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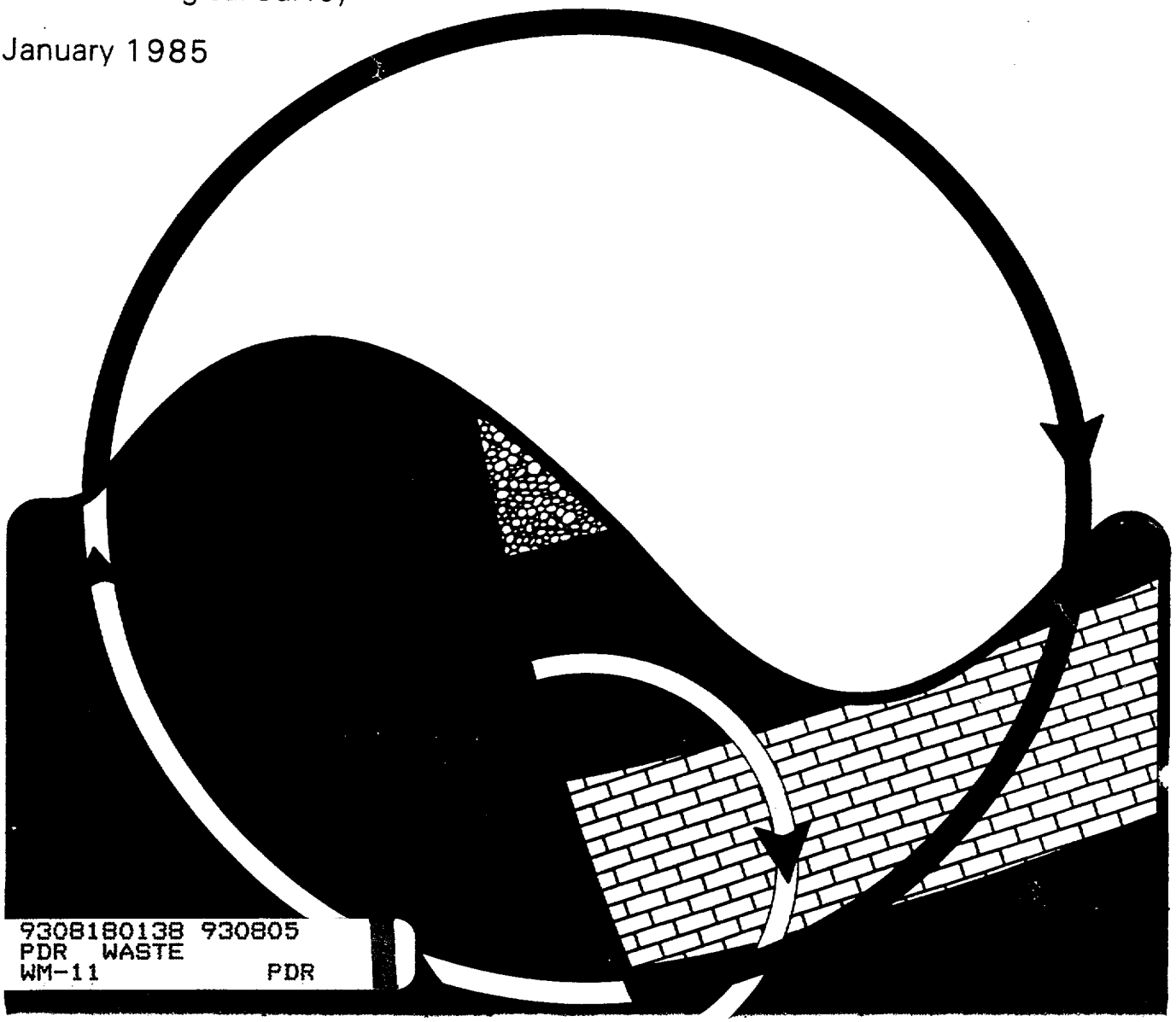

BRITISH GEOLOGICAL SURVEY

Natural Environment Research Council

Report FLPU 85-1

THE GEOMICROBIOLOGY OF EUROPEAN MINES RELEVANT TO RADIOACTIVE WASTE DISPOSAL

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January 1985

This report describes work carried out for the Department of the Environment (acting on behalf of the Secretaries of State for the Environment, Scotland and Wales) and the Commission of the European Communities as part of their research programmes into radioactive waste management. The results will be used in the formulation of Government policy, but at this stage they do not necessarily represent Government policy.
(DoE reference number DOE/RW/85.010 and contract number PECD 7/9/131 – 42/83)

KEYWORTH ENGLAND

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PREFACE

This report is the seventh in a series presenting results from the microbiological investigations of geological formations relevant to radioactive waste disposal. It presents the results of a survey of relevant underground sites in Europe and thus complements work previously done on sites in Britain.

Relevant BGS Reports

1. West, J. M., McKinley, I. G. and Christofi, N. 1982. Geomicrobiology and its relevance to nuclear waste disposal — An annotated bibliography. Rep. Inst. Geol. Sci., ENPU 82-8.

2. Christofi, N., West, J. M., Robbins, J. E. and McKinley, I. G. 1983. The geomicrobiology of the Harwell and Altnabreac boreholes. Rep. Inst. Geol. Sci., FLPU 83-4.

3. Philp, J. C., Christofi, N. and West, J. M. 1984. The geomicrobiology of calcium montmorillonite (Fuller's Earth). Rep. Brit. Geol. Survey FLPU 84-4.

4. Christofi, N., West, J. M., Philp, J. C. and Robbins, J. E. 1984. The geomicrobiology of used and disused mines in Britain. Rep. Brit. Geol. Survey FLPU 84-5.

5. West, J. M., Hooker, P. J. and McKinley, I. G. 1984. Geochemical constraints on the microbial contamination of a hypothetical UK deep geological repository. Rep. Brit. Geol. Survey FLPU 84-8.

6. West, J. M. and Arme, S. C. 1984. Geomicrobiology and its relevance to nuclear waste disposal — A further annotated bibliography Rep. Brit. Geol. Survey FLPU 84-9.

EXECUTIVE SUMMARY

Samples for microbiological analysis were taken from experimental mines, which are being used to investigate the technology for radioactive waste disposal, in Belgium, Federal Republic of Germany and Sweden. In total four mines were examined from the three countries. These were Mol (Belgium), in a Tertiary (Boom) clay deposit; Asse (FRG), within salt formations; Konrad (FRG), within iron-ore deposits and Stripa (Sweden), in granitic rocks. The samples were in the form of water from test boreholes, stagnant pools and flowing streams, and percolating water from gallery roofs. In the case of the Mol mine, clay cores were also analysed.

The physical and chemical conditions of the different samples were as diverse as their microbial content. Generally, the number and types of microorganisms increased as the salinity of the water samples decreased. Higher populations were present in the least saline waters of Stripa. Here exposed gallery stream-water contained aerobic and anaerobic bacteria as well as autotrophic and heterotrophic types. Appropriate conditions for the growth of autotrophic (phototrophic) organisms was illustrated by mats of cyanobacteria growing in shallow pools; light for photosynthesis was supplied by permanent artificial lighting strips. Chemosynthetic bacteria would also be active in this water given adequate electron acceptors and donors. Indeed autotrophic nitrifying and sulphur-oxidising bacteria (organisms implicated in the biodeterioration of concrete and metal structures) were demonstrated in the stream samples. Water from a deep borehole, drilled into the Stripa granite from within the 360 m level gallery (V1), contained a predominance of anaerobic heterotrophic bacteria. Activity monitored by gas chromatographic techniques showed that this population was organic carbon limited. A shallow borehole sample (M3) contained mainly aerobic heterotrophic bacteria which were also carbon limited.

Samples from the mines in the FRG and Belgium contained small or no populations of bacteria determined by cultural techniques. The Mol Boom clay itself, a potential backfill material, contained no determinable microbial content, and nutrients extracted from it were unable to support environmental isolates. For this reason it is considered a superior backfill material to Fuller's Earth (calcium montmorillonite). In samples where microorganisms were detected, the groundwaters were generally organic carbon deficient, limiting heterotrophic activity. In one of the Asse samples, however, a small heterotrophic activity was detected. In this sample an organic carbon content of >1100 mg per l was determined. Using infra-red spectrophotometry and liquid chromatography only traces of low molecular weight organic carbon was delineated. In this, and possibly other water samples, oligotrophic bacteria may be important. Such organisms, able to grow in nutrient-poor environments, may be

successful in deep groundwaters whose nutrients may be supplemented by the use of backfill/buffer materials. It is recommended that further research is carried out on oligotrophic bacteria.

north to the south. The iron deposit forms part of the Grifhorner Trough and is sedimentary oolitic ore of Jurassic age. It is located in this area at depths between 800 and 1200 m below ground level. Overlying the ore are Cretaceous formations eg claystones, limestones.

Shafts (1 and 2) were driven into the mine between 1960 - 1962 with levels at 1000 m bgl (3rd level), 1100 m bgl (4th level) and 1200 m bgl (5th level). Mining of the ore continued until 1976. After this date the mine was turned over to research use. (GSF final report 1980/81).

d) Stripa, Sweden

Stripa mine is a disused iron ore mine dating from the mid fifteenth century. It is situated between Kopparberg and Lindesberg, west of Stockholm. The target rock for investigations is a small intrusive body of granite of Precambrian age. It appears at the surface in a belt of older supracrustal rocks of which leptite, a strongly metamorphosed sedimentary rock, is the dominant rock type. It is within the leptite that the ore bodies are located. The mine is thus used in order to provide access to the granite at 350 m depth. Boreholes have been drilled into the granite and a complex of tunnels and rooms excavated for experimental purposes. For more details of the very wide-ranging Stripa project see Carlsson et al. (1983).

