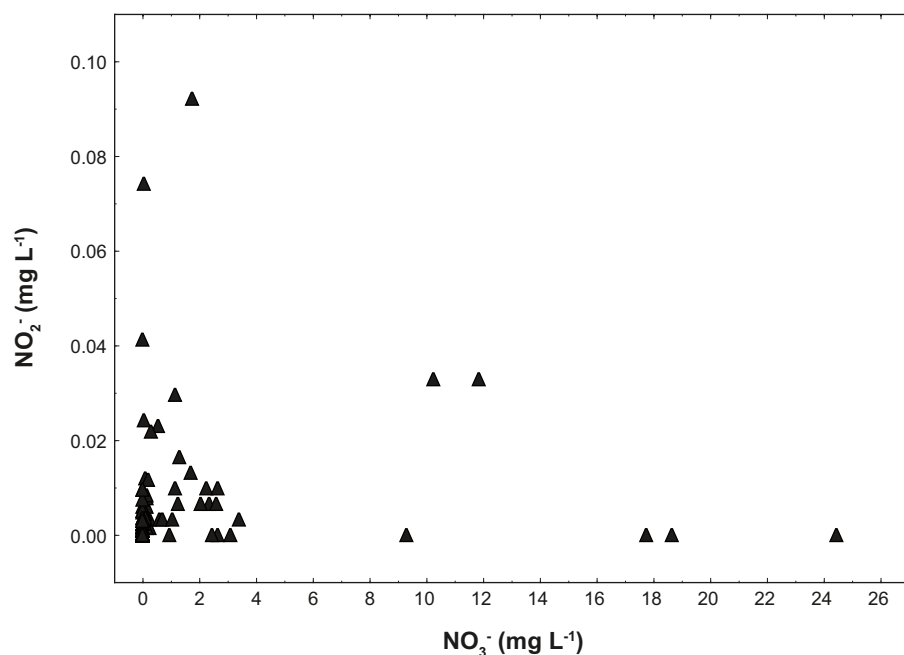


Figure 3-9. Nitrate versus nitrite in groundwater from soil pipes in Laxemar.

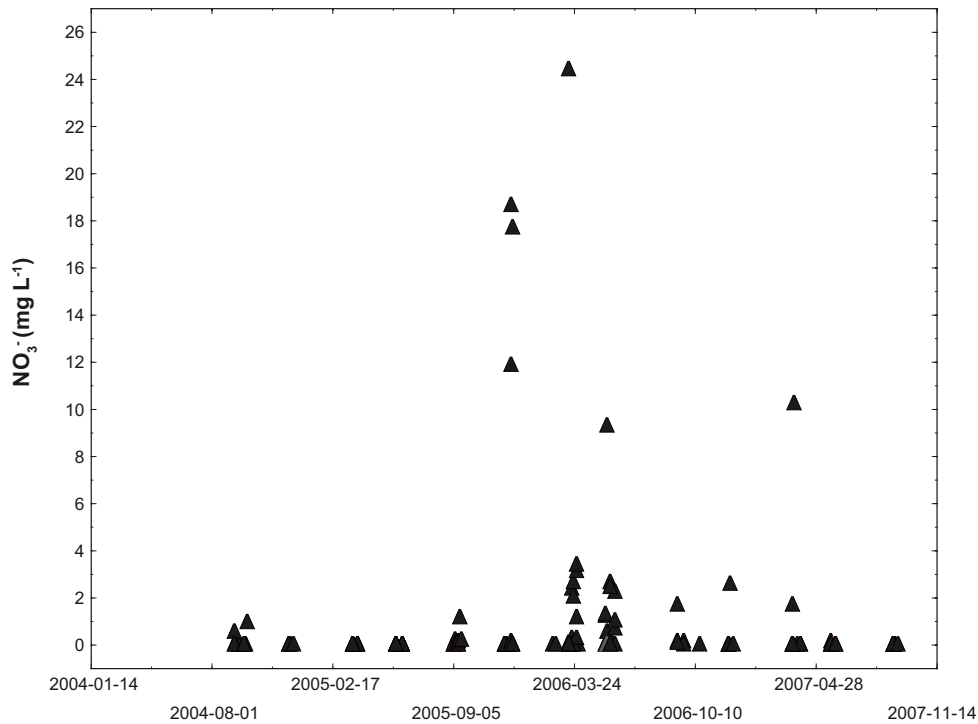


Figure 3-10. Nitrate versus sampling date in groundwater from soil pipes in Laxemar.

3.2.2 Iron and manganese reduction

The groups of anaerobic respiring bacteria that follow the nitrate-reducing bacteria after the nitrate is depleted are the iron-reducing and manganese-reducing bacteria. The activity of these groups of microorganisms is dependent on the presence of solid ferric iron or manganese phases, and results in elevated ferrous iron and manganese levels in the groundwater. Figure 3-11 shows the

