

Nagra

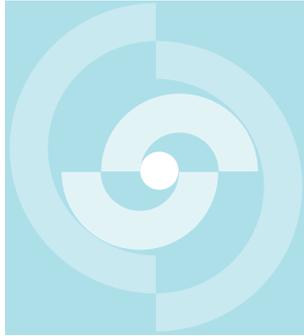
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TECHNICAL REPORT 87-16

Trace Element and Microbiological Studies of Alkaline Groundwaters in Oman, Arabian Gulf: A Natural Analogue for Cement Pore-Waters

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July 1987

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PREFACE

Further confirmatory evidence is being sought to demonstrate the beneficial influence of a highly alkaline cementitious repository environment on the solubilities of many radionuclides and on the inhibition of microbial growth. The decreases in solubilities are predicted theoretically by chemical thermodynamic modelling, but uncertainties occur in the data and ionic species relevant to the highly alkaline environment. There are few direct laboratory or natural observations of microbial viabilities at high pH. Furthermore, because repository safety analysis involves prolonged timescales, the evolution of the responses to high alkalinity in a natural environment must be considered.

This report describes a study, funded jointly by UK Nirex Ltd., and Nagra, of some rare occurrences of highly alkaline natural groundwater. Both trace element abundances and microbiology were investigated. Although the study highlights many practical and analytical difficulties in obtaining conclusive evidence from natural systems, it also indicates that further such 'natural analogue' investigations could give valuable data to constrain and complement theoretical predictions.

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EXECUTIVE SUMMARY

The microbiology and trace element chemistry of some highly alkaline groundwaters in Oman in the Arabian Gulf have been examined. The significance of the study to the understanding of processes in and around a radioactive waste repository lies in its value as a natural analogue of the high-pH sustained by cement in its pore-waters (it has been suggested that pH 11-13 might be maintained for up to a million years) and in the near-field aqueous environment. Cement and concrete may occur in the repository as components of waste, immobilisation matrix and backfill and as engineering materials. In addition to being highly alkaline, the near-field of a sealed repository will also become chemically reducing due to early-stage oxidation/corrosion reactions in the repository. Thus the safety analysis must, amongst other things, consider the chemical and microbiological influences on radionuclide behaviour in this environment.

Microorganisms will inevitably be introduced into a repository, and could participate in reactions which cause difficulties in predicting performance: e.g. deterioration of metal and of concrete which are accelerated by sulphur and iron bacteria, and production of gases by biodegradation of waste material, notably methane generation in which methanogenic bacteria are active. However it has been thought that microbial proliferation is strongly inhibited in a highly alkaline environment such as that predicted for a cement-dominated repository, and is further constrained by the rapid development of strongly reducing conditions. This natural analogue study has sought more evidence of the extent of microbial viability and growth in alkaline groundwaters.

Equilibrium chemical thermodynamics provides the usual approach for evaluating elemental solubilities and speciation in aqueous solution. However, application to the radionuclide trace elements of importance in nuclear waste disposal (i.e. actinides and their daughters and radionuclides which are produced by fission or activation) is limited by the availability of relevant data and uncertainty over the character of aqueous ionic species. The natural analogue approach has been applied here in an attempt to check some of these predictions against observed elemental abundances in a natural environment.

There are few natural environments, or artificial situations sustained over long time-scales, in which evidence of the effects of high pH on chemical and microbiological behaviour of the system can be obtained. Aged concrete structures obviously present one possibility, whilst alkaline lakes, both natural and artificial, could also be studied. The present study has investigated the natural environments created around the discharge points of hyperalkaline

groundwaters which occur only under rare geological conditions. This type of 'natural analogue' study provides evidence which is complementary to that gained in laboratory-based studies, in that the natural environment might mimic the long-term development of chemical and microbial conditions which cannot be achieved in short-term experiments.

The alkaline springs in Oman were selected for the present investigation because the hydrochemistry and some associated mineral precipitates closely resemble the inferred conditions in cement pore-waters, even to the extent of portlandite ($\text{Ca}(\text{OH})_2$) precipitate having been observed. Highly reducing conditions also occur in these groundwaters and hydrogen gas is evolved at some of the springs, thus increasing their value as a natural analogue for the repository environment. Samples were collected from several of these alkaline springs in order to isolate and identify the bacterial populations as far as possible and to test some of the predicted constraints on solubilities of radionuclides in the highly alkaline environment.

The microbial genera found in the spring-waters are similar to those in less extreme soil and water environments. The wide range of both aerobic and anaerobic bacteria isolated was enhanced by the local variations in conditions at specific sampling points, particularly with respect to redox conditions and nutrient availability. Indeed, at least some of the heterotrophic microorganisms could be attributed to contamination of the spring-waters by animals. Several heterotrophic bacteria were isolated which were able to grow beyond pH10 and some beyond pH11. Obtaining indubitable proof of viability above these values, however, presents considerable experimental difficulties. Most strains were found to be alkalotolerant. By standard criteria, the two alkalophilic strains isolated were shown to be strict aerobes and thus may be of little importance to the anaerobic repository environment.

Of particular interest is the demonstration of the presence of sulphate-reducing bacteria (SRB) in particular samples of both water and sediment. SRB are of relevance to waste disposal because of their participation in reactions which corrode steel and which may affect concrete integrity. They may not be present in large numbers but their existence shows that they are able to colonise, albeit in a dormant or near-dormant state, environments with a pH in excess of 11. The SRB are unable to grow at high pH in nutrient-rich culture, and this suggests that pH is the growth-limiting factor under experimental conditions. However, activity measurement data suggest that the high pH is not the principal constraint on microbial growth in the alkaline spring-waters. The hydrochemical data on the spring-waters indicate

that microbial populations may be limited by supply of nutrients, especially carbon, nitrogen and phosphorus. It should be borne in mind that the phosphorus content of most Portland cements is much higher than was encountered in the spring-waters. The low levels of organic carbon present in the spring-waters are similar to those in many natural environments which, although at the lower limit for uptake and utilisation by most bacteria and fungi, can support oligotrophic activity. Heterotrophic bacteria, which predominate in the alkaline spring-waters and which could be present in a waste repository, can lead to production of organic acids by hydrolysis of cellulose materials in the waste inventory. These organic acids could speed up neutralisation of the high alkalinity and the organic chelating/complexing species formed may solubilize radionuclides or may act as further substrates for microbes.

The trace elements investigated for comparison of measured solution concentrations with equilibrium values predicted by thermodynamic modelling were Se, Pd, Sn, Zr, Ni, U and Th. Predicted equilibrium solution concentrations under these highly alkaline, reducing conditions are considerably lower than state-of-the-art analytical detection limits for Pd and Sn, and close to detection limits for Ni, Th and U (though there are discrepancies between predictions for U made using databases from different sources). The observed solution concentrations of these trace elements were mostly below detection limits, with significant values only for some Zr and U determinations. Data for Pd and Ni are considered to be unreliable due to analytical interferences. The measured data for Se, Zr and possibly Ni are significantly lower than predicted equilibrium concentrations, and could indicate that appropriate solid phases or thermodynamic data have been omitted from the model. However the interpretation and significance of these observations is dependent on there being natural sources of the elements which buffer concentrations at solubility limits. For example, geochemical evidence suggests that Zr may be particularly depleted in the source rocks. Ni is perhaps the most interesting in this respect since the known rock geochemistry suggests a reasonable supply of Ni. Below-detection-limit analyses for Th are at least consistent with model predictions of low concentrations. Comparison of similarly low U analyses with predicted values varies widely, depending on the database used and the redox state; this example highlights some significant discrepancies between the databases which would benefit from further investigation and renewed attempts at validation.

The abundances and isotope compositions of U and Th in alkaline groundwater samples passed through different filter sizes suggests that, in one case, U and daughter nuclides may be associated with colloidal material. Colloids are acknowledged to be of potential importance in repository safety analysis, but tend not to be stable at high pH or high salinity. Therefore the indication of U and daughter Th being present in stable colloidal form here,

though based on limited data, is potentially interesting and worthy of further investigation.

Therefore positive quantitative or semi-quantitative 'validation' of the predictive models has not been achieved in general, although the attempt has highlighted some potentially significant points of comparison between natural solute abundances and the predictions. It has also shown that this comparison of concentrations and species distributions may be feasible analytically for some elements such as Se, Zr, Ni, U and Th, though natural elemental sources and their reaction rates will inherently limit the extent to which models can be validated.