3. MATERIALS AND METHODS

a) Sampling

i) Mol

Sampling at Mol took place on October 11th 1983. Four 250 ml water samples were collected aseptically from the base of the main shaft where it was percolating from the overlying sand layers and the surface. The passage of water from the surface was estimated to take 48 hours (fluorescein dye test). One sample was inoculated on site for aerobic heterotrophs and sulphate reducing bacteria and the rest transported in a cool box (5°C) to the UK for chemical and microbiological analysis. Solid frozen clay samples from the new shaft being excavated were also collected using a pneumatic drill. An unfrozen clay sample was also removed for analysis and sent to Napier College in January 1984.

ii) Konrad and Asse

Sampling at Konrad and Asse was carried out on September 29th - 30th 1983. All samples were transported to the UK, no inoculations on-site were

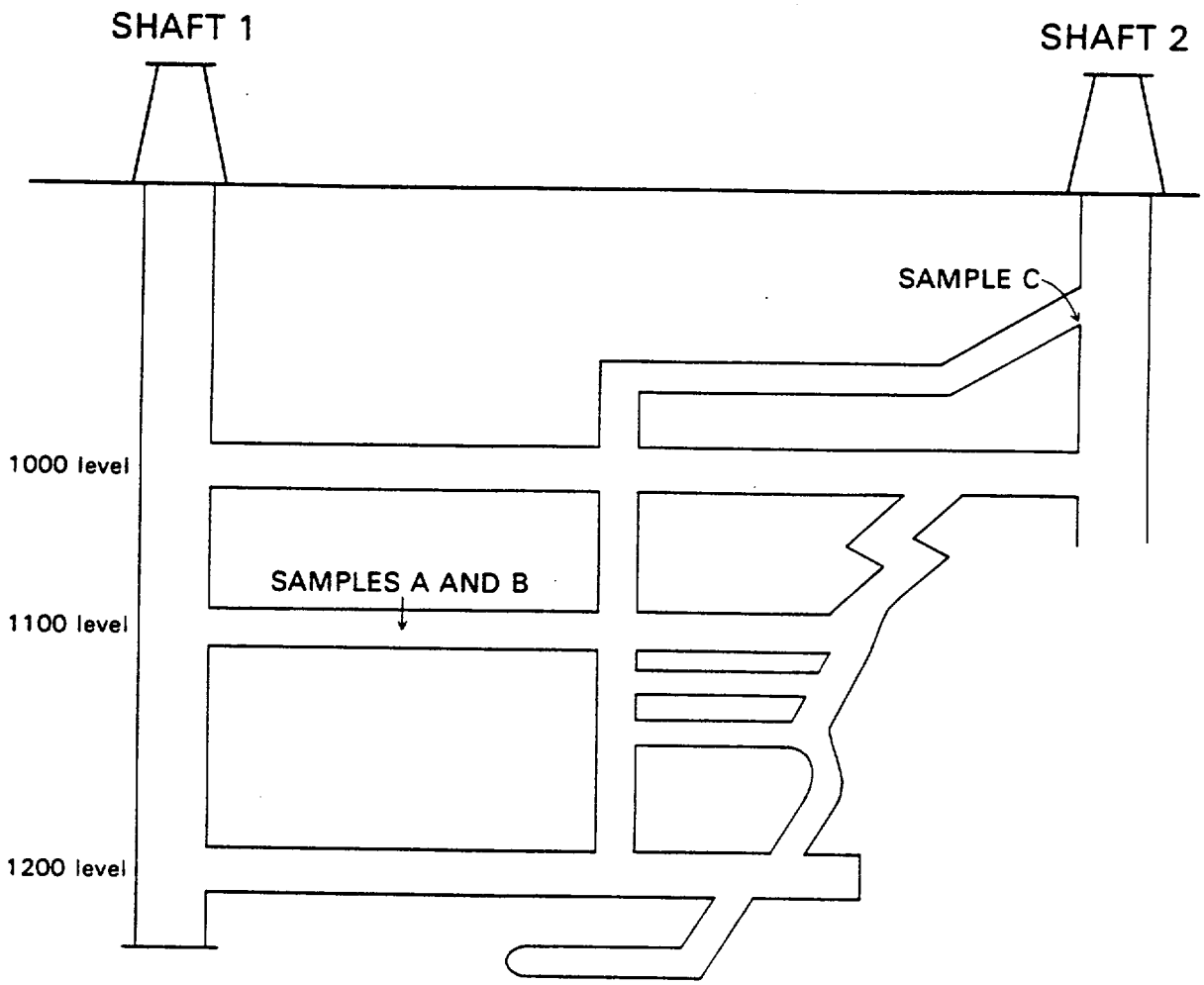


Figure 1 Diagram of Konrad mine showing sampling areas (not to scale).

attempted. Three areas were sampled at Konrad (A, B, C), indicated on Figure 1. Two 250 ml samples were abstracted from each and a further 500 ml was taken at site A for chemical analysis. At Asse two samples (D and E) totalling 500 ml were taken from two stagnant salt pools for microbial analysis. A further 500 ml were taken from E for chemical analysis.

iii) Stripa

Two experimental boreholes (M3 and VI) and a stream within a gallery were sampled for complete microbiological analyses during November 1983. 500 ml was abstracted from each site. Inoculations for total aerobic heterotrophic bacteria and sulphate reducing bacteria were done on site. For SRB, both freshwater and saline media were utilised.

In addition water samples were also removed from boreholes F2, V21/1 and V2/4 for total aerobic heterotrophic bacteria and SRB. Inoculations for these were again carried out on site.

Details of the sampled boreholes are given in Table 1.

Table 1 Details of sampled boreholes at Stripa

Borehole	
V1	Vertical 505 m deep Ø76 borehole; section 100-505 m. ¼" nylon flowline to site surface.
F2	Subhorizontal (20° down) Ø76, 250 m long, only single section over the entire holes.
V2	Vertical 822 m deep Ø56, sealed off in 4 sections; 382-423 m, 424-499 m, 500-561 m, 562-822 m. Flowline to surface.

All sections are artesian with varying flow rates between 10-200 ml min^{-1} (Norlander)

b) Chemical Analyses

These results are given in Table 2 and are compiled from chemical analyses carried out specifically for the sampling programme or from data already available. The sources of the information are indicated.

c) Microbiological Analyses

i) Microbial Enumeration/Enrichment

A standard routine established during the examination of borehole micro-organisms (Christofi et al., 1983) and Fuller's Earth (Philp et al., 1984) was used in the present study for examining rock-surface and water microbiota. Emphasis was not placed on the quantitative determination of microorganisms but rather on the identification and isolation of groups present.

ii) Microbial Activity Measurement

Heterotrophic microbial activity in the mine waters was assayed using C_{12} evolution studies. The method for this work is similar to denitrification using gas chromatographic techniques and is described in Christofi et al. (1984). Amended and unamended minewater samples were utilised in the activity tests. Amendments were usually carried out using yeast extract in order to determine whether the minewater samples were nutrient limited.

Table 2 Chemical analyses of minewaters sampled

Sample	MOL	KONRAD	ASSE	STRIPA	
		A	E	M3*	VI*
Depth (m)	190	1100	750	340	
pH	12.7	6	-	8.84	9.54
Eh (mv)	-	-	-	-120	-130
Chemical analysis (μg/ml-1)					
Na	350	64,400	84,450	46	270
Cl	2800	132,000	307,700	40	570
Ca	1700	12,600	31.4	14	152
Fe (total)	<0.1	47	4.84	0.006	<0.1
Mg	<0.05	2,640	95,060	0.23	0.6
NO_3	<1	<1	32	0.6	<0.1
HCO_3	7	<5	203	86	11
SiO_2	<2	6	2.8	11.8	13.7
T.O.C.	29	<50	1115	1.1	1.6
PO_4^{3-}	<0.5	<1.2	<0.1	<0.03	
NH_4	8	71	37	0.001	0.03
SO_4^{2-}	<18	820	25,980	4.9	110
K	140	300	140	0.59	2.6

* Analysed by SGU

d) Microbiology of the Mines

i) Mol, Belgium

Table 3 presents data for the Mol mine. Samples transported to the UK in an ice-box processed did not give evidence of the presence of microorganisms. Only aerobic heterotrophic bacteria and SRB were demonstrated on media inoculated on site. This signifies that transportation of these particular samples had some deleterious effect on the microorganisms. It is recommended that future microbiological work for the whole range of microorganisms be carried out on site.

However, synthetic laboratory media, whose ionic content differs from that of the environment in which they are established, are unable to detect microorganisms. The fact that organisms were detected in the field on media

Table 3 Geomicrobiology of the Mol (Boom Clay) mine samples

Sample	A Sept 1983	B Sept1983	C Jan 1984
Depth (m)	190	223	223
Sampling site description	Water percolating to 223 m level; water-bearing sand layers to a depth of 190 m	Frozen consolidated Tertiary clay (Boom Clay sample)	Defrosted consol-idated Tertiary clay sample
Microbial content			
Aerobic heterotrophs	1.2×10^3*	ND	ND
Anaerobic heterotrophs	ND	ND	ND
Aerobic sporeformers	ND	ND	ND
Anaerobic sporeformers	ND	ND	ND
Fungi	ND	ND	ND
Denitrifying bacteria	ND	ND	ND
MPND	ND	ND	ND
MOB	ND	ND	ND
SRB	+	+	ND
Thiobacilli	ND	ND	ND
Nitrifying Bacteria:-			
Autotrophic)-NH_4^+-oxidisers	ND	ND	ND
)-NO_2^--oxidisers	ND	ND	ND

All figures in colony forming units (CFU) ml^{-1}
* From inoculations on-site
ND Not detected
\+ Present

reconstructed with natural groundwater suggests that media formulations are critical in culturing microorganisms from diverse groundwater environments.