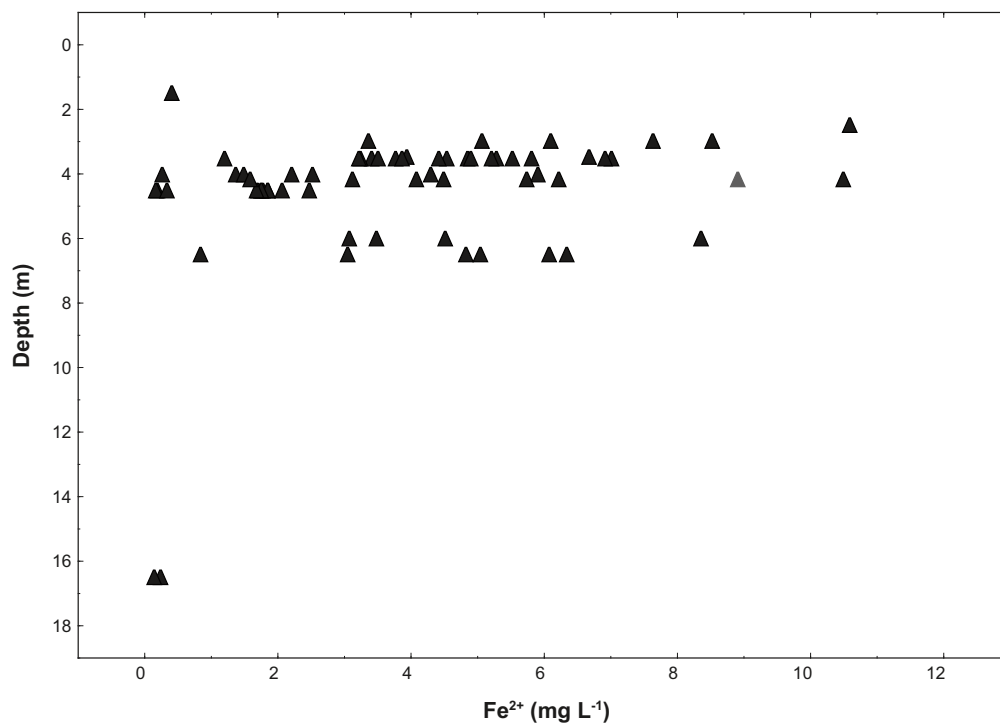


Figure 3-11. Ferrous iron versus depth in groundwater from soil pipes in Laxemar.

ferrous iron concentration versus depth and Figure 3-12 the manganese concentration versus depth for groundwater samples from soil pipes in Laxemar. There was no obvious trend in either the ferrous iron or manganese concentrations versus depth, except that concentrations of both species were low at 16.5 m. The concentrations of both iron and manganese were higher than the values measured in Forsmark, the highest iron value in Laxemar being 11 mg L^{-1} and the highest manganese value 1.5 mg L^{-1} . The concentrations seem to be more soil pipe related than depth related. This is obvious in Figure 3-13, where ferrous iron is plotted against manganese.

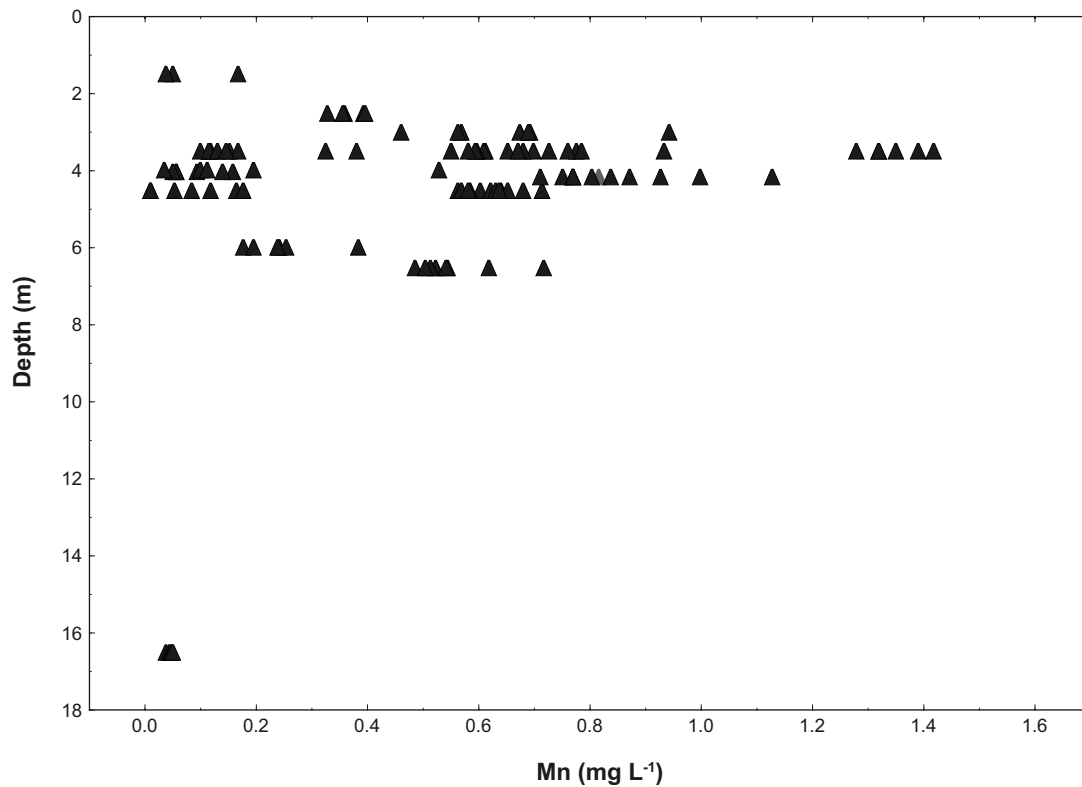


Figure 3-12. Manganese versus depth in groundwater from soil pipes in Laxemar.

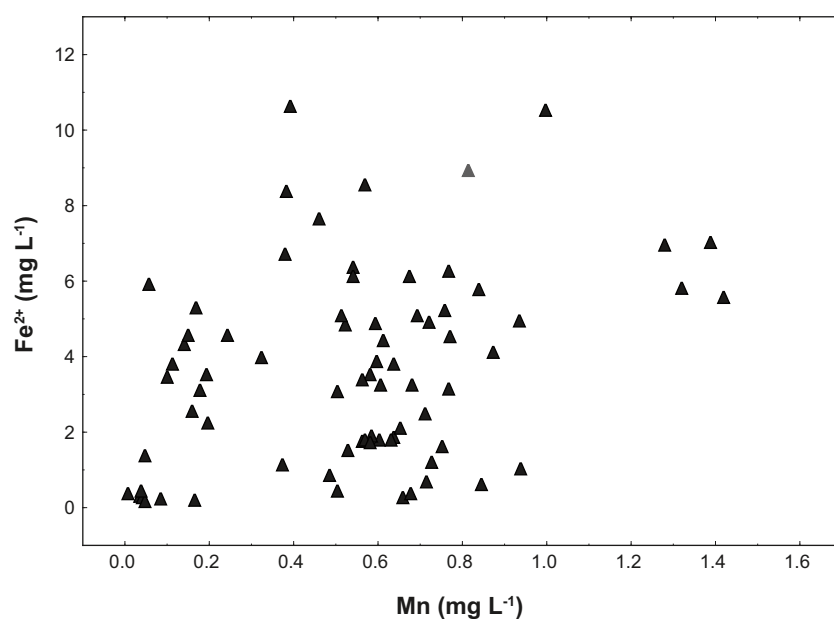


Figure 3-13. Iron versus manganese in groundwater from soil pipes in Laxemar.