R E S U M E

La microbiologie et le chimisme des éléments traces de quelques eaux souterraines fortement alcalines à Oman, ont été analysées. Ces études sont très importantes pour la compréhension des processus se déroulant à l'intérieur et autour d'un dépôt final pour déchets radioactifs. Leur valeur réside notamment dans les analogies naturelles qu'elles représentent pour l'environnement fortement alcalin dans les eaux interstitielles du ciment (on a supposé des valeurs de pH de 11 à 13 sur une période allant jusqu'à un million d'années) et dans l'environnement aquifère du champ proche du dépôt final.

Le ciment et le béton peuvent se manifester dans le dépôt final sous diverses formes: en tant que composants du déchet, matrice de solidification, colmatage et matériaux de construction. Le champ proche d'un dépôt final scellé sera non seulement fortement alcalin, mais aussi chimiquement réducteur en raison de réactions à l'oxydation et à la corrosion intervenant très tôt dans le dépôt. Ainsi l'analyse de sûreté doit-elle considérer, entre autres, les influences chimiques et microbiologiques s'exerçant sur le comportement des radionucléides dans cet environnement.

Des micro-organismes s'introduiront nécessairement dans un dépôt final; leur réactivité pourrait alors rendre les prévisions sur le comportement des composants du dépôt difficiles, comme par exemple, la détérioration du métal et du béton, accélérée par des bactéries de soufre et de fer, ainsi que la formation de gaz lors de la biodégradation des déchets, notamment le méthane dans lequel les bactéries méthanogènes sont actives. On a toutefois postulé que la prolifération microbienne est fortement ralentie dans un milieu fortement alcalin, tel que celui qui a été prévu pour un dépôt final dominé par le ciment, et est en plus affaiblie par le développement rapide de conditions fortement réductrices. Cette étude sur les analogies naturelles a tenté d'acquiescer de plus larges informations sur la viabilité et la croissance microbiennes dans des eaux souterraines alcalines.

Les méthodes d'équilibre thermodynamique permettent habituellement d'évaluer et/ou de prévoir la spéciation de composantes solubles. Mais en ce qui concerne les éléments traces des radionucléides (actinides et éléments de filiation, produits de fission et d'activation), importants dans le dépôt final, les données de base thermodynamiques leur étant nécessaires sont relativement incertaines et d'étendue plutôt limitée. Dans la présente étude, la comparaison entre solubilités prévues et mesurées a servi à mettre en évidence ces incertitudes.

Il existe peu d'environnements naturels ou de conditions artificielles se maintenant sur de longs espaces de temps, qui fournissent des informations sur l'influence d'un pH élevé sur le comportement chimique et microbiologique d'un système. Une possibilité consiste manifestement dans l'analyse d'anciennes structures de béton, une autre dans l'étude des eaux alcalines (lacs salés tant naturels qu'artificiels). On a donc procédé ici à l'analyse de l'environnement naturel autour de points de décharge d'eaux souterraines hyper-alcalines, situation que l'on ne ren-

contre que dans de rares conditions géologiques. Des expériences à court terme réalisées en laboratoire ne sont pas nécessairement en mesure de simuler le développement à long terme de conditions chimiques et microbiologiques. Mais on suppose que ce type d'"analogie naturelle" peut compléter judicieusement les résultats d'expériences en laboratoire.

Les sources alcalines d'Oman ont été sélectionnées pour la présente étude, parce que l'hydrochimie et quelques précipités minéraux associés sont très semblables aux conditions supposées dans les eaux interstitielles du ciment, même dans la mesure où des précipités de portlandite (Ca(OH)_2) ont été observés. Des conditions fortement réductrices se manifestent aussi dans ces eaux souterraines et du gaz hydrogène se dégage de plus d'une source, augmentant ce faisant leur valeur en tant qu'analogie naturelle pour l'environnement du dépôt. Des échantillons de plusieurs de ces sources alcalines ont été recueillis afin d'isoler et d'identifier les populations bactériennes ainsi que de contrôler les solubilités de radionucléides prévues dans un environnement fortement alcalin.

Les espèces microbiennes identifiées dans les eaux de source sont semblables à celles décelées dans des environnements de sol et d'eau moins extrêmes. Le large éventail de bactéries isolées, tant aérobies qu'anaérobies, indique des fluctuations locales dues surtout à des variations des conditions rédox et de l'apport nutritionnel. En fait, certains micro-organismes hétérotrophes pourraient provenir de la contamination animale des eaux de source. Plusieurs bactéries hétérotrophes, qui ont pu se développer avec un pH >10 et dans quelques cas avec un pH >11 , ont été isolées. Il est cependant extrêmement difficile d'acquérir la preuve indubitable de la viabilité au-delà de ces valeurs de pH en recourant à des expériences en laboratoire. La plupart des espèces se sont avérées tolérantes aux alcalis. Partant des critères d'évaluation standard, deux espèces isolées tolérantes aux alcalis se sont avérées strictement aérobies. Elles ne sont que de peu d'importance pour l'environnement anaérobie du dépôt final.

La preuve de la présence de bactéries réductrices de sulfate (SRB) dans certains échantillons d'eau et de sédiment est d'un grand intérêt. Les SRB sont importantes pour l'élimination des déchets, vu qu'elles participent à des réactions qui favorisent la corrosion de l'acier et affectent l'intégrité du béton. Elles peuvent ne pas se présenter en grand nombre, mais leur existence montre qu'elles sont capables de coloniser des environnements au pH >11 , même si ce n'est que dans un état inactif ou quasi inactif.

Les SRB sont incapables de croître dans un environnement alcalin et riche en substances nutritionnelles. Cela signifie que, dans des conditions expérimentales, le pH est le facteur qui limite la croissance. Toutefois, des données de mesure sur l'activité microbienne dans les eaux de source alcalines donnent à penser que le pH élevé n'est pas le facteur le plus important. Il ressort en effet des données hydrochimiques sur les eaux de source, que la croissance des populations microbiennes peut aussi être limitée par l'apport de substances nutritionnelles (spécialement carbone, nitrogène et phosphore). Mais il ne faut ici pas oublier que la teneur en phosphore de la plupart des ciments de Portland est bien plus élevée que celle des eaux de source. Les faibles

teneurs de carbone organique observées dans les eaux de source sont semblables à celles identifiées dans maints environnements naturels. Bien que ces faibles teneurs représentent la limite inférieure pour l'absorption et l'utilisation par la plupart des bactéries et champignons, elles peuvent supporter une activité oligotrophique. Les bactéries hétérotrophes, qui prévalent dans les eaux de source alcalines et pourraient aussi être présentes dans un dépôt final, peuvent contribuer à la production d'acides organiques par l'hydrolyse de substances de déchets contenant de la cellulose. Ces acides organiques pourraient accélérer la neutralisation de l'alcalinité élevée et les acides organiques en résultant solubiliser divers radionucléides ou servir à nouveau de substance nutritionnelle pour d'autres bactéries.

Dans les eaux de sources, les éléments traces Se, Pd, Sn, Zr, Ni, U et Th ont été analysés en vue de la comparaison entre solubilités mesurées et solubilités prévues. Dans les conditions réductrices fortement alcalines données, les concentrations d'équilibre prévues pour Pd et Sn sont beaucoup plus basses que les limites de détection analytiques; pour les éléments Ni, Th et U les prévisions sont proches des limites de détection. Dans le cas de l'uranium, certaines difficultés sont apparues déjà au niveau de la prévision, en raison des différentes bases de données utilisées.

A l'exception de quelques valeurs significatives pour Zr et U, les concentrations des éléments traces mesurés sont inférieures aux limites de détection. En raison de la méthode analytique, les données sur Pd et Ni doivent être évaluées avec prudence (interférences avec d'autres éléments). Les valeurs mesurées pour Se, Zr et peut-être aussi Ni sont nettement plus basses que les valeurs prévues. Cela signifie que, dans la base de données thermodynamiques utilisée, on n'a peut-être pas tenu compte des phases solides appropriées. Mais une telle interprétation suppose que l'élément observé est présent dans la roche dans des proportions suffisantes. Des résultats géophysiques indiquent par exemple que les formations rocheuses entrant en question peuvent être particulièrement pauvres en zirconium. A cet égard, Ni est peut-être plus intéressant depuis que la géochimie connue suggère une présence de nickel suffisante. Pour Th au moins, les prévisions ne sont pas en contradiction avec les mesures. Toutes les mesures sont ici inférieures aux limites de détection et aux prévisions. Pour l'uranium, la situation est plus compliquée. Les concentrations prévues sont nettement supérieures aux valeurs mesurées et montrent une grande variabilité, fonction de la base de données utilisée et du potentiel rédox de l'eau de source. Cet exemple met en évidence les problèmes que ce genre de prévisions peut poser et indique dans quel domaine de nouvelles recherches sont nécessaires.