Heterothrophic activity (Figure 2) showed that microorganisms were present in sample A (not delineated by cultural techniques) and that these were stimulated by organic carbon additions indicating that the water sample was carbon-limited.

Belgian minewater sample (Sample A) heterotrophic activity.

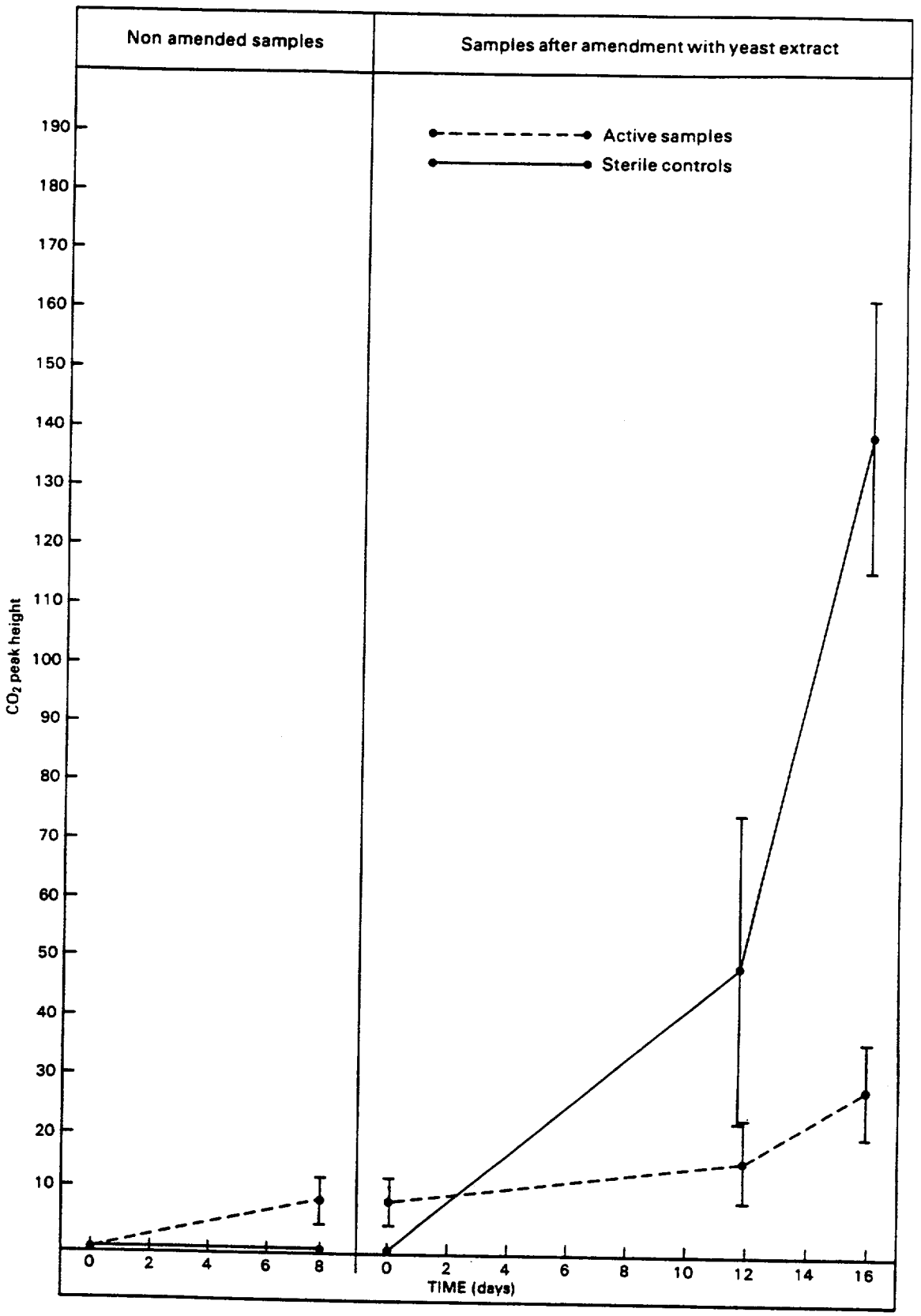


Figure 2 Belgian minewater sample (A) heterotrophic activity

Sulphate-reducing bacteria were demonstrated in the frozen clay sample. As this sample was removed from the surface of a shallow borehole, the presence of SRB could be due to contamination. This is a likely proposition as in unfrozen clay core-samples (recovered in January 1984) deeper into the clay formation, avoiding atmospheric contamination, SRB could not be detected. It therefore appears that the clay stratum may be naturally microbial-free but that water from overlying sand layers finding its way into the clay region could contaminate it. It remains to be shown whether such introduced microorganisms will be active within the clay deposits. Work is underway to determine whether the Boom-clay contains essential nutrients for the growth of introduced microorganisms. From carbon and nitrogen analysis of clay samples it seems that these elements are low and would probably not support a high microbial biomass (Carbon content = 1.25 %, Nitrogen content = 0.078 %) (percentage of dry clay).

ii) Mines in FRG (Konrad and Asse)

The techniques used during this study failed to isolate significant populations of microorganisms in the water samples of both Konrad and Asse (Table 4). Only Konrad samples B and C gave evidence of microorganisms using cultural techniques. In sample B 7.5×10^{-2} CFU (colony forming unit) per ml were isolated on nutrient-rich media (heterotrophic bacteria) incubated aerobically. It is possible that these heterotrophs were not active in the water samples as the aerobic spore count was not significantly different.

In Konrad sample C only small populations of anaerobic denitrifying bacteria and sulphate-reducing bacteria (SRB) were detected. These groups of organisms are implicated in corrosion of metal structures. The SRB from this sample have been isolated in pure culture and are being used in corrosion studies.

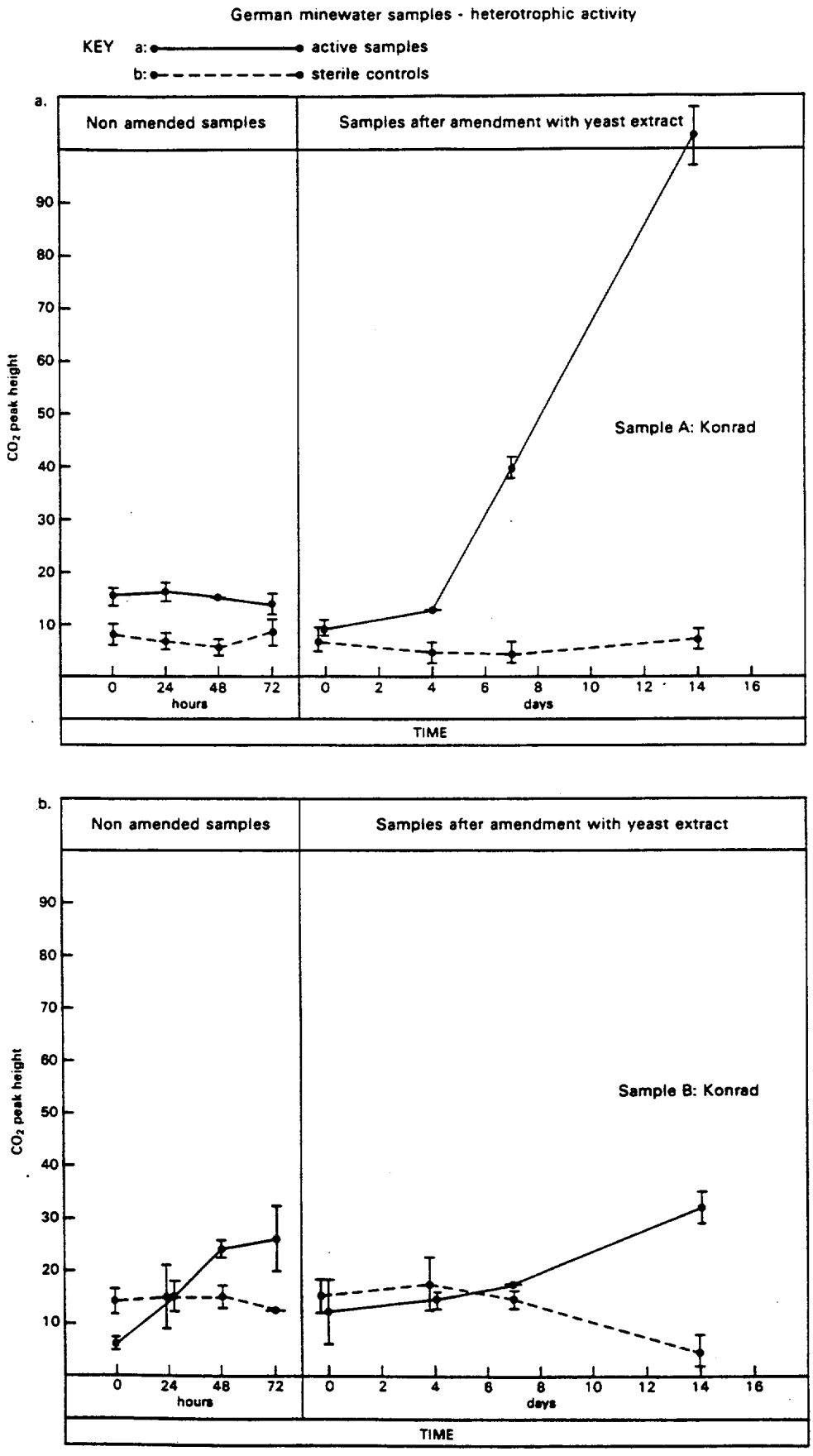
Although microorganisms could not be cultured in Konrad sample A and Asse samples D and E that does not preclude their presence. For example, heterotrophic bacteria may be present but are unable to grow in the nutrient-rich media utilised in the laboratory. These oligotrophic bacteria (Kuznetsov et al. 1979) are adapted to grow in the presence of low organic substances and it would have been more appropriate to attempt their isolation in unamended natural groundwater media as used for the UK mine, South Crofty (Christofi et al. 1984b). Unfortunately it was not possible to transport large volumes of mine water during these pilot studies in Europe, and there were no microbiological