It can be seen in this plot that both species were related in several of the soil pipes. If iron and manganese data from the selected soil pipes were plotted against each other, the soil pipe-specific pattern was even clearer (Figure 3-14). Here the data are distributed into three groups: groundwater in SSM000027 and SSM000030 have the lowest manganese values between 0.05 and 0.2 mg L⁻¹; groundwater from SSM000034 has high manganese concentrations, between 1.2 and 1.5 mg L⁻¹; while groundwaters from the other soil pipes have manganese values between 0.6 and 1.1 mg L⁻¹. Iron values varied in most of the soil pipes, with the largest variation evident in groundwater from SSM000042. To determine whether this variation was related to seasonal variations, the ferrous iron values were plotted against the sampling date (see Figure 3-15). This figure indicates no obvious relationship with the season, but the ferrous iron concentration did vary over the year.

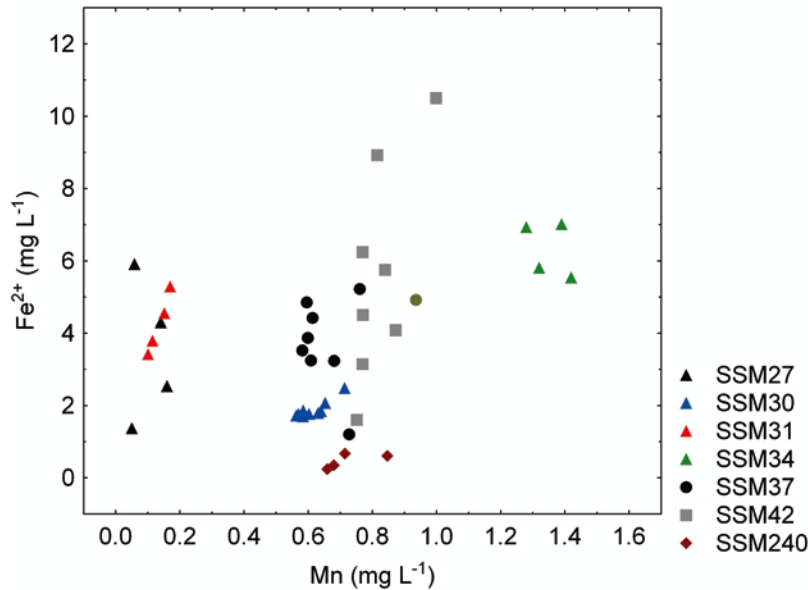


Figure 3-14. Iron versus manganese in groundwater from selected and identified soil pipes in Laxemar.

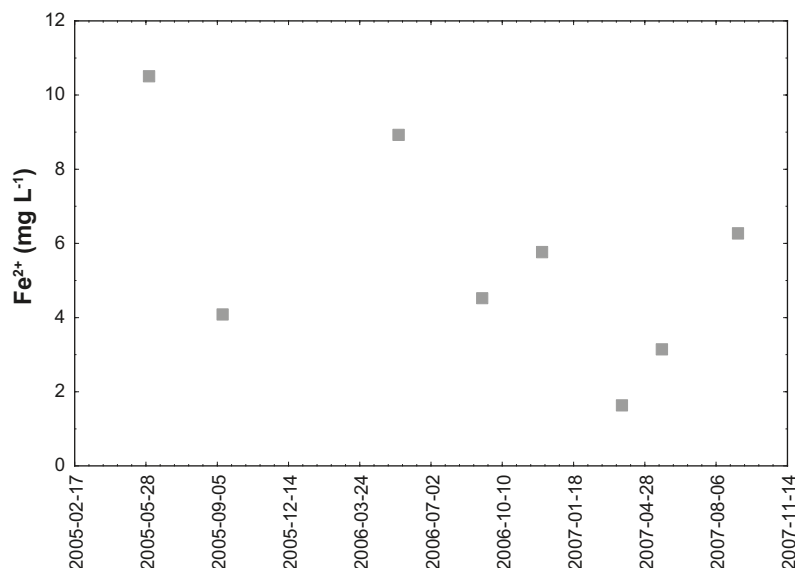


Figure 3-15. Ferrous iron versus sampling date for groundwater in soil pipe SSM000042 in Laxemar.

The microbial investigation of shallow groundwater in Olkiluoto, Finland, demonstrated that iron- and manganese-reducing bacteria decreased in number with depth, but that between depths of 300 and 350 m, the numbers increased to approximately 1,000 cells mL⁻¹. The concentrations of ferrous iron decreased with depth /Pedersen 2008/.

Samples from the investigation of deeper groundwater in Laxemar displayed high numbers of iron- and manganese-reducing bacteria at depths of 300–600 m (see Figure 3-16). There are few sampling points from more shallow parts in Laxemar. The sample from KLX08, at a depth of 150 m, contained 13 iron-reducing microbes per millilitre compared with around or above 100 mL⁻¹ at depths of 300–600 m.

High concentrations of ferrous iron indicate an anoxic environment because of the rapid reaction of ferrous iron and oxygen. The shallow groundwater system in Laxemar has high ferrous iron values for most of the year and is therefore probably anoxic most of the time.

3.2.3 Sulphate reduction

Sulphate-reducing bacteria often follow the iron and manganese reducers when oxidised iron and manganese phases are depleted. These two groups of microorganisms utilize the same carbon and energy sources, i.e. short-chained organic acids or hydrogen. The sulphate reducers, for example, are found in high-salinity environments that often include high sulphate concentrations. The sulphate concentrations in groundwater from soil pipes in Laxemar are shown in Figure 3-17.