Dans un cas, les expériences de filtration réalisées avec différentes dimensions de filtres montrent que l'uranium et ses nucléides-filles peuvent être associés au matériel colloïdal. Dans l'analyse de sûreté du dépôt final, les colloïdes sont reconnus être d'une importance potentielle, mais on suppose qu'ils tendent à l'instabilité en cas de pH ou de salinité élevé. En conséquence, l'indication que U et ses filles pourraient être présents dans ces eaux alcalines sous une forme colloïdale stable est très intéressante et mérite des recherches plus approfondies.

Bien qu'une "validation" positive quantitative ou semi-quantitative des modèles de prévision n'ait en général pas été réalisée, l'essai a mis en évidence quelques points importants de comparaison entre solubilités mesurées et prévues. Il a aussi montré qu'une telle comparaison peut être réalisable analytiquement pour quelques éléments tels Se, Zr, Ni, U et Th, bien que le manque de connaissances sur des répartitions minérales naturelles et des vitesses de réaction rende une validation des modèles extrêmement difficile.

ZUSAMMENFASSUNG

Die Mikrobiologie und der Chemismus der Spurenelemente von einigen stark alkalischen Grundwässern in Oman (Arabischer Golf) sind untersucht worden. Für das Verständnis von Prozessen, welche in einem Endlager für radioaktive Abfälle ablaufen können, sind derartige Untersuchungen von grossem Interesse. Insbesondere könnten solche Systeme als natürliche Analoga für die stark alkalische Umgebung in Zementporenwässern (pH-Werte von 11 bis 13 über eine Zeitspanne von 1'000'000 Jahren wurden vorgeschlagen) und für die Lösungsschemie des Endlager-Nahfeldes dienlich sein.

Zement und Beton kommen im Endlager in verschiedenen Formen vor, als Abfallkomponenten, Verfestigungsmatrix, Verfüllung und als Baumaterialien. Das Nahfeld eines versiegelten Endlagers wird nicht nur stark alkalisch, sondern wegen bereits sehr früh einsetzenden Oxidations- und Korrosionsreaktionen auch chemisch reduzierend sein. Der Einfluss dieser speziellen chemischen (und mikrobiologischen) Bedingungen auf das Verhalten der Radionuklide muss in der Sicherheitsanalyse berücksichtigt werden.

Die Zufuhr von Mikroorganismen in ein Endlager ist unvermeidlich. Die Reaktivität solcher Organismen könnte Voraussagen zum Verhalten der Endlagerkomponenten stark erschweren. Beispiele sind der Abbau von Metall und Beton durch Eisen- und Schwefelbakterien, sowie die Gasbildung (Methan, Biodegradierung von Abfallmaterial) durch methanogene Bakterien. Gleichzeitig wurde aber auch postuliert, dass mikrobielle Vermehrung in einer hoch alkalischen Umgebung (wie für ein zement-dominiertes Endlager vorausgesagt) stark gehemmt ist und durch den schnellen Aufbau stark reduzierender Bedingungen zusätzlich unterdrückt wird. Es war ein Hauptziel dieser Analogstudie, weitergehende Informationen über die Lebensfähigkeit und das Wachstum von Mikroben in alkalischen Grundwässern zu erhalten.

Üblicherweise kann die Speziation löslicher Komponenten mit Hilfe thermodynamischer Gleichgewichtsmethoden berechnet und/oder vorausgesagt werden. Für endlagerrelevante Spurenelemente (Aktinide und Tochterelemente, Spaltprodukte, Aktivierungsprodukte) sind aber die dazu notwendigen thermodynamischen Basisdaten relativ unsicher und in ihrem Umfang eher beschränkt. In der vorliegenden Studie wurde der Vergleich zwischen vorausgesagten und gemessenen Löslichkeiten dazu benutzt, diesbezügliche Unsicherheiten aufzuzeigen.

Es gibt nur wenige natürliche, oder sich über lange Zeiträume erstreckende künstliche Umweltbedingungen, welche Informationen über den Einfluss von hohem pH auf das chemische und mikrobiologische Verhalten eines Systems liefern. Eine Möglichkeit ist die Untersuchung alter Betonstrukturen, eine andere die Untersuchung von alkalischen Gewässern (natürliche und künstliche Salzseen). In der vorliegenden Studie wurde die natürliche Umgebung um Ausströmstellen hyperalkalischer Grundwässer untersucht, eine Situation die nur unter seltenen geologischen Verhältnissen vorkommt. Kurzfristige Laborexperimente sind nicht notwendigerweise in der Lage, die Langzeitentwicklung von chemischen und mikrobiologischen Bedingungen zu simulieren. Es wird aber angenommen, dass sol-

che "Analogstudien" die Erkenntnisse aus Laborexperimenten sinnvoll zu ergänzen vermögen.

Die alkalischen Quellen in Oman wurden für diese Studie ausgewählt, weil die Hydrochemie (und damit assoziierte Mineralausfällungen) den aus Zementporenwässern abgeleiteten Bedingungen sehr ähnlich ist (es wurde sogar eine Portlandit (Ca(OH)_2)-Ausfällung beobachtet). Stark reduzierende Bedingungen im Grundwasser, und bei manchen Quellen sogar die Abgabe von Wasserstoffgas, erhöhen ihren Wert als Analoga für ein Endlagerumfeld. Im Hinblick auf die Isolierung und Identifizierung von Bakterienpopulationen und im Hinblick auf die Überprüfung der vorausgesagten Nuklidlöslichkeiten wurden von mehreren dieser alkalischen Quellen Proben gesammelt.

Die in den Quellwässern identifizierten mikrobiellen Gattungen sind denen in weniger extremen Wasser- und Bodenumgebungen ähnlich. Das breite Sortiment sowohl aerober als auch anaerober Bakterien zeigt lokale Schwankungen, vor allem bedingt durch Variationen in den Redoxbedingungen und im Nährstoffangebot. Einige der heterotrophen Bakterien konnten auf tierische Kontamination der Quellwässer zurückgeführt werden. Mehrere heterotrophe Bakterien, die bei $\text{pH} > 10$ (und in manchen Fällen bei $\text{pH} > 11$) wachsen können, wurden isoliert. Es ist jedoch sehr schwierig, unzweifelhafte Evidenz für die Lebensfähigkeit oberhalb dieser pH-Werte durch Laborexperimente zu gewinnen.

Die meisten Arten wurden als alkalotolerant identifiziert. Ausgehend von den üblichen Bewertungskriterien haben sich zwei der isolierten alkalotoleranten Gattungen als streng aerob erwiesen. Diese sind für eine anaerobe Endlagerumgebung nur von untergeordneter Bedeutung.

Von grossem Interesse ist der Nachweis, dass sulfatreduzierende Bakterien (SRB) in gewissen Wasser- und Sedimentproben vorhanden sind. Die SRB sind für die Abfallbeseitigung relevant, da sie an Reaktionen teilnehmen können, welche die Stahlkorrosion fördern und die Integrität von Beton beeinflussen. Obwohl sie nicht in grossen Populationen zu erwarten sind, zeigt ihre Existenz, dass sie Umgebungen mit einem $\text{pH} > 11$ besiedeln können, wenn auch nur in einem inaktiven oder nahezu inaktiven Zustand.

In einer nährstoffreichen, alkalischen Umgebung ist das Wachstum der SRB gehemmt. Dies deutet darauf hin, dass (unter experimentellen Bedingungen) der pH der wachstumskontrollierende Faktor ist. Messdaten über die mikrobielle Tätigkeit in den alkalischen Quellwässern zeigen jedoch, dass der hohe pH nicht den wichtigsten Faktor darstellt. Aus den hydrochemischen Daten geht nämlich hervor, dass das Wachstum der Populationen auch über das Nährstoffangebot (vor allem C, S, und P) kontrolliert sein kann. Dabei soll aber nicht vergessen werden, dass der Phosphorgehalt in den meisten Portlandzementen viel höher ist als derjenige in den Quellwässern. Die tiefen Gehalte an organischem Kohlenstoff in den Wässern sind denjenigen in vielen natürlichen Umgebungen ähnlich. Obwohl diese tiefen Gehalte eine untere Grenze für die Aufnahme und die Verwendung durch die meisten Bakterien und Pilze darstellen, können sie oligotrophe Aktivität unterstützen. Heterotrophe Bakterien, welche in den alkalischen Quellwässern dominieren und welche möglicherweise auch im Endlager vorhanden sind, könnten durch Hydrolyse von cellulosehaltigem Abfallmaterial zur Produktion von organischen Säuren beitragen. Die-

se Säuren könnten die Neutralisierung der hohen Alkalinität beschleunigen, die resultierenden organischen Säuren könnten für verschiedene Radionuklide solubilisierend wirken oder für andere Bakterien wiederum als Nährstoff dienen.