Table 4 Microbiology of Konrad and Asse Mines

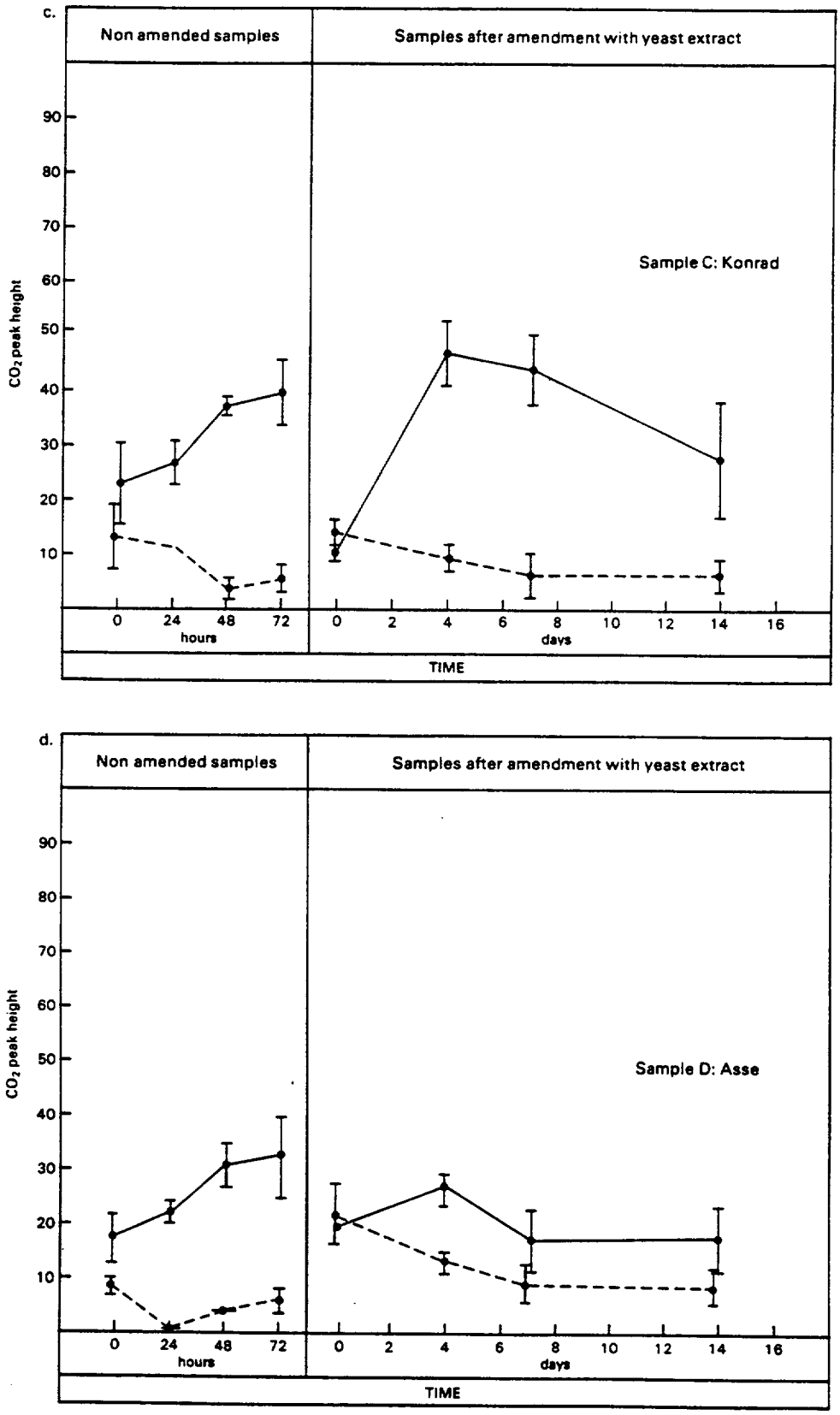
	KONRAD			ASSE	
Sample	A	B	C	D	E
Depth (m)	1100	1100	830	750	750
Sampling site description	Mixture of groundwater and water from drilling processes. (Level 4 - Pump No 6)	Water precolating through gallery room via salt stalactites. (slow drips - level 4)	Uncontaminated water above a pumping station. (Level 2 - Pump No 2)	Stagnant salt pool. 20 years old (Position 8)	Large stagnant salt pool. 50 years old (Position 13)
MICROBIAL CONTENT:					
Aerobic heterotrophs	ND	750	ND	ND	ND
Anaerobic heterotrophs	ND	ND	ND	ND	ND
Aerobic sporeformers	ND	550	ND	ND	ND
Anaerobic sporeformers	ND	ND	ND	ND	ND
Fungi	ND	ND	ND	ND	ND
Denitrifying bacteria	ND	ND	2.3[i]	ND	ND
MPNB[ii]	ND	440	ND	ND	ND
MOB[iii]	ND	ND	ND	ND	ND
SRB[iv]	ND	ND	+[v]	ND	ND
Thiobacilli	ND	ND	ND	ND	ND
Nitrifying bacteria					
) NH_4^+-oxidisers	ND	ND	ND	ND	ND
) NO_2^--oxidisers	ND	ND	ND	ND	ND

Counts CFU ml^{-1}

Most-Probable number and method used (see Christofi et al, 1983)
i Metallo-precipitating non-oxidising bacteria
ii Metallo-oxidising bacteria
v Sulphate-Reducing bacteria
Positive enrichment
ND Not detected



Figures 3a. b Heterotrophic activity in German mines (Konrad and Asse)



Figures 3c, d. Heterotrophic activity in German mines (Konrad and Asse).

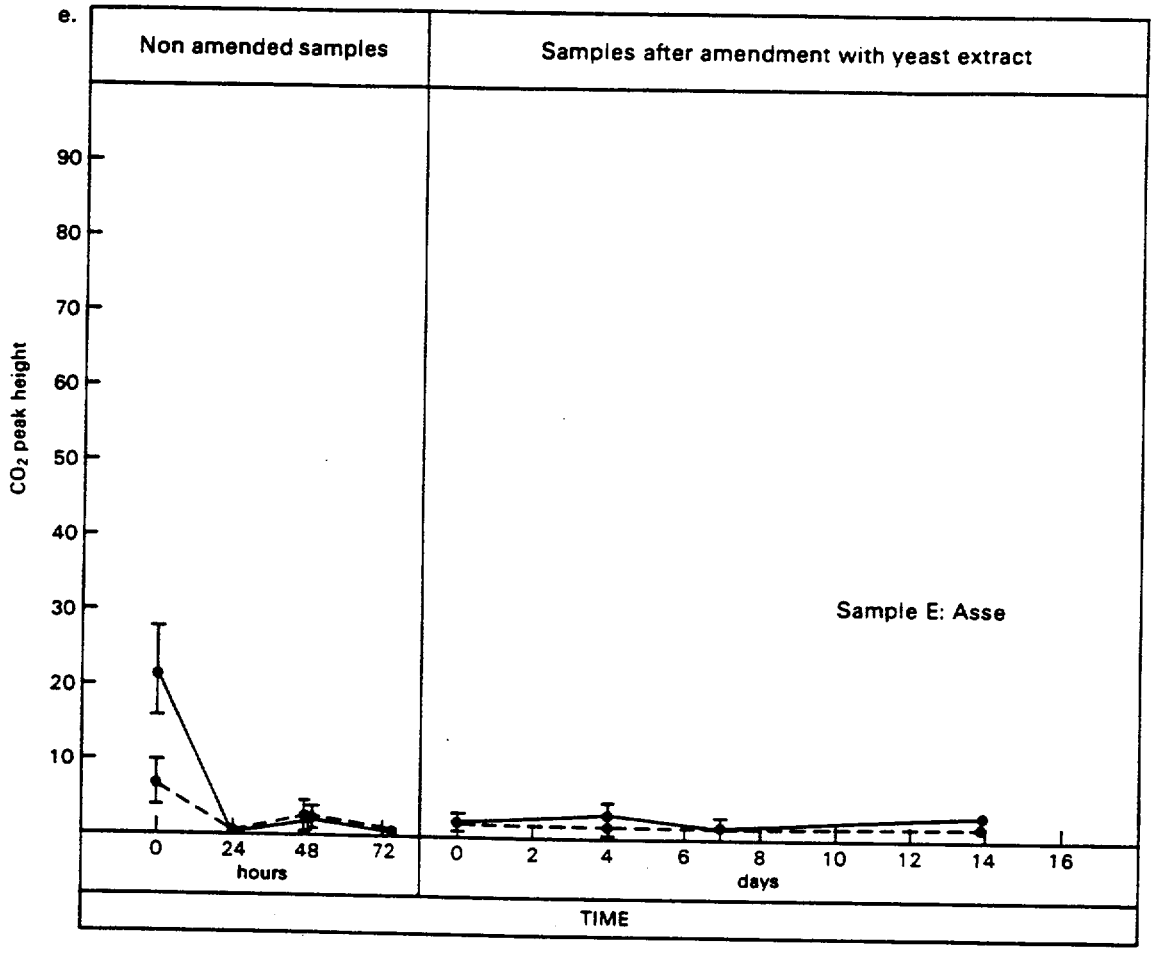


Figure 3e Heterotrophic activity in German mines (Asse).

facilities on site. Nevertheless, measurements whereby heterotrophic activity was assessed using carbon dioxide evolution gave some evidence on the presence of heterotrophic microorganisms capable of growth when supplied with low concentration of organic carbon. During these tests, natural groundwaters were incubated for 72h during which time CO_2 production was monitored using gas chromatography. After 72h organic carbon (yeast extract) was added at 0.033 % of the final concentration used in the cultural method for aerobic heterotrophs and changes in CO_2 monitored. The data are presented in Figures 3a - e.