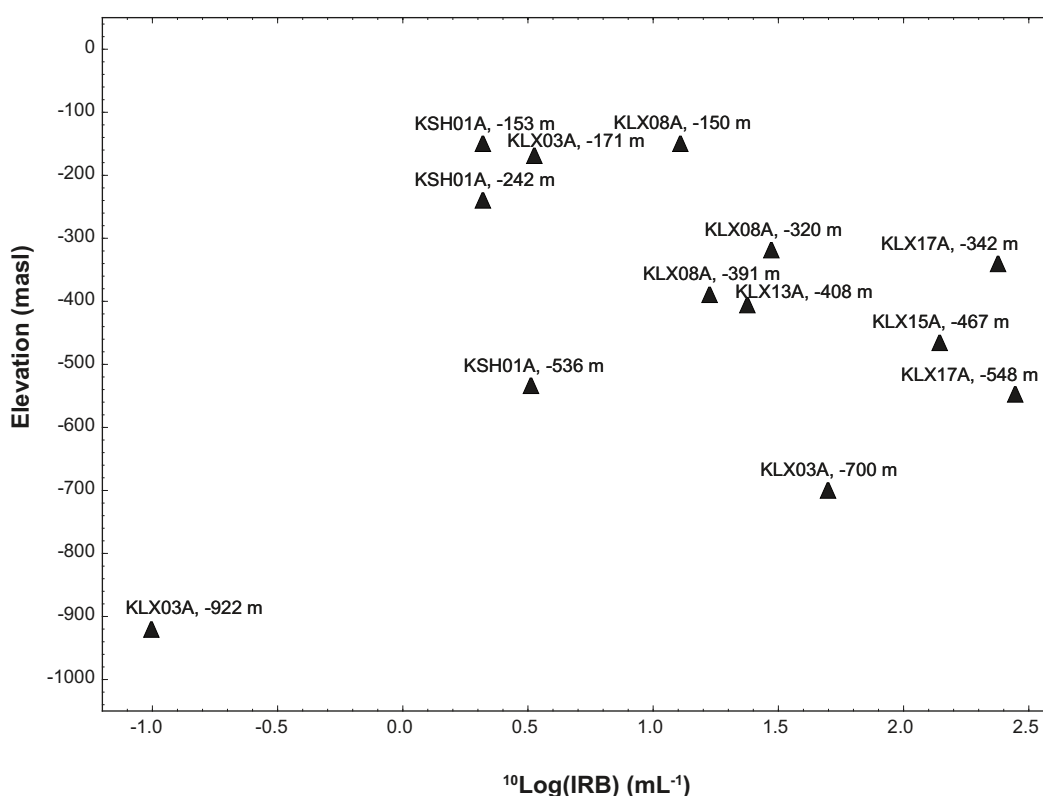


Figure 3-16. Most probable number (MPN) of iron-reducing bacteria (IRB) in groundwater from core-drilled boreholes in Laxemar.

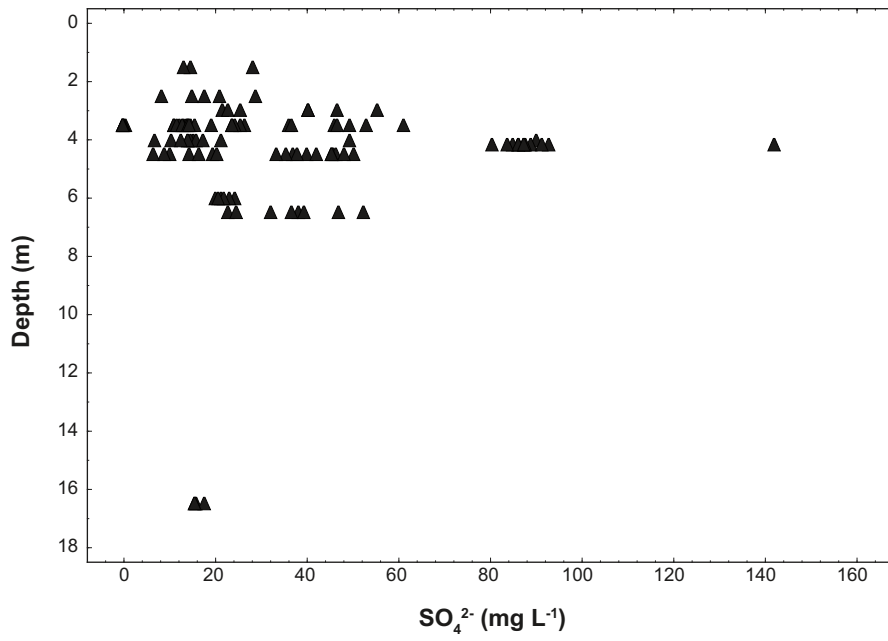


Figure 3-17. Sulphate versus depth in groundwater from soil pipes in Laxemar.

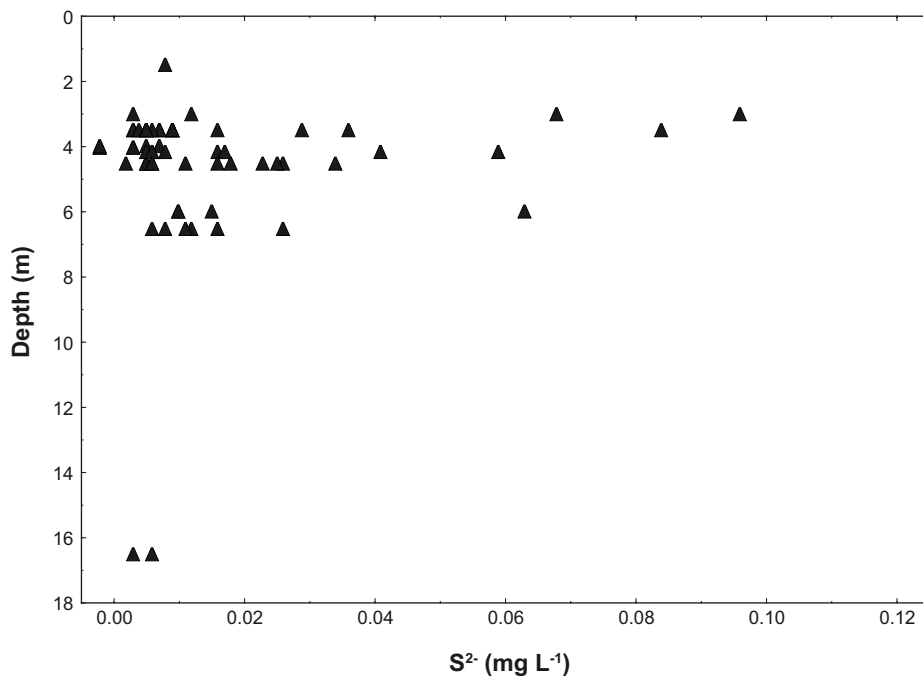


Figure 3-18. Sulphide versus depth in groundwater from soil pipes in Laxemar.