In den Quellwässern wurden die Spurenelemente Se, Pd, Sn, Zr, Ni, U und Th für den Vergleich zwischen gemessenen und vorausgesagten Löslichkeiten untersucht. Unter den gegebenen hochalkalischen, reduzierenden Bedingungen liegen die vorausgesagten Gleichgewichtskonzentrationen für Pd und Sn eindeutig tiefer als die analytischen Nachweisgrenzen, für die Elemente Ni, Th und U liegen die Voraussagen nahe bei der Nachweisgrenze. Im Falle des Urans ergaben sich durch die Verwendung verschiedener Datenbasen bereits bei der Voraussage gewisse Schwierigkeiten.

Mit Ausnahme von einigen Werten für Zr und U liegen die Konzentrationen der gemessenen Spurenelemente unter der experimentellen Nachweisgrenze. Aufgrund der analytischen Methode sind die Daten über Ni und Pd vorsichtig zu bewerten (Interferenz mit anderen Elementen). Die Messwerte für Se und Zr (möglicherweise auch Ni) sind bedeutend tiefer als die vorausgesagten Werte. Dies deutet darauf hin, dass in der verwendeten thermodynamischen Datenbasis relevante Festphasen möglicherweise nicht berücksichtigt sind. Eine solche Interpretation setzt aber voraus, dass das beobachtete Element in genügendem Ausmass im Gestein vorhanden ist. Beispielsweise deuten geochemische Befunde darauf hin, dass die in Frage kommenden Gesteinsformationen besonders Zirkon-arm sein könnten. In dieser Hinsicht ist vielleicht Ni von grösserem Interesse, da die bekannte Geochemie ein ausreichendes Nickel-Angebot vermuten lässt. Zumindest beim Th stehen die Voraussagen nicht im Widerspruch zur Messung. Alle Messungen liegen hier unter der Nachweisgrenze und unter der Voraussage. Beim Uran sind die Verhältnisse komplizierter. Die vorausgesagten Konzentrationen liegen deutlich über den gemessenen Werten und zeigen eine grosse Variabilität, abhängig von der verwendeten Datenbasis und vom Redoxpotential des Quellwassers. Dieses Beispiel zeigt klar die Problematik dieser Art Voraussagen auf und gibt Hinweise, auf welchem Gebiet neue Untersuchungen erforderlich sind.

In einem Falle deuten Filtrationsexperimente mit verschiedenen Filtergrössen darauf hin, dass das U und seine Töchter mit kolloidalem Material assoziiert sein könnten. In der Endlager-Sicherheitsanalyse sind die Kolloide als potentiell bedeutend anerkannt, es wird aber vermutet, dass sie bei hohem pH oder bei hoher Salinität zu Instabilität neigen. Der Hinweis, dass U und Töchter in diesen alkalischen Wässern in stabiler Kolloidform vorliegen könnten, ist von grossem Interesse und verdient weiter untersucht zu werden.

Obwohl eine positive quantitative oder halbquantitative "Validierung" der Modelle im allgemeinen nicht erreicht wurde, hat der Versuch wichtige Punkte im Vergleich zwischen gemessenen und vorausgesagten Löslichkeiten aufgezeigt. Es wurde auch gezeigt, dass ein solcher Vergleich für Elemente wie Se, Zr, Ni, U und Th analytisch durchführbar ist, obwohl fehlende Kenntnisse über natürliche Mineralverteilungen und Reaktionsgeschwindigkeiten eine Validierung von Modellen stark erschweren.

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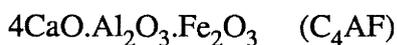
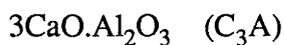
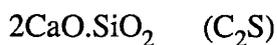
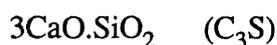
1. INTRODUCTION

1.1 Cement in the Nuclear Waste Repository Environment

Cement and concrete will form major components of many nuclear waste repositories. A front-runner for the conditioning of intermediate level radioactive waste is encapsulation in a cement-based matrix. A cement-based backfill could also be used as a packing matrix around drums emplaced in the repository. Concrete itself will also constitute a waste for disposal, particularly when it arises from decommissioning. Finally, concrete is a major structural component of many repository designs; therefore its long-term behaviour in a groundwater environment, and conversely the influence it has on solute mobilisation and transport within the repository and on groundwater chemistry and mineralogy in the neighbouring host rock, are fundamental to the repository integrity.

The favourable properties of cement-based matrices are their mechanical strength, low permeability, and maintenance of a high-pH environment in and around the matrix for a considerable length of time. This latter property is of particular importance in radioactive waste confinement since the solubility, and therefore potential mass transport, of most radionuclides is substantially lower in a strongly alkaline environment. A further beneficial characteristic of the high-pH environment is the severe inhibition of microbial activity which occurs in extreme pH conditions.

The chemical environment of cement or concrete is governed by the reactions between the solid phases and water, the distribution of these phases, the mobility of water and solutes within the system, and the rates of the various reactions in the system. Ordinary Portland Cement (OPC) comprises the following principal components in the anhydrous system $\text{CaO-Al}_2\text{O}_3\text{-Fe}_2\text{O}_3\text{-SiO}_2$:



as well as minor amounts of free CaO, alkali sulphates, gypsum, and other compounds. Hydration of these compounds, C_3S and C_2S reacting most rapidly, initially produces an

amorphous or colloidal calcium silicate gel and portlandite, Ca(OH)_2 . Maturation of these early products and continued reaction with other components results in progressive hardening of the cement; late-stage maturation reactions are very slow at normal temperatures, and the solid and solution phases remain out of long term equilibrium. The detailed chemistry of these processes is reviewed by Lea (1970), and summarised by Milodowski et al. (1985). Pore solutions within and adjacent to a setting cement mixture initially contain high concentrations of Ca^{2+} , K^+ , Na^+ , OH^- and SO_4^{2-} derived by dissolution and reaction between alkali sulphates and lime. As the reactions progress, SO_4^{2-} diminishes in solution as it is incorporated into the hydrated aluminate phases, and the pore-waters tend towards NaOH/KOH solutions saturated with Ca(OH)_2 . These solutions are highly alkaline with pH values up to 13. Estimates of the possible rates of leaching of Ca(OH)_2 out of a hypothetical repository due to groundwater flow suggest that the Ca(OH)_2 -dominated regime with pH above 12 might persist for up to 10^5 years followed by progressive decay of pH to around 8-10 after 10^6 years (Atkinson, 1985; Berner, 1986).

A further characteristic of the post-closure repository environment is the predicted onset of strongly reducing conditions due to early-stage oxidation/corrosion reactions in the waste-container-leachate system. The low redox potential, Eh, is another factor in depressing the solubilities of many radionuclides and therefore attenuating their release (Kemakta, 1985). A model of a hypothetical steel canister - concrete backfill system has suggested that highly reducing conditions will be established throughout the backfill in a period of about 100 years after closure (Sharland et al., 1986).

1.2 Natural Analogue Studies

This report describes a field-based study aimed at verifying and demonstrating the confidence to be placed in the beneficial properties arising from an alkaline environment. The study comprises a 'natural analogue' for the artificial cement-based matrix, in that a natural groundwater environment has been investigated where the water-rock reactions generate a water with the extreme alkalinity and chemical composition corresponding to equilibrium with, and precipitation of, cementitious minerals. Thus the system forms a natural analogue of cement-influenced pore-waters which has been stable for sufficient length of time to represent a reasonable approach to equilibrium. This sort of study is complementary to direct studies of cement mixtures and of water in contact with cement/concrete blocks in which the

influence of cement on the chemical and microbial environment may be modified in the course of time.

The essential role of natural analogue studies is to confirm that predictions based on theoretical or laboratory-based data are qualitatively and/or quantitatively appropriate for the natural environment (Chapman et al., 1984). This is of particular value in nuclear waste disposal safety assessments where chemical processes in the repository environment over extended timescales have to be considered. Natural analogues provide opportunities to assess the evolution of complex geochemical systems over appropriate timescales and to compare these with predictive models constructed from accumulated experimental and/or theoretical data.

In addition to the geochemical analogue, this study has provided an opportunity to examine the natural microbial populations in an accessible alkaline groundwater environment, whereas previous predictions have been based mostly on laboratory evidence of microbial tolerances of these extreme conditions. The viability and growth of microorganisms in a repository environment might be a significant factor in governing the rates of waste breakdown and leaching, of containment degradation, and of ultimate radionuclide transport through the near-field into the geosphere (West et al., 1984).