It is seen that sample A contained biological activity; CO_2 concentrations in the headspace of the test systems increased with organic carbon additions. Heterotrophic activity could not be stimulated in samples D and E from Asse although a significant increase in CO_2 was monitored over a 72h incubation in unamended sample D. This may suggest that even the small additions of organic carbon to this sample inhibited the growth of microorganisms adapted to low

organic environments. Konrad sample C also gave evidence of heterotrophic activity without carbon additions. This activity increased after organic carbon amendments but decreased after approximately 4 days (Figure 3c). Overall it appears that in the various water samples from Asse and Konrad, microorganisms are present which are either adapted to growth in the presence of low organic carbon and are inhibited by organic carbon additions or microorganisms are present which are capable of increased metabolism with increased organic carbon input. The highly saline Asse sample E was probably sterile.

iii) Stripa, Sweden

Two experimental boreholes (M3 and V1) and a stream within a gallery were sampled for microbiological analysis. The shallow borehole M3 gave a relatively high count of aerobic, heterotrophic bacteria and smaller populations of anaerobic bacteria including denitrifying bacteria (Table 5). All other groups of microorganisms could not be detected.

The deeper groundwater sample from the V1 borehole was interesting in that the microorganisms demonstrated were predominantly anaerobic or facultative and included denitrifying bacteria and SRB. A population of 30 CFU per ml aerobic heterotrophs was counted and it is likely that the waters in the borehole are permanently anoxic. The waters in M3 on the other hand contain oxygen for the aerobes.

Highest populations of microorganisms were detected in the stream where the waters are constantly in contact with air. Aerobic autotrophic bacteria such as thiobacilli and nitrifiers, and "Iron bacteria" were demonstrated, whereas these were absent in the V1 sample considered anoxic. The stream also contained high populations of heterotrophic bacteria again including denitrifiers and SRB. Low spore counts suggest that adequate nutrients are present in the stream for the organisms to remain active. Indeed activity measurements (Figure 4) showed that heterotrophs are active, producing CO_2 in unamended stream water but that organic carbon addition stimulates a higher heterotrophic activity. This was also the case for borehole M3 and V1 waters.

A further interesting observation during the sampling at Stripa was the presence of photosynthetic microorganisms in shallow pools within a gallery of the mine. Photoautotrophic growth was possible due to the permanent artificial lighting in the gallery. Such an observation implies that chemoautotrophic

Table 5 Geomicrobiology of Stripa mine water samples

Samples**	Borehole M3	Borehole V1	STREAM ST
Depth (m)	330 level	360 level	360 level
Sampling site description	Borehole depth interval sampled 3 - 10 m	Vertical borehole from 360 m level sampled at depth between 100 - 505 m -90-95% of water derived from crushed zone at 450 - 500 m depth	Stream water running along gallery floor
Microbial content			
Aerobic heterotrophs	2.4×10^4	30	1.3×10^5
Anaerobic heterotrophs	1×10^2	1.2×10^5	1.3×10^5
Aerobic sporeformers	ND	ND	1×10^2
Anaerobic sporeformers	ND	ND	ND
Fungi	ND	ND	10^2*
Denitrifying bacteria	1.5×10^2	2.1×10^2	3.2×10^3
MPNB	ND	ND	+
MOB	ND	ND	+
SRB	ND	+	+
Thiobacilli	ND	ND	+
Nitrifying bacteria:-			
Autotrophic) NH_4^+-oxidisers	ND	ND	+
) NO_2^--oxidisers	ND	ND	+

Counts as CFU ml^{-1}

* Interference from spreading fungal colonies

** Groundwater samples from boreholes F2; V2/1 and V2/4 containe an estimated aerobic heterotrophic population of 10; 75 and 23 CFU ml^{-1} respectively, V2/1 and V2/4 contained SRB.

ND Not detected

\+ Present

bacteria deriving their energy from the oxidation of reduced inorganic compounds such as sulphide, ammonium etc. and organic carbon through CO_2 fixation could be successful in these mine waters provided oxygen was supplied. Carbon dioxide (as HCO_3) concentration was high in both M3 and V1 borehole waters (Table 2).

4. DISCUSSION

During this microbiological survey emphasis was placed on the detection of microorganisms present in contaminated and 'uncontaminated' groundwaters. In the case of the Mol experimental mine, clay samples were also analysed. The data show that microorganisms are present in all relevant mines.

The samples ranged from highly saline Asse and Konrad minewaters (chloride content $>1.32 \times 10^5$ mg per 1) through to Mol water (chloride content 2.8×10^3 mg per 1) to Stripa borehole waters (chloride content $<5.7 \times 10^2$ mg per 1). It is seen that populations and diversity of microorganisms are highest in the least saline groundwaters from Stripa. The Mol water sample (a mixture of water from the overlying sandstone and seepage water from ground level) had a measured pH of 12.7 (Table 2). Few organisms are able to proliferate in such an alkaline environment (Langworthy, 1978). These include the strict aerobic chemolithotrophic/mixotrophic nitrifying bacteria e.g. Nitrosomonas and Nitrobacter (Meek and Lipman, 1922). It is interesting to speculate that the inability to detect heterotrophic bacteria in this water sample, when analyses were carried out in the UK, may have been due to the hostile (high pH) environment, having a deleterious effect on microorganisms introduced to these waters from the surface. This assumes that the water percolating into the 223 m chamber level contains introduced and not native (sandstone formation) microorganisms. It is still possible that the media utilised were not appropriate for the isolation of alkalinophilic microorganisms. The SRB demonstrated in the initial Mol clay sample are considered contaminants; firstly the SRB from this sample were isolated in freshwater media and secondly in deeper clay cores SRB were not detected.

In the three water samples from the Konrad mine, only samples B and C were shown to contain microorganisms using cultural techniques. A small population of aerobic heterotrophic bacteria was present in sample B (750 CFU per ml). Pasteurisation of this water sample and culturing showed a population of sporeformers not significantly different from the total aerobic heterotrophic count, indicating that the total heterotrophic bacteria were present as spores and are therefore inactive. Such an observation indicates the presence of aerobic sporeformers of the genus Bacillus and indeed the demonstration of metallo-precipitating non-oxidising bacteria (MPNB; Table 4), a number of which have been shown to be Bacillus sp. (Clark et al. 1967), suggests this.

In the Konrad sample C, only facultative (denitrifying bacteria) and strict anaerobes (SRB) were demonstrated. Again these are likely to be contaminants as they grew only in freshwater media.

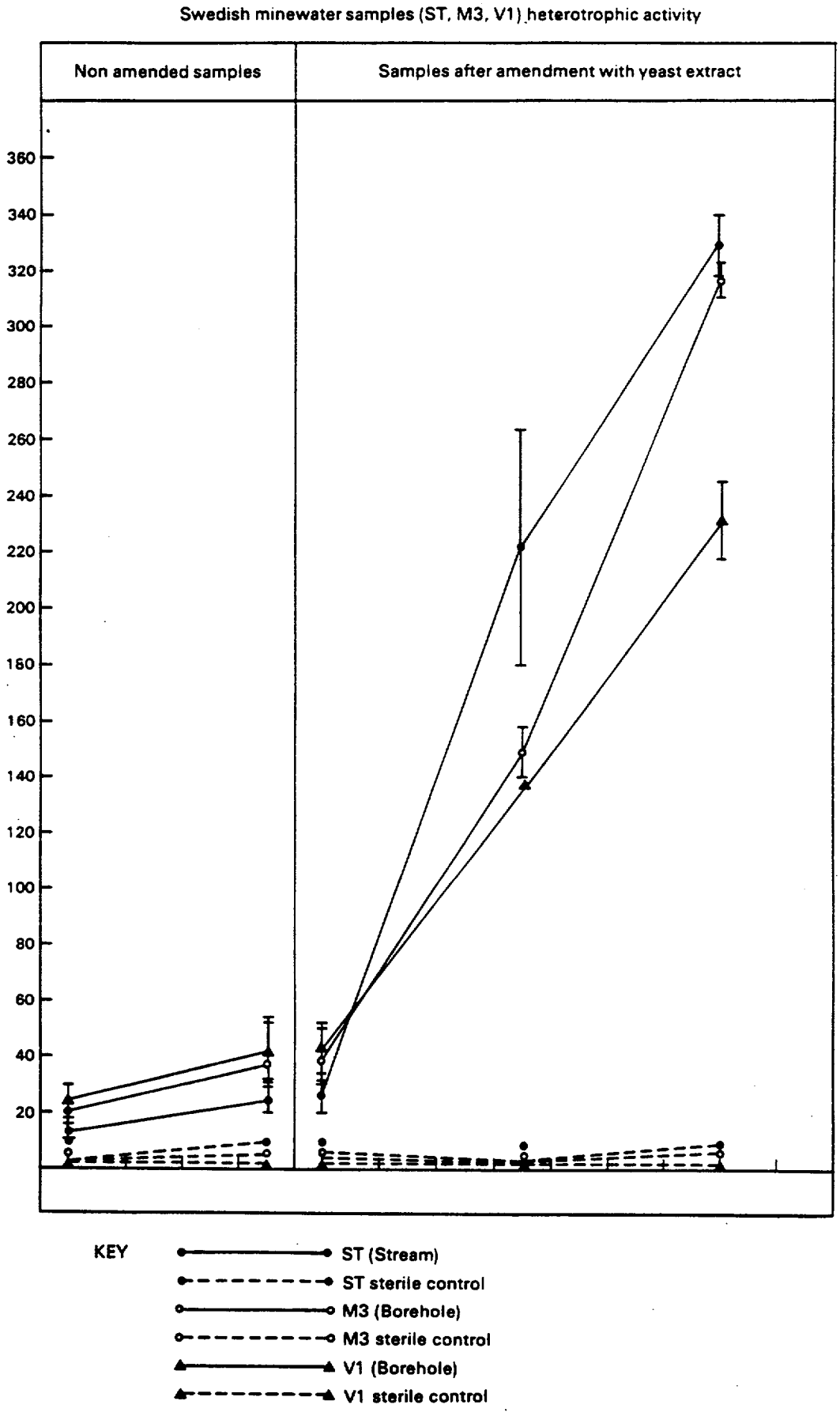


Figure 4 Heterotrophic activity in Swedish samples.