The concentration varied between close to zero and up to 150 mg L⁻¹ sulphate. Figure 3-18 shows the sulphide concentration versus depth in groundwater from soil pipes in Laxemar. Even though the concentrations were low, all below 1 mg L⁻¹, there were measurable amounts of sulphide. The presence of sulphide indicated that the groundwater conditions in this soil pipe were reducing. During the Forsmark and Laxemar site investigations and the studies in the Äspö tunnel, it was obvious that sulphide is a parameter that is sensitive to pumping /Hallbeck and Pedersen 2008/. Since the sulphide is produced by microorganisms, the pumping rate and volume can cause a “washout” of both microorganisms and sulphide. The sulphide concentrations will then be lower than in an undisturbed system. Depending on the number of sulphate-reducing bacteria, the volume of the sulphide-producing water, and substrate inflow, producing enough sulphide to reach the initial concentrations can take up to several months. Sulphide data should therefore be interpreted with caution, because the levels they indicate are probably lower than they would be under undisturbed conditions.

During sulphate reduction, fractionation of the sulphate isotopes takes place, leaving heavy sulphate isotopes in the groundwater. Figure 3-19 shows the $\delta^{34}\text{S}$ in sulphate, compared with that in a standard, Canon Diablo meteoritic troilite (CDT), versus sulphate in groundwater from soil pipes in Laxemar. The figure indicates large variation in the parameter, the $\delta^{34}\text{S}$ varying from -20 to +30. The marine sulphate signature is +20 while sedimentary sulphides can vary from -40 to +30. Some shallow groundwater has a marine signature of +20, while other shallow groundwater displays a signature affected by sulphate reduction and therefore heavier, up to +30. On the other hand, in some groundwater a light sulphate signature indicates that biogenic sulphides have oxidised.

It can be seen from Figure 3-20 that the $\delta^{34}\text{S}$ signature also varied in the selected and identified groundwater samples. In groundwater from soil pipe SSM000042, the sulphate signature was light, between 0 and -10. The signatures from soil pipes SSM000027 and SSM000031 were also lighter than that of marine sulphate but the concentrations were lower than in groundwater from SSM000042. The heaviest groundwater was found in SSM000240 and SSM000037 with $\delta^{34}\text{S}$ values between +6 and +21. In groundwater from SSM000037, the isotopic signature varied between +8 and +21 while the sulphate concentration varied between 23 and 61 mg L⁻¹. The variation in sulphate concentration could be a result of dilution from precipitation. If so, the chloride concentration would display the same pattern of variation. The chloride concentration did vary, but not in correlation with the sulphate (data not shown). Distinguishing between different processes would require thorough investigation involving measurements of both chemical and microbiological parameters.

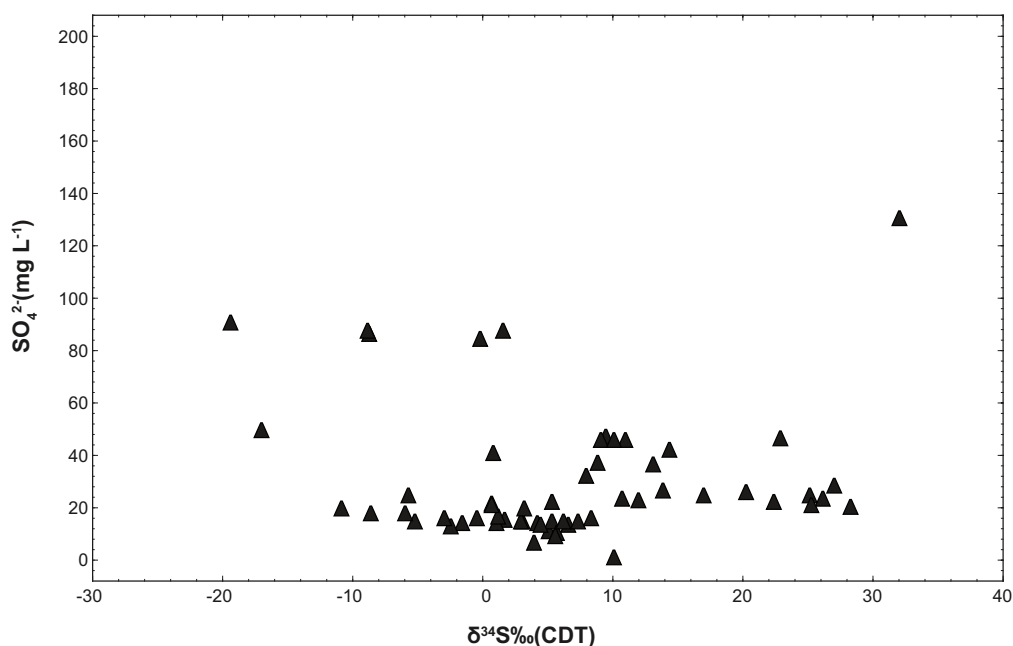


Figure 3-19. The stable ³⁴S isotope in sulphate versus sulphate in groundwater from soil pipes in Laxemar:

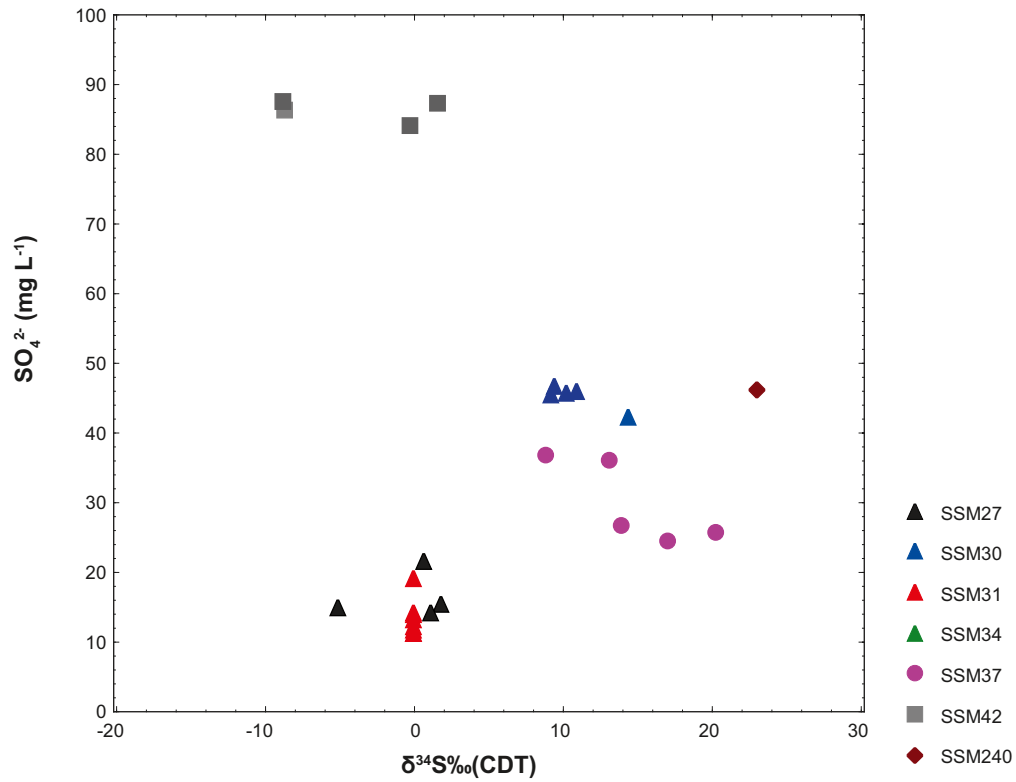


Figure 3-20. The stable $\delta^{34}\text{S}$ isotope in sulphate versus sulphate in groundwater from selected and identified soil pipes in Laxemar.

Figure 3-21 shows the sulphate concentrations in groundwater from percussion-drilled boreholes in Laxemar. The concentration varied from close to zero to 120 mg L^{-1} , except for one value of 260 mg L^{-1} found in groundwater from borehole HLX07. The isotopic sulphate signatures of groundwater from percussion boreholes were between +10 to +40 (Figure 3-22).

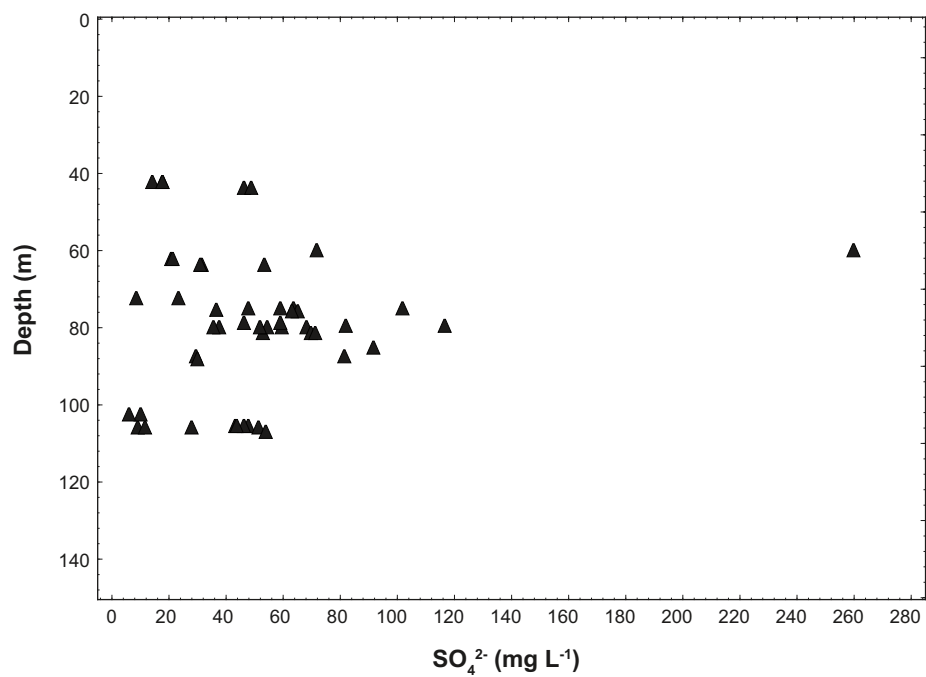


Figure 3-21. Sulphate versus depth in percussion- drilled boreholes in Laxemar.

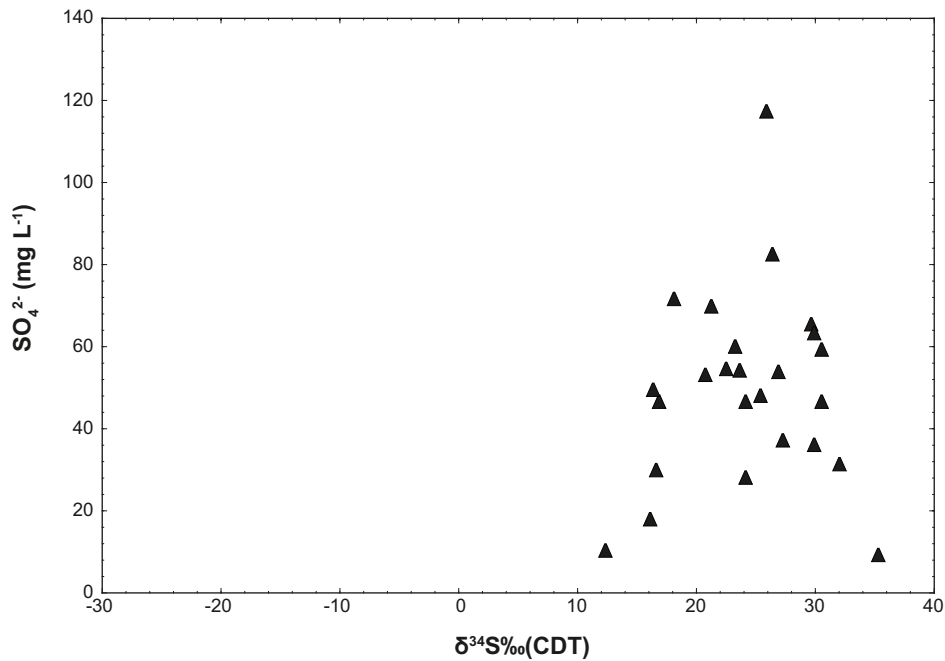


Figure 3-22. The stable $\delta^{34}\text{S}$ isotope in sulphate versus sulphate in groundwater from percussion-drilled boreholes in Laxemar.