1.3 Oman Alkaline Springs

Highly-alkaline groundwaters in the natural environment are extremely rare. Most natural groundwaters have their pH values controlled by reactions with carbonate and silicate solid phases, having evolved from meteoric waters in which pH is determined principally by abundance of CO₂ in the soil zone environment. However groundwater discharges in which alkaline (pH>10) conditions persist have been identified in various geological localities around the globe. The best documented occurrences are in California (Barnes et al., 1972), Oman (Barnes et al., 1978; Neal and Stanger, 1983 and 1985), Cyprus (Pantazis, 1976), and Jordan (Barnes et al., 1982; Khoury et al., 1985). Most of these occurrences are associated with ultramafic rocks, i.e. sequences of ancient igneous (volcanic and/or intrusive) rocks whose compositions are within the general category of basaltic, although the alkaline water in Jordan is thought to originate in naturally calcined bituminous marl. Ultramafic rocks are relatively low in their silica contents and enriched in magnesium and iron (the "mafic" elements). In these cases, the process associated with the generation of extreme alkalinity is the alteration or "serpentinization" of these highly-reactive rocks by the

flux of ground-water. Present-day groundwaters exhibit alkalinity either due to the buffering by alkaline precipitates (e.g. portlandite, $\text{Ca}(\text{OH})_2$) from a past phase of serpentinization which commenced when the rocks were uplifted into a subaerial environment, or due to active serpentinization at the present. Extreme reducing conditions (i.e. low redox or Eh values) in these spring-waters are parallel features of the environment and are another significant factor in its value as a natural analogue.

The alkaline springs in Oman were selected for the present investigation because the hydrochemistry and some associated precipitates closely resemble the inferred conditions in cement pore-waters; there was also the advantage of previous studies of hydrochemistry and mineralogy (Neal and Stanger, 1983, 1984a, 1984b, 1985) coupled with knowledge of the sites and of logistics required for an efficient field operation.

1.4 Microbiological Analogue - Background

The natural bacterial populations indigenous to these alkaline springs provide an indication of what microbial activity might be viable in the cement-influenced reducing environment of a repository. Review information has indicated that in general microbial activity should be inhibited strongly above about pH 10 (Zajic, 1969). More recent studies have identified alkalotolerant and alkalophilic bacteria belonging to a number of genera, some being inhabitants of normal soil and water environments (Grant and Tindall, 1980; Krulwich and Guffanti, 1983; Langworthy, 1978; Souza et al., 1974). Several alkalotolerant photosynthetic microbial species have been isolated (Grant et al., 1979; Kallas and Castenholz, 1982) but these would not be of importance in sealed repositories. Amongst obligate alkalophiles, *Bacillus* species appear to be the most common (Clejan et al., 1986; Guffanti et al., 1980; Guffanti et al., 1986) and are easily recovered from most soils, presumably including those in the vicinity of a waste disposal site.

Experimental investigation of the potential microbial degradation of concrete is very limited so far (Grogan, 1987). It is possible that sulphur-oxidising bacteria can colonise at intermediate pH conditions under aerobic conditions, resulting eventually in the metabolic production of sulphuric acid which both lowers the pH further and attacks the concrete. The experimental evidence of microbial degradation of concrete under anaerobic conditions is very limited. The sulphate-reducing bacteria (SRB) are of particular interest to waste disposal for several reasons. They participate in steel corrosion (Hamilton, 1985; Philp et al., 1987) and may affect concrete integrity by H_2S production (Moosavi et al., 1985; Moosavi,

personal communication). Furthermore, they have the ability to withstand relatively high pH (Langworthy, 1978) and are generally tolerant of extreme environments (Herbert and Stott, 1983; Stott and Herbert, 1986; Willingham and Quinby, 1971; ZoBell and Morita, 1957).

In this study, samples were taken from various alkaline spring-waters in Oman in order to isolate and identify the bacterial populations as far as possible. These are compared to those likely to be found in a waste repository, with interest focussing on facultative and strict anaerobes in view of the likely reducing conditions in a repository. Ultimately, it would be important to know how such organisms will function in the cementitious environment of a waste repository and how they will affect radionuclide containment. Nutrient availability might also be a constraint on microbial viability in natural hyperalkaline waters. Theoretical calculations have shown how these geochemical constraints might limit the proliferation of microorganisms (West et al., 1984; Grogan, 1987). The present study therefore included some analyses of nutrients in the hyperalkaline spring-waters so that data on populations can be interpreted in the context of limiting factors in addition to the high pH.

1.5 Trace Element Analogue for Radionuclide Behaviour - Background

The hyperalkaline, chemically reducing, groundwaters have been used to test some of the predicted constraints on solubilities of radionuclides in a cement-influenced environment. Equilibrium chemical thermodynamics provides the usual approach for evaluating elemental solubilities and speciation in aqueous solution. However, application of this approach to the radionuclide trace elements of importance in nuclear waste problems is limited by the availability of relevant data and uncertainty over the applicability of this approach to natural groundwaters (Lindberg and Runnells, 1984).

A further complication for this specific application is that data derived mainly under normal aqueous solution chemistry (neutral to acidic) must be extrapolated to the very alkaline environment expected. Relatively few laboratory data are available which would allow validation of model predictions - particularly under strictly reducing conditions - and this limits the weight which can be placed on such predictions.

At first sight, examination of groundwater from Oman in order to test thermodynamic geochemical models might seem a rather contrived use of analogues in a situation where a laboratory experimental approach is more relevant. Although certainly not a substitute for laboratory work, the analogue study is complementary as:

- a) The chemical environment involved (high pH, very reducing conditions) is very difficult to simulate in the laboratory and is readily perturbed (e.g. by low-level CO₂ or O₂ contamination). Special approaches to ensure stable conditions e.g. pH or redox buffers, potentiostats etc. may introduce additional complications into the system (e.g. Meyer et al., 1984).
- b) Experimental timescales may be insufficient for equilibrium to be reached, particularly for complex systems in which several solid phases are involved. Although natural groundwaters may not represent a complete equilibrium, they have reaction times much closer to those considered in a safety analysis context.

It must be noted that a major limitation to the analogue approach to testing solubility limits of trace species is uncertainty of the sources within the geological system and hence of whether observed concentrations reflect trace solubility limits or if natural abundances or release rates are insufficient to reach saturation concentrations. Thus, if predicted solubilities are above measured concentrations, the model is not necessarily conservative (in a safety analysis sense) and may simply reflect input constraints, but if predictions are below measurements the model is certainly in error (non-conservative).

2 DESCRIPTION OF ALKALINE SPRINGS IN OMAN

2.1 Geological Setting

The Semail Ophiolite Nappe of northern Oman is a complete cross-section of up to 15km of oceanic crustal and upper mantle rocks which have been tectonically thrust into its present subaerial position, forming mountainous terrain with elevations of 500-1800m (Lippard et al., 1986). Penetration of meteoric water during and since uplift has caused an alteration process known as serpentinization in which the ultramafic (high-Mg, high-Fe) minerals are hydrated to serpentine minerals with concomitant release of iron which forms iron oxides. These serpentinization reactions (described in more detail in Section 2.2) result in the circulating water becoming both strongly alkaline and highly reducing, with the result that hyperalkaline springs, some of which degas substantial amounts of hydrogen gas, flow from the lower parts of the mountainous section of ophiolite rocks (Stanger, 1986). Individual flows vary from seepages to springs of up to 10 l s⁻¹. The springs are found mostly along the contact between the crustal and mantle sequences of basic and ultramafic rocks; the former comprises complex undeformed crystalline units of gabbros and basalts whereas the

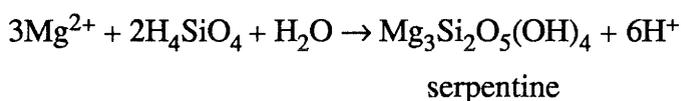
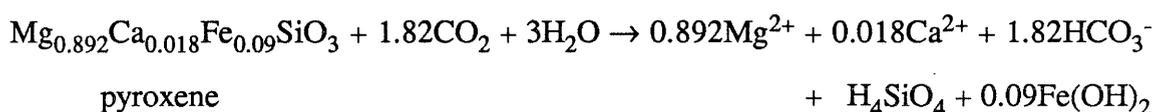
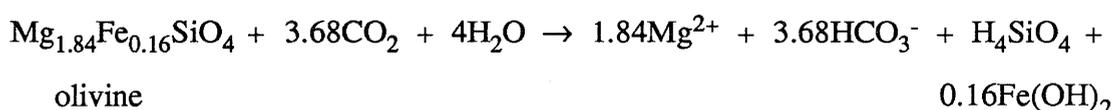
latter is partly serpentized dolerites and harzburgites which are intensely fractured, thus permitting widespread groundwater penetration.