In the highly saline Asse waters (Samples D and E) no microorganisms were cultured both on freshwater and saline (2.5 % NaCl) media. Although microorganisms were not cultured, a low microbial activity was evident in sample D possibly due to an exacting group of microorganisms. Such organisms may be oligotrophic types (Kuznetsov et al., 1979). Four groups of oligotrophic bacteria have been described by Kuznetsov and coworkers (1979). These are (a) bacteria which grow only on first cultivation on sterile media but cannot be recultivated; (b) bacteria which on first cultivation grow on nutrient-poor media and not on rich media, but can be recultivated on rich media; (c) bacteria which can be isolated and cultivated on special poor media, and (d) bacteria which can only be detected under electron microscopy and cannot be grown under laboratory conditions.

The most diverse group of microorganisms in terms of numbers and types were demonstrated in the Stripa 360 m stream. Here autotrophic and heterotrophic forms as well as aerobes and anaerobes were cultured. Without doubt microorganisms in such a habitat will contaminate boreholes drilled within the galleries at Stripa. In the case of the shallow borehole, sample M3, aeration conditions were such that aerobic microorganisms predominated. The presence of oxygen, however, was not indicated by the measured Eh of -120 mV, an observation which is difficult to explain accepting that Eh measurements are a good indication of the aeration status of such waters. The total anaerobic population possibly consisted of facultative organisms as the population was not significantly different from the number of denitrifying bacteria (organisms which are basically aerobes but can grow in oxygen-free environments in the presence of nitrate). Nitrate levels in M3 were higher than in other groundwaters examined (600 ug per 1). In terms of inorganic nitrogen such a system is considered eutrophic (nutrient rich; see Vollenweider, 1968). The waters of the deeper borehole, V1, contained a predominantly anaerobic population as high as that delineated in the stream sample. Such waters are likely to be oxygen-free hence the selection of anaerobes. As these boreholes were sealed and had been sealed for a number of years, the relatively high microbial content is probably maintained by available nutrients in the groundwater.

Microorganisms in natural environments require an adequate supply of nutrients for growth. It is unlikely that microorganisms will grow optimally in natural environments. Optimal growth appears only to be achieved in laboratory culture under controlled physico-chemical conditions (Harder and Dijkhuizen, 1983). In nutrient-poor environments such as deep groundwaters, we cannot discount microbial growth because microorganisms can adapt to such environments. The chemistry data (Table 2) reveal that in the various groundwaters the total organic carbon (TOC) content varies between 1.1 - 1115 mg per 1. The rate of active transport of organic substances into

cells which requires energy is stabilised at organic concentrations of 0.5 mg per 1 and relies on the presence of phosphates in the medium (see Kuznetsov et al., 1979). Although the lowest TOC concentration of the various groundwater examined is higher than this value, one must appreciate that the quality and not the quantity of the organic substance is important. At present the quality of this organic carbon is unknown.

In addition phosphate levels in the groundwaters are low and likely to affect microbial growth even though microorganisms cope with phosphate limitation by diverting the available phosphate to essential processes (Harder and Dijkhuizen, 1983). Crystalline rocks, however, are known to contain phosphates (Mayfield and Barker, 1982).

In the growth experiments carried out using the abstracted groundwaters measurable heterotrophic activity only occured after organic carbon additions, particularly yeast extract. This implies that the microorganisms in these waters require unidentified substances present in yeast extract. There was however a small heterotrophic activity in unamended groundwater from an Asse pool which may have been due to oligotrophic bacteria. These organisms need further investigation utilising techniques more sensitive than gas chromatographic ones, perhaps using radioisotopes. Because of the difficulty in culturing oligotrophic bacteria, their demonstration may be possible by immersing glass slides or EM grids into relevant groundwater systems and examining their colonisation by light and electron microscopy.

In a sealed repository nutrient sources will include those in groundwater and those present in backfill/buffer materials. As deep groundwaters move slowly and are nutrient-poor, the activity of microorganisms may be controlled by nutrients in backfill materials such as clays. In Fuller's Earth (calcium montmorillonite) that is freshly excavated from surface environments there are high populations of microorganisms (Philp et al., 1984). These populations are maintained in Fuller's Earth by endogenous nutrients as well as exogenous inputs from nutrient-rich surface waters. The use of such clay material to backfill and seal deep repositories will introduce nutrients. A highly compacted clay will restrict water movement and any additional nutrient inputs. The clay environment will then act as a batch system which will ultimately become nutrient limiting. Aerobic organisms may be active until the dissolved oxygen has been utilised. The population succeeding these (or indeed occuring at the same time in oxygen-free microzones) will be facultative anaerobes utilising nitrate as an electron acceptor. A reduction in Eh and nitrate will than favour strict anaerobic organisms such as sulphate-reducing bacteria (using SO_4 as an electron acceptor) and methanogens (using CO_2). Such organisms are heterotrophic and require

an adequate source of organic carbon. Methanogens, however, can grow autotrophically (see Thauer et al., 1977). It may be that activity of corrosion important organisms such as SRB will not be important as readily assimilative organic substances will be scarce. Any such activity will be reliant on lysis products of previous populations or mineralisation of humic or other organic material including microbial biomass (see Burns, 1982). Mineralisation under anaerobic conditions is lower than under aerobic conditions (Alexander, 1973). The ability of anaerobic mineralisation to support the growth of strict anaerobic organisms, and the extent of their growth and effects on waste isolation materials are unknown.

'Aerobic' conditions may arise due to release of radionuclides and radiolysis of water. Depending on the concentration of radiolysis products such as superoxide and hydrogen peroxide, aerobic organisms may be able to counteract the toxic effects of these radicals, with enzymes such as superoxide dismutase and catalase, by forming O_2. Success of aerobes under such conditions may enhance mineralisation of organic materials and the formation of labile growth substances, and also enhance organic carbon production from CO_2. The latter is a function of chemolithotrophic microorganisms such as Thiobacillus and Nitrosomonas species demonstrated in the Stripa mine. This situation may then enhance heterotrophic activity (Christofi et al., 1984a). Indeed the growth of thiobacilli which produce sulphuric acid may lead to biodeterioration of concrete and steel, potential waste isolation materials.

Boom clay from the Mol experimental mine is another potential backfill material. The use of this native clay to seal waste emplaced in a repository will restrict microbial activity. We have tentatively shown that Boom clay samples recovered aseptically are microbe-free. Organisms introduced to the clay repository will have to rely on endogenous nutrients in the clay. Total carbon and nitrogen analysis of Boom clay shows that as a percentage of dry weight 1.25% is carbon and 0.078% is nitrogen. Nutrients extracted from Boom clay by autoclaving volumes of clay with distilled water were unable to support environmental isolates such as the aerobic/facultative anaerobe Pseudomanas fluorescens and the strict anaerobic sulphate-reducer of the genus Desulphovibrio (unpublished data). It appears that the Mol Boom clay may be an ideal backfill material.

Other interesting scenarios related to clay are considered. Firstly, if dry clay is used to backfill a waste chamber, the rewetting of this clay may release soluble carbon compounds bound to the clay surface and may enhance microbial activity (cf. nutrient release from clay soils; Lynch, 1982). Lynch (1982) also showed that irradiation of clay soils had a similar effect. Secondly if wet clay is used, heat generated by the waste canister may dry and crack it increasing the movement of

water to and from the waste canister. Problems with clay dessication are considered by Daniel (1984). In a fissured or cracked crystalline rock formation, therefore, increased groundwater movement bringing nutrients to the waste-form environment, may enhance the effect of microorganisms on isolation materials. The concentration of nutrients and hence their effect on the microorganisms will depend on the groundwater system (which includes depth and formation) utilised.

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POTENTIAL FOR THE RAPID TRANSPORT OF PLUTONIUM IN GROUNDWATER AS DEMONSTRATED BY CORE COLUMN STUDIES

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INTRODUCTION

The mobility of radionuclides in groundwater flow systems can be significantly altered by processes such as complexation, sorption on particulates, or hydrolysis, precipitation and the formation of colloids. Such processes provide potentially significant pathways for transport of radionuclides in the biosphere. Previous soil column studies[1] demonstrated that radiocesium could be transported by particulates and that the process was likely mediated by micro-organisms. That observation provided a plausible mechanism to explain the anomalously rapid transport of small amounts of cesium-137 released from glass blocks buried below the water table at the Chalk River Nuclear Laboratories[2].