In Olkiluoto, Finland, the numbers of sulphate-reducing bacteria range from zero to $1,000 \text{ mL}^{-1}$ in groundwater from soil pipes and shallow boreholes to depths of 25 m /Pedersen 2008/. This indicates that the presence of various microbial groups is more site specific, i.e. soil pipe or borehole related, than generally depth related. This is also evident in Figure 3-23, which displays the most probable number (MPN) of sulphate-reducing bacteria in groundwater from core-drilled boreholes in Laxemar. The highest number of sulphate reducers, $3.5 \times 10^3 \text{ mL}^{-1}$, was found in the borehole KLX17A at a depth of 548 m.

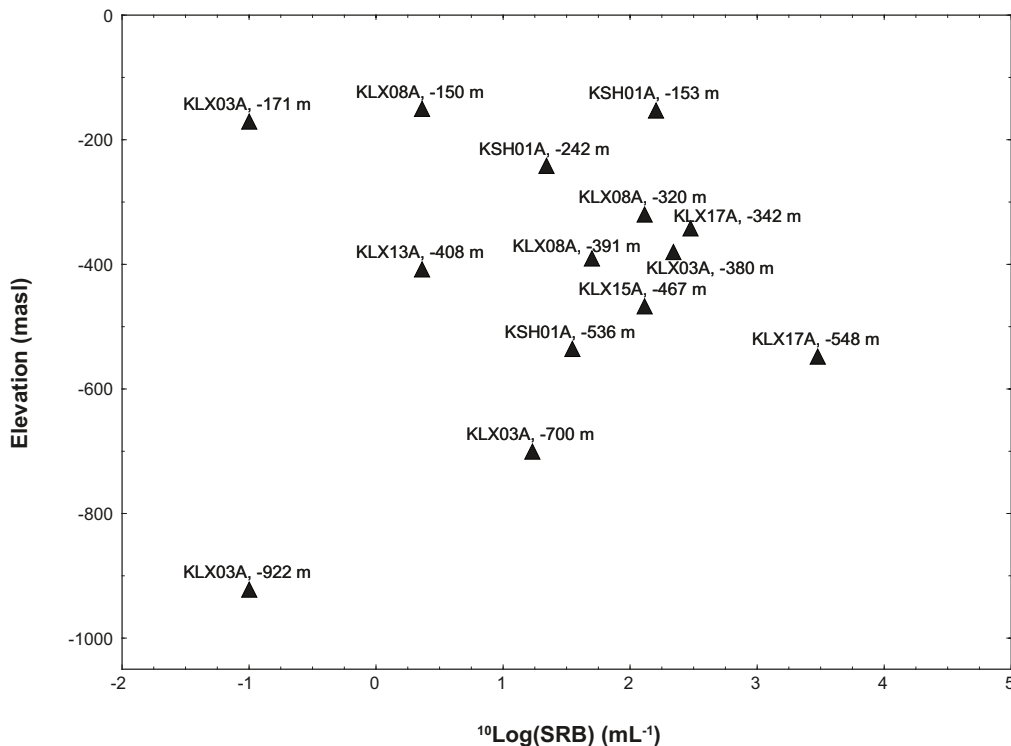


Figure 3-23. The most probable number of sulphate-reducing bacteria in groundwater from core-drilled boreholes in Laxemar.

3.3 Methanogenesis and acetogenesis

Methanogens and acetogens are important organisms that merit study in the context of oxygen consumption. Their metabolic products, methane and acetate, are readily consumed by aerobic microorganisms by means of oxygen reduction. Microorganisms that oxidise methane are called methanotrophic microorganisms and comprise a specialised group that can use methane and other one-carbon compounds, such as formaldehyde. Acetate is an intermediate molecule in most microbial metabolisms and is consumed rapidly by both aerobic and anaerobic microorganisms. Both methane and acetate are oxidised with oxygen but by different organism groups.

In an ecosystem in which all possible electron acceptors are present, for example, sea or lake sediment, the last microbial process that takes place is methanogenesis. In this process, fermentatively produced hydrogen is oxidised, with carbon dioxide as the electron acceptor and methane as the end product (see also Chapter 1). There is also another group of microorganisms, the acetogens, which use the same components as methanogens do but produce acetate instead of methane. In some environments (e.g. moss and bog environments), methanogenesis and fermentation are almost the only microbial processes that take place. *Sphagnum* moss produces large amounts of organic matter, but the environments in which it grows contain nearly no available electron acceptors with which aerobic or anaerobic microorganisms can degrade this organic material. The fermenting organisms produce short organic molecules together with hydrogen, which the methanogens use in their metabolism. Methane concentrations are therefore high in groundwater from mosses.

The groundwater of the Fennoscandian Shield contains relatively high amounts of hydrogen produced by various processes in the deep bedrock /Apps and Van de Kamp 1993/. Studies of microbiology in deep groundwater in Forsmark and Laxemar and in shallow and deep groundwater in Olkiluoto, Finland, have demonstrated that acetogens are present in all sampled groundwater. Methanogens, on the other hand, were also present in groundwater in Olkiluoto, Forsmark and Laxemar but in much lower numbers /Pedersen 2008/.

The investigation of shallow groundwater in Olkiluoto also examined the presence of methanotrophic bacteria, the distribution of which is shown in Figure 3-24 /Pedersen et al. 2008/. This indicates that methane oxidisers are part of the oxygen-consuming microbial population in the shallow groundwater system in Olkiluoto and possibly in Forsmark as well.