2.2 Previous Studies and Geochemical Evolution of the Alkaline Springs

The previous studies of alkaline groundwaters in northern Oman by Neal and Stanger (1983, 1984a, 1984b, 1985) were concerned with their overall hydrogeochemical evolution, in particular the special geological and mineralogical conditions which give rise to the extreme alkalinities and also to the extreme reducing conditions. The hydrogeological context has been described in detail by Stanger (1986).

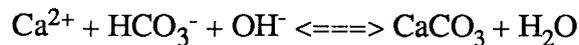
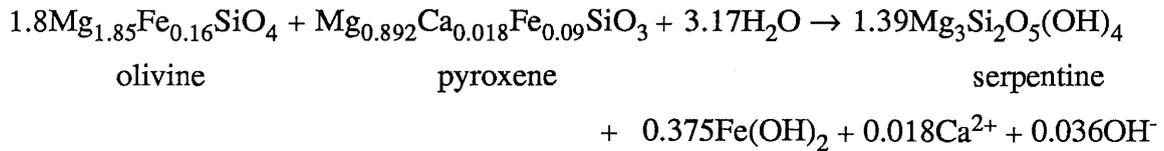
The chemical evolution of groundwaters in the Oman ultramafic environment from dilute Ca-HCO₃ or Mg-HCO₃ surface and shallow groundwaters at recharge to moderately saline Na-Cl-Ca-OH solutions in the springs is determined by the low temperature weathering of the two primary minerals in the host rock, olivine and pyroxene, and by the leaching of soluble sea salts trapped during the emplacement of the ophiolite from the ocean floor.

In the near surface zones, the reactions are dominated by weathering involving the introduction of atmospheric CO₂ and by evaporative processes in the arid climate. This promotes the generation of Ca and Mg bicarbonate-bearing waters and the formation of secondary components such as serpentine and the clay minerals. These reactions are typically:

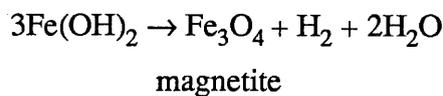
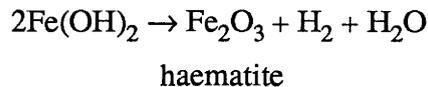


As these surface and near-surface waters enter the groundwater zone, olivine and pyroxene decomposition continues resulting in Ca²⁺ and OH⁻ ions being released to solution. Initially this process results in the precipitation of CaCO₃ until all of the introduced inorganic carbon from the surface and near-surface zones is consumed, after which further Ca²⁺ and OH⁻

release leads to the production of hyperalkaline conditions together with the precipitation of serpentine and iron oxides/hydroxides. This process can be described by the reactions:



Under the most alkaline conditions, $\text{Ca}(\text{OH})_2$ also precipitates. Na^+ and Cl^- are introduced by leaching of entrained marine salts during the decomposition stage. The release of iron from the olivine and pyroxene leads to the production of highly reducing conditions; hydrogen gas is generated by the concomitant oxidation of Fe^{II} iron and decomposition of water, which may be catalysed by transition metal hydroxides under these conditions (Neal and Stanger, 1983):



On complete consumption of the available pyroxene, olivine hydration continues to generate serpentine but with additional precipitation of brucite $\text{Mg}(\text{OH})_2$; the previously formed Ca^{2+} and OH^- remain stable in solution. Finally, with the exhaustion of the available olivine, the rock is completely serpentized, and the groundwater reverts to a $\text{Mg}^{2+}\text{-HCO}_3^-$ type while the previously-formed brucite redissolves or reacts to form magnesite MgCO_3 and/or dolomite $\text{CaMg}(\text{CO}_3)_2$ and/or huntite $\text{Mg}_3\text{Ca}(\text{CO}_3)_4$.

The chemical composition of the hyperalkaline groundwater emerging at spring sites can be modified in the vicinity of the springs by two processes. Firstly, the waters are highly reactive with CO_2 from the atmosphere, leading to the precipitation of CaCO_3 and the generation of Ca^{2+} -depleted, CO_3^{2-} -bearing waters. Secondly where the near-surface

HCO₃⁻-bearing waters mix with the hyperalkaline groundwaters, CaCO₃, brucite Mg(OH)₂ and serpentine precipitate. This precipitation leads to the generation of waters which are either CO₃²⁻ or OH⁻ bearing but are Ca²⁺ depleted.

2.3 Locations of Present Samples of Alkaline Groundwater

Seven springs were sufficiently accessible to be sampled within the limitations of a short field visit; their locations are shown in Figure 1. All seven are seepages with flowrates of ≤ 1 l s⁻¹. Of these sites, five (Sites 1, 3, 4, 5 and 7) were sampled for full hydrochemical analysis and three (Sites 1, 2 and 3) were chosen for comprehensive microbiological investigation, although other samples were also tested for microbial groups such as SRB.

Site 1: Nizwa Jill 1 (grid reference 5534 25287). Sediment and water samples were taken: the water samples for a range of microbial isolations and the sediment for SRB isolation only. At this site, a channel had been made by locals to carry water from the spring to the village. Water samples were taken at various points (1A to 1G) along the stream for field chemical analyses which showed no systematic change in pH, Eh and total alkalinity. The sample for full chemical analysis was taken near to the discharge point (1A). The samples for microbiology were taken from a point towards the end of the flow path (1F) where the water flow was low and where fine sediments had collected on the bottom of the channel (Plates 1 and 2).

Site 2: Nizwa Jill 2 - Birkat (grid reference 5534 25298). A small irrigation reservoir at the edge of a date plantation. Water and sediment samples were taken as above, and field chemical analyses were performed. The water was at pH 10.69 and +7mV Eh, although -580mV was measured in the sediment. In addition to being less reducing, the water here is also different in having a significant amount of carbonate alkalinity (66 mg l⁻¹) due to influx of atmospheric CO₂. This site was particularly suitable for microbiological studies because of a high input of organic matter from falling date leaves, which formed part of a thick sediment (Plates 3 and 4). Disturbance of sediment gave rise to much gas evolution and there was a faint but definite smell of hydrogen sulphide.

Site 3: Karku (grid reference 6067 25833). Karku springwater is used by locals but is not channelled. A relatively clean site upstream of the inhabited area was chosen for water sampling. A fine sediment of CaCO₃ was present (Plate 5).

Site 4: Jebel Awq (grid reference 5460 25220). Samples of water and sediment were taken for SRB enrichment. Some field chemistry was done on the water samples.

Site 5: Nidab (grid reference 6164 25678). Water and sediment samples were taken for SRB enrichment, and water for field chemical analyses.

Site 6: Hamaydayin (grid reference 5833 25238). Red and black material which appeared biological in origin was removed for examination.

Site 7: Bahla (grid reference 5296 25420). This proved an interesting site in that despite the high pH and reducing nature of the waters, it supported fish life. A sediment of CaCO_3 was present. At one position, gas (previously identified as H_2) was freely evolving.

3 CHEMICAL COMPOSITIONS OF GROUNDWATER SAMPLES

3.1 Field Measurements

Temperature, pH, alkalinity and Eh were measured on each sample at the time of collection in the field. pH was measured using a 91-62-00 Orion combination electrode and Model 230 meter; this was calibrated with pH 7 and pH 13 buffer solutions (the latter being saturated $\text{Ca}(\text{OH})_2$ for which appropriate temperature correction was made). An Orion 97-78-00 combination redox electrode was used for Eh measurements. Potentiometric alkalinity titrations were carried out in the field using a Hach digital titrator with a 1.6N H_2SO_4 acid cartridge. The hydroxide end-point was detected as an inflection point on a plot of pH against volume of acid titrated; there was no corresponding inflection which would indicate the presence of carbonate.