The behaviour of plutonium in soils is apparently covered largely by processes which influence the chemistry of the Pu (IV) ion. Wildung et al[3] maintained that higher oxidation states are reduced to Pu (IV) which can then undergo extensive hydrolysis to produce insoluble products. Such reactions should effectively inhibit Pu transport in the biosphere. However, complexation with organic and inorganic ligands can stabilize Pu (IV) against hydrolysis and increase its solubility. Analysis of surface soils that contain Pu has shown that a small percentage of the Pu remains in solution predominantly as colloidal particles[4] and therefore is available for transport. This study has examined the potential for the transport of Pu in a groundwater flow system through the use of laboratory columns prepared from "undisturbed" horizontal cores taken from saturated zone soils adjacent to the glass block site. The columns were spiked with ^{239}Pu, continuously eluted with groundwater from the glass block site, and the activity and physical-chemical characteristics of the ^{239}Pu in the effluent determined following various manipulations of the column elution conditions.

MATERIALS AND METHODS

Pu Solution - The Pu solution contained 76.6, 19.7, 2.9, 0.5 and 0.1 atom % ^{239}Pu, ^{240}Pu, ^{241}Pu, ^{242}Pu, and ^{238}Pu respectively. In the text the predomi-

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nant isotope, ^{239}Pu, is used when referring to this mix of isotopes.

Soil Columns - Soil columns were prepared from undisturbed horizontal cores of uncontaminated saturated zone sand taken upgradient of the glass block site. The columns (4.5 cm x 11 cm, void volume ~54 mL) were equilibrated for 1 to 3 months with groundwater, taken from a well adjacent to the site, with an upward flow of approximately 45 $cm \cdot d^{-1}$ (approximately 3 times the groundwater flow rate at the site). Columns were loaded with 80 µg (10 µCi or 3.1×10^5 Bq) of ^{239}Pu, obtained by a 100-fold dilution of a stock ^{239}Pu solution (8 M HNO_3), and eluted with groundwater. The column effluent was collected for analysis.

Effluent Analysis - ^{239}Pu in the column effluent was determined as either total Pu or as particulate Pu. Total ^{239}Pu was determined by evaporating 10 mL of effluent directly onto a stainless steel planchet and α-counting. Particulate ^{239}Pu was determined by filtering an aliquot of the column effluent through 0.45 µm Millipore filters and then counting the filtrate and filter. The reported ^{239}Pu activities have a statistical accuracy of ±12% or less with 95% confidence limits.

Geochemistry - The geochemical characteristics of the groundwater, before and after equilibration in the laboratory, and of the column effluent were measured. Redox and pH measurements were made in line using a flow cell. Dissolved anions in the groundwater were measured by ion chromatography using a Dionex system while cations were measured by atomic absorption spectroscopy. Dissolved oxygen was measured on a YSI Model 57 dissolved oxygen meter. Alkalinity was determined by potentiometric titration[5] of the sample following 0.45 µm filtration.

Temperature Cycling - Columns were routinely eluted at 22±2°C. For low temperature conditions (~0°C) the column and the eluant were kept in an ice bath during the experiment.

Antibiotic Treatment - The columns were eluted in the normal manner at ~22° with groundwater containing 50 $mg \cdot L^{-1}$ streptomycin sulfate, 50 $mg \cdot L^{-1}$ penicillin G, plus 100 $mg \cdot L^{-1}$ chloramphenicol. The antibiotic solution was shielded from light and changed after 2 days. Recovery was achieved by re-eluting with untreated groundwater.

Ultrafiltration - The standard protocol for ultrafiltration was as follows; Amicon ultrafiltration membranes (UM05 and YM 10, apparent exclusion limits of 500 and 10,000 molecular weight, respectively) were presoaked overnight in double-distilled deionized water, the membranes were placed in the ultrafiltration cell and washed with 1 cell volume of double-distilled deionized water, the cell was then rinsed with an aliquot of the sample which was then

discarded, the sample (prefiltered through a 0.05 μm Millipore filter) was then loaded into the cell and concentrated to ~10% of the cell volume (the first ~6 ml of ultrafiltrate containing some distilled water from the membrane rinse was discarded). The filtrate and retentate were accurately measured for the mass and activity balance calculations. UM05 membranes and YM 10 membranes were run at N_2 pressures of 60 and 20 psi (414 and 138 kPa) respectively.

Chemical Speciation - The oxidation states of the Pu in the column effluents were determined using modifications of the methods reported by Foti and Freiling[6].

NdF Coprecipitation - The NdF_3 coprecipitation was conducted using a ^{236}Pu (IV) tracer which was prepared by adding 100 mg of $NaNO_2$ to 400 μL of ^{236}Pu tracer in 8 M HNO_3, evaporating to dryness, and then resuspending in 5 mL of distilled H_2O. The coprecipitation was carried out by adding 5 mL of tracer solution to 10 mL of column effluent containing ^{239}Pu. This solution was adjusted to 1 M in HNO_3, 100 μg of Nd (III) carrier in 1 M HNO_3 (1 mL) was added and dispersed, and then 2 mL of 49% HF was added to precipitate NdF_3. A NdF_3 bed was prepared by mixing 50 μg Nd (III), 5 mL of 1 M HNO_3, and 1 mL of 49% HF and filtering through a 0.1 μm Tuffryn filter. The sample was similarly filtered, washed, and then mounted on a stainless steel planchet for determination Pu (III), (IV) or (V) by α-spectrometry. The filtrate which contained any Pu (VI) present was then reduced with $NH_2OH \cdot HCl$ plus Fe and any reduced Pu (VI) was coprecipitated on CeF_3 and determined by α-counting. The tracer yield in the above procedure was 85 to 90%.

TTA Extraction - A 10 mL sample of column effluent was extracted sequentially with 2-thenoyltrifluoracetone (TTA) in toluene at pH's 0.4 and 4.3. 10 mL of column effluent was adjusted to pH 0.4 with hydrochloric acid, extracted with 10 mL of 0.4 M TTA for 20 minutes, and the phases separated. The aqueous phase was then adjusted to pH 4.3 with ammonium acetate, extracted for 20 minutes with 0.4 M TTA, and the phases were again separated. The organic phases were each washed twice with 10 mL of distilled water for 10 minutes and the Pu was then stripped out by extracting twice with 6 M hydrochloric acid. These acid extracts were pooled, evaporated just to dryness, resuspended in nitric acid and dryed twice, and finally resuspended in nitric acid, heated, and then transferred to stainless steel planchets for evaporation and α-counting.

RESULTS AND DISCUSSION

Low levels of plutonium were continuously released from the core columns

TABLE 1

GEOCHEMICAL DATA

Water Source	pH	E_H	DO	HCO_3^-	NO_3^-	SO_4^{2-}	Ca^{2+}	Mg^{2+}	K^+	Na^+	Si^+	Al^+
Groundwater at Well Head	5.3	445	2.5	8.7	0.2	18.8	5.5	1.6	0.9	1.9	5.0	0.2
Groundwater in Laboratory	5.6	490	8.5	8.2	0.2	17.4	5.1	1.6	0.9	2.0	5.0	0.2
Column Effluent	6.7	520	n·a[b]	9.5	0.2	18.5	6.1	1.6	0.9	2.0	4.0	0.5

a All data in $mg \cdot L^{-1}$, except E_H(mV)
b n·a = not analyzed

Significant alterations in the various geochemical parameters characterizing the well water, Table 1, were not noted during the course of these experiments. Fe and Mn concentrations were both at the limits of detection (0.05 $mg \cdot L^{-1}$). The well water is relatively oxidizing as it contains appreciable dissolved oxygen and its oxidizing potential was only marginally elevated by laboratory equilibration and passage through the column.

Size Distribution of Plutonium Species - The size characteristics of the Pu species released from the column have been examined by filtration and ultrafiltration techniques. For the purposes of this study particulate species are those excluded by a 0.45 μm (450 nm) filter, the remaining species are considered "dissolved". The dissolved species are further subdivided into colloidal and soluble components. Colloidal material is that excluded by a YM 10 (10,000 molecular weight exclusion limit, effective pore diameter of 2.8 nm) ultrafiltration membrane as proposed by Schindler and Alberts[7]. Soluble components are those passing through a YM 10 ultrafiltration membrane. Two size classes of colloids were determined since ultrafiltration studies were always performed on samples which had been prefiltered through 0.05 μm filters. The soluble components were similarly divided into two size classes, one comprising intermediate molecular weight (500-10,000) species and the other (<500 molecular weight) including ionic and small organic or inorganic complexes.

We have determined the size distribution for the Pu species in the effluent from the column illustrated in Figure 1. Table 2 contains results for samples taken prior to the attainment of steady state conditions (85 void volumes), column A of Table 2, and for samples taken at steady state (250 and 350 void volumes), column B of Table 2. The size distribution at both steady state sampling points were very similar and have been averaged. Under conditions of steady state release (column B, Table 2) 46% of the Pu was associated with

into the groundwater used for column elution. Figure 1 shows the ^{239}Pu release rates observed on a column over a period of ~8 months during which time ~800 void volumes of solution were passed through the column; low levels of Pu were detected in the effluent within 1 day of loading. The transport of Pu associated with particulates is shown in the lower curve of Figure 1 which represents the activity of plutonium in solution that was retained on 0.45 µm filters. The amount of Pu associated with particulates increased gradually with time and, at steady state approximately 50% of the plutonium released was associated with particulates.