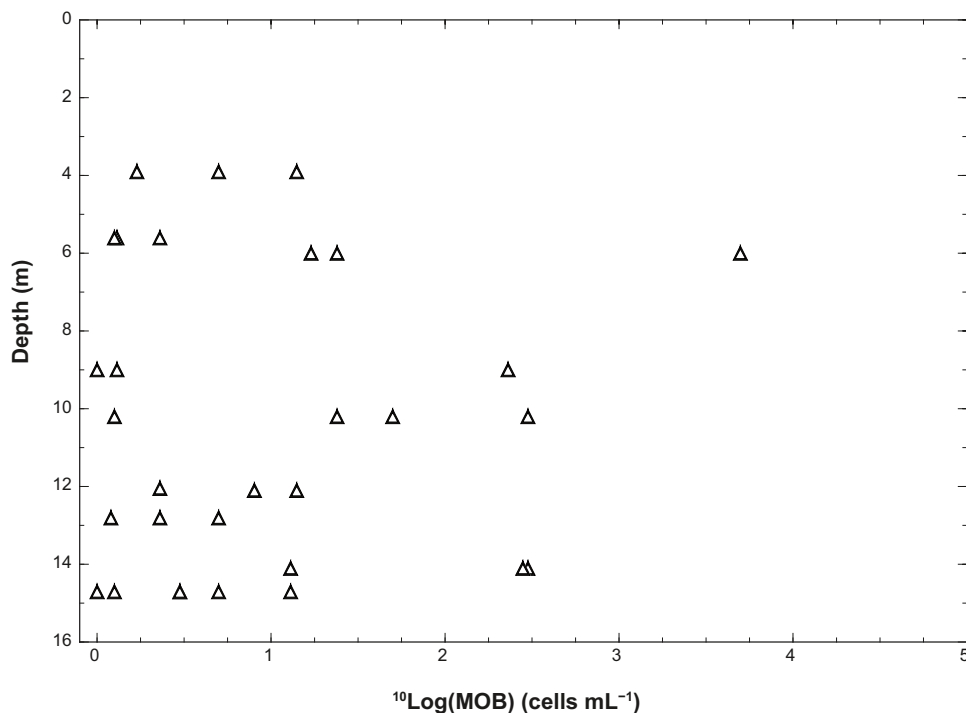


Figure 3-24. The distribution of methane-oxidising bacteria (MOB) versus depth in shallow Olkiluoto groundwater /from Pedersen et al. 2008/.

4 Conclusions

Investigating and evaluating the available data from shallow groundwater in Laxemar demonstrated that: 1) different soil pipes had individual chemical characteristics in terms of the chemical species related to particular microbial activities, and 2) microbial activity could not be linked to the class of soil pipe, i.e. recharge, discharge or intermittent.

Because no oxygen analyses or ORP measurements were made, it is difficult to draw any conclusions as to the presence of oxygen. Based on the presence of the reduced species ferrous iron and sulphide, however, it can be concluded that reduced conditions prevailed at most sampled depths. No obvious seasonal variation controlled by the DOC amounts could be found. Soil pipes located in similar biotopes displayed similar chemical signatures regarding DOC, while soil pipes in forested areas generally displayed evidence of high DOC levels.

This study aimed to answer the following questions (the proposed answers are given in italics):

- Is it possible to determine from area-specific chemistry and the results of the microbe investigation in Olkiluoto, Finland, what microbial processes are active in different parts of the Forsmark area?

Active microbial processes could not be conclusively identified, but the chemistry gave an indication that DOC had been consumed by aerobic microorganisms and that various anaerobic processes had taken place. Autotrophic anaerobic processes, such as methanogenesis or acetogenesis, may be ongoing, but the proper microbial data on which to base conclusive statements were not available.

- Which process or processes dominates/dominate the degradation of organic matter? Do different processes dominate in different areas and/or depths? Could the area be divided into sub-areas based on depth, recharge/discharge areas, or other factors?

With no available oxygen and ORP data, it was impossible to draw any conclusions regarding this matter. Microbial activity and processes seemed mostly to be linked to the specific characteristics of the studied boreholes and to the biotope in which they are situated. A direct relationship between microbial processes and depth and recharge/discharge area could not be identified.

- Is it possible to establish a depth limit for the presence of oxic groundwater? Does this limit vary over the course of the year or between years? Where is the oxygen consumed, if it is not transported down to the bedrock?

A depth limit for oxygen penetration could not be established from this dataset, because of the missing oxygen data and because there was only one soil pipe deeper than 6.5 m, SSM000224. The samples from the percussion-drilled boreholes started at a depth of 28.5 m; notably, they were missing for the 7–28.5 m depth interval, the interval where the depth limit for oxygen intrusion was established from data from Olkiluoto, Finland. Oxygen data were also missing for greater depths, since neither oxygen nor ORP was measured in percussion-drilled boreholes. The particular soil pipes and boreholes sampled varied from sampling occasion to sampling occasion, and the parameters measured on the different occasions varied as well. This variability in the analytical program created a very problematic discontinuity in the dataset, which severely hampered the interpretation and conceptual analysis. In addition, many of the percussion-drilled boreholes were not packed off, so the sampled sections were too extended to give useful information.

- Are we still missing some chemical parameters necessary for a full understanding of the microbiology in the oxic/anoxic zone in Laxemar?

*Precise and reproducible measurements, made over seasonal time scales, of the oxygen content and redox potential of groundwater from selected soil pipes and boreholes over the whole depth range, including the 10–25 m interval, could return a more accurate model of oxygen reduction with depth in Laxemar. It is crucial that the measurements cover the **whole depth range** from the ground surface to a depth of approximately 100 m, where the deep CHEMNET investigations start. All parameters related to microbial processes, such as oxygen, ferrous iron, sulphide, and DOC, are sensitive to sample disturbance. They must be measured with confidence and reproducibility on repeated sampling occasions over the course of a year to amass a complete dataset. In addition, gas data should be gathered on the same sampling occasions, preferably together with isotopic composition.*

There is generally no need for more soil pipes or percussion-drilled borehole. The existing ones can be used if sampling points are carefully chosen and a careful sampling procedure is devised. It may be necessary to get new soil pipes to cover the 7–28 m depth interval. To gather useful information from percussion-drilled boreholes, distinct fractures must be packed off.

It is strongly recommended that future sampling programs in shallow groundwaters should be hypothesis driven, rather than oriented towards the mass production of data that end up harbouring great uncertainties. Hypotheses can then be constructed concerning questions such as: What microbial processes are expected, and how can they be mapped with respect to rates and extent? What processes merit investigation with respect to repository safety (e.g. oxygen intrusion and biogeochemistry related to radionuclide migration processes)?

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