Samples for laboratory analyses of major and trace element chemistries were collected in ultraclean 500ml FEP™ (fluoro-polymer) bottles after filtration sequentially through $1\mu\text{m}$, $0.45\mu\text{m}$ and $0.1\mu\text{m}$ membrane filters. The used filters were retained for residue analysis. In addition, a 500ml sample at each site was reacted in bulk with a mixture of cation and anion exchange resins (10-20% by bulk volume of IR-120 and IRA-400, suitably conditioned in alkaline $\text{NaCl}/\text{Ca}(\text{OH})_2$ solution) in an attempt to separate charged from uncharged trace

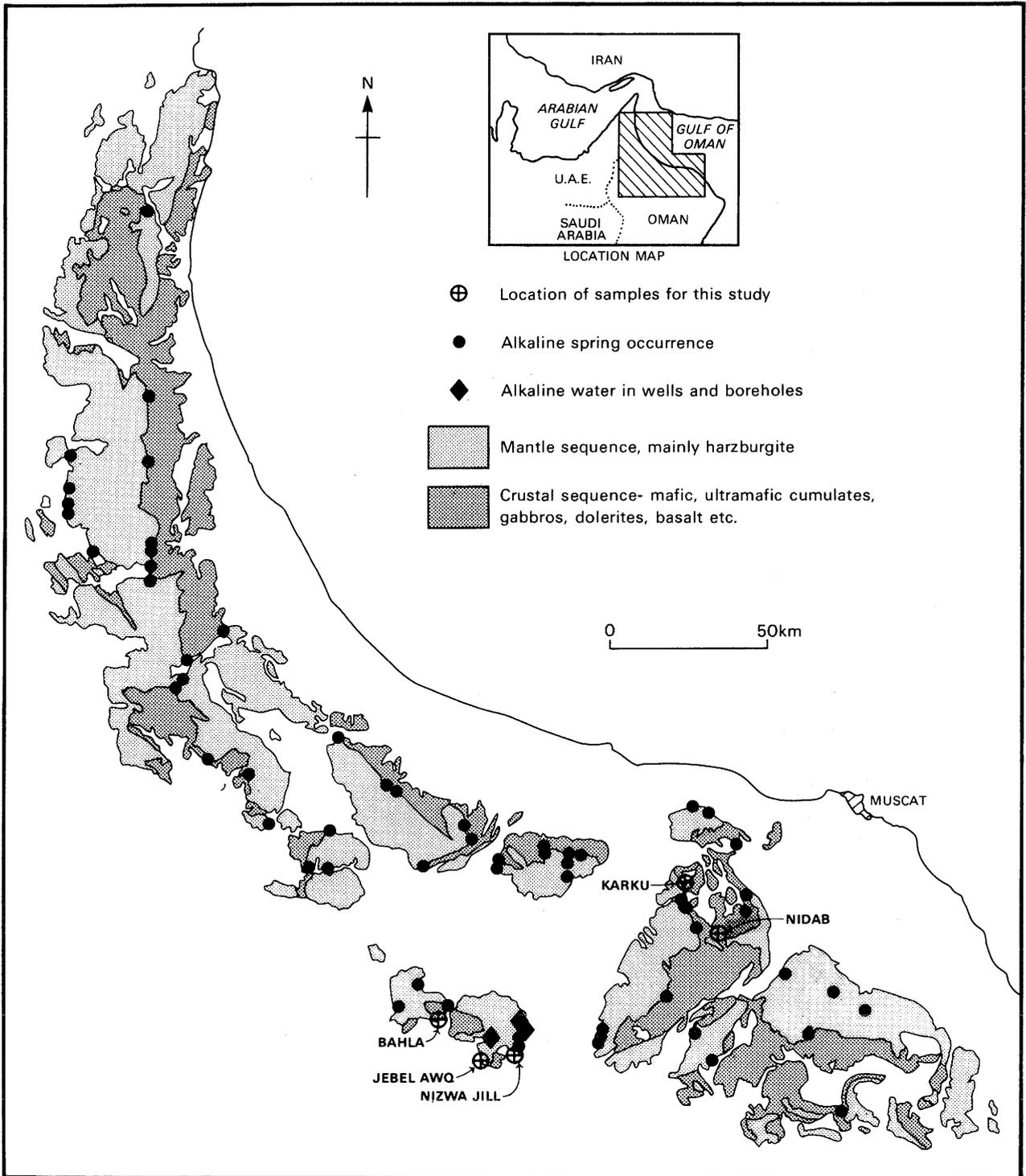
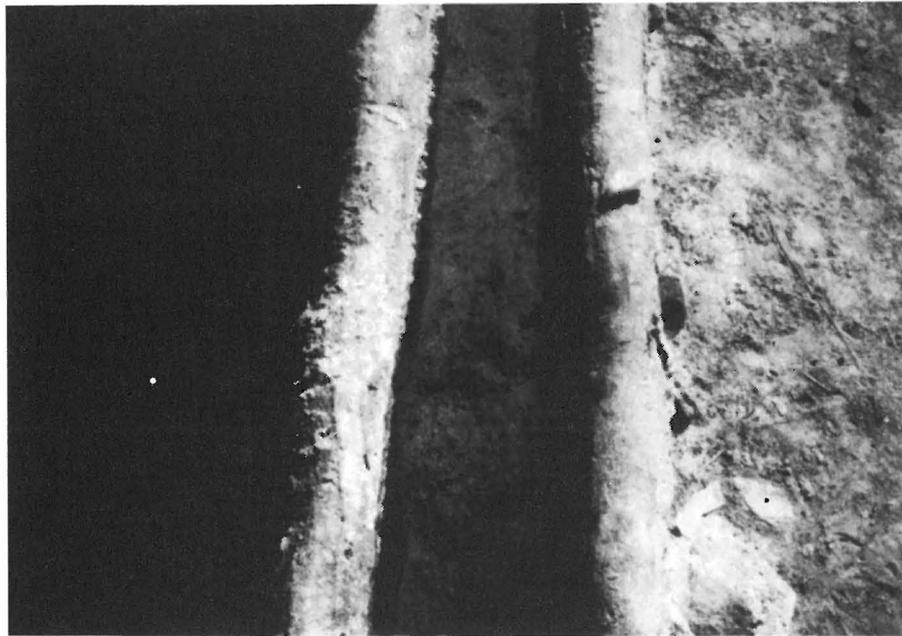
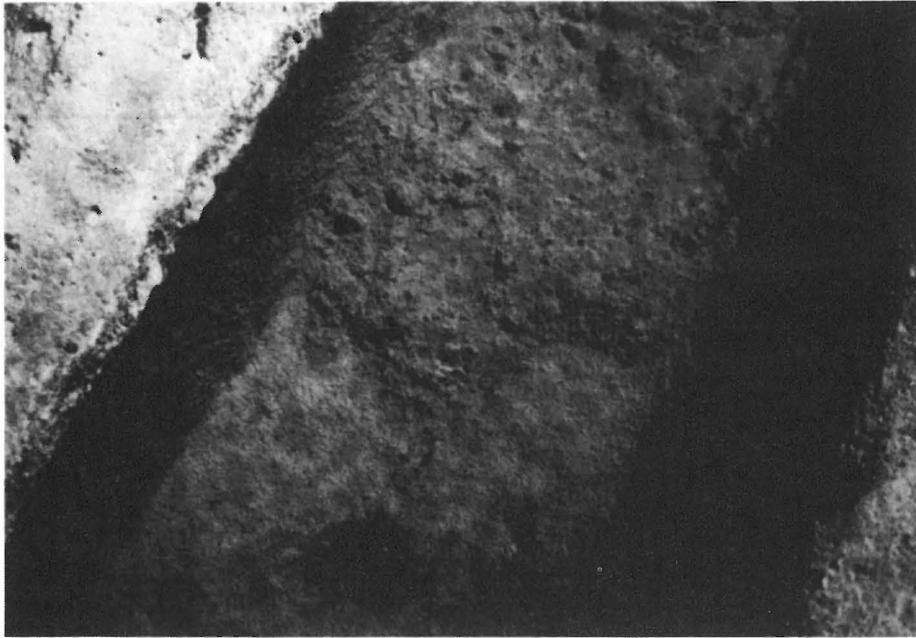


Figure 1. The distribution of ophiolites and hyperalkaline springs in northern Oman, showing the locations of sites from which samples were collected for this study.



Plates 1 and 2. Various points along a stream carrying spring-water at Nizwa Jill 1 (Site 1).



Plate 3. Distant view of Nizwa Jill 2 - Birkat (Site 2).

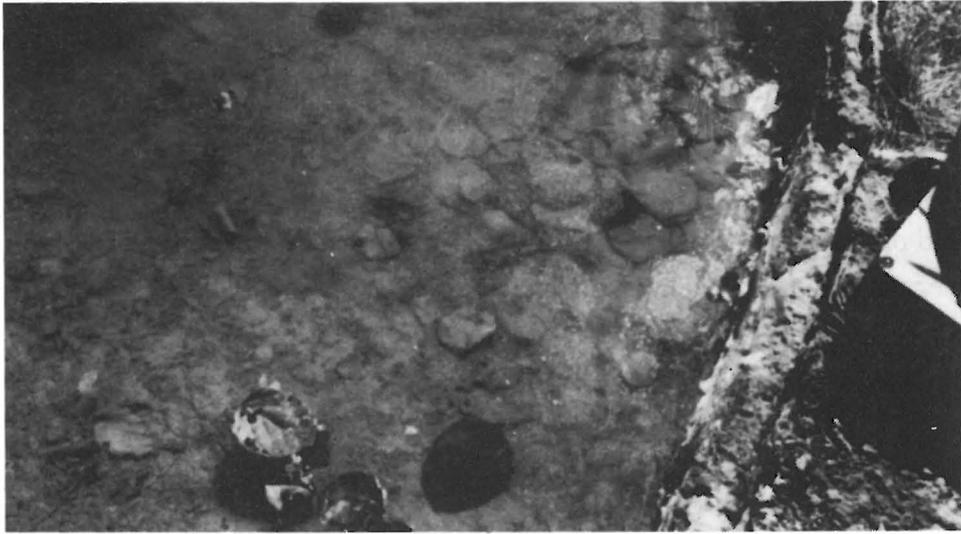


Plate 4. Close-up view of Nizwa Jill 2 - Birkat, showing leaves, sources of organic material to the spring-water and sediment.