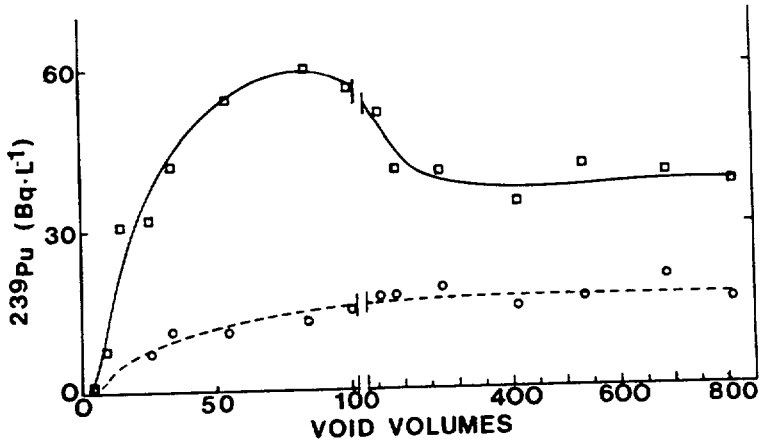

Figure 1 Release of plutonium from Soil Column. The column was eluted at 220 mL·d^{-1} and the total, □————□, and particulate, o--------o, ^{239}Pu activity in the effluent determined as described in Methods.

Steady-state releases from individual columns have varied from approximately 40 Bq·L^{-1} for the column shown in Figure 1 (release of the total Pu inventory would require ~115 years) to a maximum steady state release rate of approximately 130 Bq·L^{-1}. In the latter case the initial peak was only 140 Bq·L^{-1}. In spite of the 3 fold higher release rate for total Pu from the latter column the percentage associated with particulates was similar; 60±10% for the latter versus 50±10% for the former. The different release rates from the different columns appear to be a function of either the mineralogy and/or the associated microbiology of the sediments since all columns were equilibrated and eluted with groundwater from the same well.

particulates, a further 23% was colloidal and 31% was soluble. Two-thirds of the colloidal species were 2.8 to 50 nm in size. Approximately 87% of the soluble species had apparent molecular weights of 500 to 10,000, the remaining 13% were present likely as small complexes with apparent molecular weights of less than 500. The data in column A (Table 2) indicates that during the approach to steady state there has been a shift in the size distribution of the Pu species, most notably in the particulate versus large colloidal fraction. The significance of this observation is as yet unclear.

TABLE 2

SIZE DISTRIBUTION OF PLUTONIUM IN COLUMN EFFLUENTS

Size Category		Percent of Pu in Column Effluent	
		A	B
Particulate (>450 nm)		21	46
'Dissolved' (<450 nm)			
Colloidal	(50-450 nm)	23	8
	(2.8-50 nm)	48[a]	15
Soluble[b]	(500-10,000 molecular weight)		27
	(<500 molecular weight)	8	4

[a] Ultrafiltration with a UM05 membrane only was performed.

[b] Ultrafiltration percentages have been corrected assuming 100% recovery. Average recoveries were 87 and 92% for UM05 and YM10 ultrafiltration respectively.

Avogadro et al[8] have also reported on Pu soil column experiments where a continuous input of Pu to the column was derived from leaching of actinide bearing glass. Under these conditions ~80% of the input Pu was not retained by the soil columns and of this only ~20% was colloidal or particulate as assayed in the column effluent, substantially less than we have observed. The generation of particulate or colloidal Pu species on the column, as opposed to that which may have been present in the column input, could not be assessed on the basis of the information presented. Our observation of a large fraction of

colloidal plus particulate material is in general agreement with observations for Pu in other systems, such as various surface soils[2]. The colloidal material in the column effluent appears to be relatively stable since no statistically significant difference in the percentage retained by a 0.05 μm filter was observed following storage at ~22°C for 4 weeks.

Distribution of Oxidation States - The oxidation states of ^{239}Pu in the column effluents were determined by a combination of NdF_3 coprecipitation and 2-thenoyltrifluoracetone (TTA) extraction. From the NdF_3 coprecipitation results, shown in Table 3, we conclude that most if not all ^{239}Pu in the column effluent was present in the lower oxidation states.

TABLE 3
NdF_3 COPRECIPITATION OF ^{239}Pu IN COLUMN EFFLUENTS

Pu Species	Percent of Activity[a]	
	^{236}Pu Tracer[b]	^{239}Pu
Pu(III), (IV) and (V)	86	81
Pu(VI)	n.d.[c]	n.d.

[a] Values are means of triplicate determinations. Bulk effluent activity was 37 $Bq \cdot L^{-1}$ ^{239}Pu

[b] ^{236}Pu(IV) was added as a tracer

[c] Not detectable.

From the results of the TTA extraction experiments (Table 4) approximately 80% of the ^{239}Pu was extracted from the column effluents by TTA at pH 0.4 and was therefore likely present as Pu (IV). The remaining 20% of the activity extracted at pH 4.3 must have been present at Pu (III) since only Pu (VI) would have coextracted with Pu (III) and we have previously shown the absence of Pu (VI). Extraction experiments on filtered and unfiltered samples yielded very similar results from which we conclude that the Pu present as or associated with particulate species and the dissolved species are equally well extracted by the procedure used. The results also show that the distribution

of oxidation states is very similar for both fractions.

TABLE 4
TTA EXTRACTION OF ^{239}Pu IN COLUMN EFFLUENT

Source	pH	Percent of ^{239}Pu Recovered in Extract[a]
Bulk Column Effluent	0.4	80
	4.5	20
0.45 µm Filtered Column Effluent	0.4	86
	4.5	14

[a]Values are means of triplicate determinations assuming 100% recovery. The mean recovery was 82%.

The overall chemical speciation results have shown that the Pu in the column effluents was present as Pu (III) and Pu (IV) in a ratio of approximately 1 to 4. The ^{239}Pu stock solution in 8 M HNO_3 likely contained only Pu (IV)[9], however upon dilution the pH change may have resulted in a change to Pu (III) and Pu (VI)[9]. The complexity of these reactions makes it very difficult to decide whether the effluent speciation is what one would expect on the basis of the input conditions. However since Pu (III) and Pu (IV) were observed in the effluent then the Pu must have been either sorbed as Pu (III) and Pu (IV) or if Pu (VI) was also present it must have undergone reduction to the lower oxidation states during residence on, or transfer through, the column.

Mechanisms of Pu Release - The mechanisms of release of the Pu have been studied by temperature cycling and treatment with antibiotics.

Figure 2 illustrates the effect of temperature on release rates for 2 columns whose steady state release rates differed by a factor of 3. After cooling to 0°C there was an approximately 4-fold decrease in the release rate of total Pu within approximately 48 hours. However the release rate of particulate Pu decreased by approximately 8-fold suggesting different mechanisms for the release of particulate versus other fractions. Within 20 days of shifting back to ~22°C both columns had re-established the original release rates for both total Pu and particulate Pu species. At present we have no explanation for the

unexpectedly long recovery time.

The influence of a suite of antibacterial agents on Pu release was also tested and the results for 2 columns are presented in Figure 3.

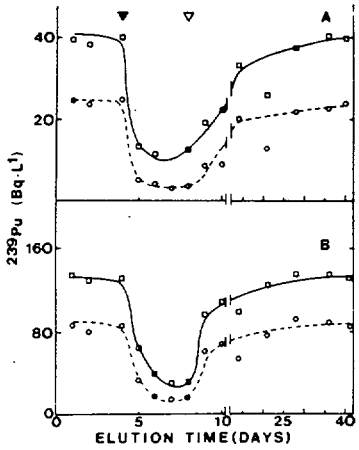

Figure 2. Temperature Effects on ^{239}Pu Release for 2 Different Columns. ▼, shift to ~0°C; ▽, shift to ~22°C.

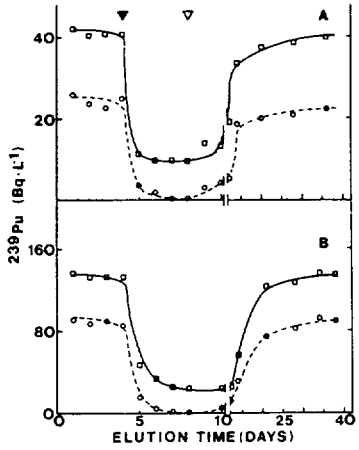

Figure 3. Effect of Antibiotics on ^{239}Pu Release for the same Columns as in Figure 2. ▼, beginning of elution with antibiotic ▽, cessation of antibiotic solution.

These results provided further support for differing mechanisms for the release of colloidal or particulate material versus soluble material. Following 3 days of continuous exposure to the antibiotic solution there was no longer any Pu in the effluent that could be removed by filtration through 0.45 µm filters, from which we conclude that the release of Pu in association with particulates was totally inhibited by the antibiotic treatment. The total Pu activity in the effluent was only 20 to 25% of the activity levels before antibiotic treatment and following recovery. If we compare these residual activity levels with the size determination data of Table 2 we see that the residual activity levels are equivalent to the percentage of the Pu in the column effluent that was present as soluble species. This comparison suggests that the antibiotic treatment inhibited the release of colloidal species as well as particulate species. This prediction will be tested in future studies. If indeed the antibiotic effects were due to the inhibition of bacterial metabolism then the slow but total recovery of Pu release rates would suggest that either the

bacterial species responsible for the release were not completely removed by the treatment and repopulated the column or that bacteria with similar metabolic properties were present in the groundwater eluent and repopulated the column.

The introduction of antibiotics at relatively high concentrations may have had effects other than removing bacteria but these effects if any have not been defined. However the authors[1] have also observed the transport of Cs in association with particulates. In that instance not only antibiotic treatment but also irradiation of the column and eluent interfered with the release of particulates, which lends further support to bacterial involvement. The nature of the particulates and of the bacterial involvement in their release is still undergoing investigation.

SUMMARY

Through the use of core columns we have demonstrated the potential for the release of low-levels of Pu from contaminated saturated zone soils. Transport of the reduced species of Pu occurs as both dissolved (soluble plus colloidal) species and as particulates. The colloidal plus particulate species account for 50 to 70 % of the total Pu released under our oxidizing conditions. The mechanisms for release of soluble versus colloidal plus particulate species appear to be different with bacterial involvement in the release of the latter species being highly probable.

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