Plate 5. A view of Karku (Site 3)

metal species. At two sites only (Sites 1 and 3), 5 litre filtered (0.45 μ m) samples were collected for uranium and U-Th isotope analysis.

3.2 Major and Minor Element Chemistries

Total alkalinity and pH were again measured on samples after return to the laboratory at BGS Keyworth. These were carried out on a Radiometer RTS 822 recording titration system at about 20°C. The results are shown in Table 1, in which they can be compared with the field measurements. The apparent discrepancy is attributable to the different temperatures of measurement, and the effect these have on the K_w value for dissociation of H_2O ($\log K_w = -14.19$ at 20°C and -13.74 at 34°C). The pH values calculated from respective values of $[OH^-]$ are ≤ 0.2 pH units higher than field pH and are very close to the lab pH values. The performance of the robust field electrode may account for the slight difference in the former values. Field pH values have been used for the trace element modelling in this report (Section 4). However the pH values calculated from $[OH^-]$ might be the more reliable in view of the 'robustness' of OH^- relative to the very subordinate H^+ in hyperalkaline solution - this is borne out by the excellent agreement of field and lab $[OH^-]$.

Anions were determined on the unacidified sample by ion chromatography (Dionex 2000i with AS4A anion separator and AG4A guard column). The results for Cl, Br, SO_4 , HPO_4 and NO_3 are shown in Table 1. Fluoride was measured by ion selective electrode. Ammonium ion was analysed with Nessler's reagent, using standard addition to overcome matrix interferences. Iodide and total organic carbon (TOC) were analysed at the Hydrochemical Laboratory of IoH Wallingford (analyst: C. Smith) by a spectrophotometric autoanalyser technique (Truesdale and Smith, 1975) and a Tocsin II analyser (Phase Separations Ltd.) respectively.

Major, minor and some trace cations were analysed by inductively-coupled plasma optical emission spectrometry (ICP-OES) using an ARL 35000 instrument at the BGS Wallingford laboratory. Solutions were acidified to 1% with HCl. The analytical results for these species are listed in Table 2. Note that the ICP method also analyses total S and P in solution, giving data (Table 1) which can be compared with the specific analyses for SO_4^{2-} and HPO_4^{2-} from ion chromatography, allowing an estimate of sulphur due to S^{2-} . The trace elements Ni, Zr, Cu, Co, Zn, Cd, Cr, Mo and La were found to be below detection limits for ICP-OES in all cases.

Table 1. Hydrochemistry of samples taken from alkaline springs in Oman, May 1986
(concentrations in mg l⁻¹ = ppm)

Site No	1 Nizwa Jill	3 Karku	4 Jebel Awq	5 Nidab	7 Bahla
pH (field)	11.24	11.44	11.39	11.16	11.41
pH (lab)	11.90	11.98	11.96	11.70	11.90
Eh (mV)	-173	-363	+33	-376	-372
Temp (°C)	33	35.7	30.6	34.9	34.1
Na ⁺	218	258	603	130	189
K ⁺	9.2	11.2	27.8	3.6	8.4
Ca ²⁺	54.7	72.0	55.2	63.5	62.2
Mg ²⁺	<0.1	<0.1	<0.1	<0.1	<0.1
OH ⁻ (field)	84	104	104	56	83
OH ⁻ (lab)	81	104	100	55	77
CO ₃ ²⁻	0	0	0	0	0
Cl ⁻	291	351	858	193	275
SO ₄ ²⁻	0.91	2.39	34.1	5.6	0.19
ΣS	0.39	26.8	11.2	18.9	<0.17
¶S ²⁻	-	26.0	-	17.0	-
HPO ₄ ²⁻	<0.1	0.95	<0.1	1.64	<0.1
§ΣP	<0.015	<0.015	<0.015	<0.015	<0.015
NO ₃ ⁻	0.38	0.43	31.2	0.20	0.18
NH ₄ ⁺	<0.25	0.31	<0.25	1.54	0.58
F ⁻	<0.05	<0.05	<0.05	<0.05	<0.05
Br ⁻	0.38	0.43	1.08	0.35	0.37
I ⁻	0.033	0.100	0.060	0.026	0.041
Tot Org C	2.5	2.1	1.5	0.5	2.3
± Charge balance-1.7%	-3.0%	-2.9%	+0.5%	-4.5%	

¶S²⁻ = difference between ΣS (total S obtained by ICP) and SO₄²⁻ (by ion chromatography).

§ΣP = analysis of total P by ICP; cf.HPO₄²⁻ obtained by ion chromatography.

3.3 Trace Element Analyses

A new ultra-sensitive analytical technique, inductively-coupled plasma source mass spectrometry (ICP-MS), was used in an attempt to quantify concentrations of some relevant analogue trace elements. The instrument used was a VG Isotopes PlasmaQuad™ in the Chemical Analysis Group at AERE Harwell. Samples were aspirated into the plasma from solution acidified to pH <2 with HNO₃. Detection limits using single ion monitoring for many heavy elements by ICP-MS are between 0.1 and 0.01 ng ml⁻¹ (ppb) and are therefore 2-3 orders of magnitude better than for ICP-OES. Although the technique is very sensitive for Ni, the measurements of this element must be considered suspect because the cone for transmitting ions into the mass spectrometer is made of nickel and therefore introduces a low but variable blank for this element. During the interpretation of raw analytical data from these samples, it was discovered that the Pd peak at m/e 105 suffers interference from SrOH⁺, and as a result the low but detectable signal at this mass is not necessarily indicative of Pd abundance. Analyses of Zr, Sn, Ce, Nd, Eu, Au, Th and U were attempted by ICP-MS, some results of which are shown in Table 3; data are generally below limits of detection, even for this very sensitive technique.

Se was analysed by the generation of selenium hydride which was measured directly in a Perkin-Elmer 403 atomic absorption spectrometer using a P-E MHS-10 hydride generator.

3.4 U and Th Isotope Measurements

Measurements of U and Th isotopes by α -spectrometry were carried out on the two large volume water samples collected at the Nizwa and Karku springs (Sites 1 and 3). These analyses were carried out by the Uranium Disequilibrium Section at A.E.R.E. Harwell. The results are shown in Table 4, from which U and Th analyses may be compared with values obtained by ICP-MS in Table 3. It should be noted that samples for ICP-MS analysis were filtered to 0.1 μ m whereas the larger water samples for α -spectrometry analysis were filtered only to 0.45 μ m. The measured U concentrations are consistent for Nizwa but for Karku the value by α -spectrometry is very significantly higher. Th concentrations measured by the two methods are consistent.

These results may be interpreted as suggesting that 'colloidal' material containing U has enhanced the total value for U in the more coarsely filtered sample (M. Ivanovich, personal

Table 2. Analyses of major, minor and trace cations by ICP-OES
(concentrations in mg l⁻¹ = ppm)

Site No	1 Nizwa Jill	3 Karku	4 Jebel Awq	5 Nidab	7 Bahla
Na ⁺	218	258	603	130	189
K ⁺	9.2	11.2	27.8	3.6	8.4
Ca ²⁺	54.7	72.0	55.2	63.5	62.2
Mg ²⁺	<0.1	<0.1	<0.1	<0.1	<0.1
ΣSi	0.29	0.42	0.81	1.29	0.13
ΣAl	<0.1	<0.1	<0.1	<0.1	<0.1
Sr ²⁺	0.27	0.28	0.62	0.27	0.14
Ba ²⁺	0.017	0.003	0.007	<0.002	<0.002
Li ⁺	0.017	0.021	0.024	<0.010	0.021
B	<0.02	<0.02	<0.02	<0.02	<0.02
ΣFe	<0.01	<0.01	<0.01	<0.01	<0.01
ΣMn	<0.002	<0.002	<0.002	<0.002	<0.002
Ni	<0.05	<0.05	<0.05	<0.05	<0.05
Zr	<0.015	<0.015	<0.015	<0.015	<0.015
Cu	<0.01	<0.01	<0.01	<0.01	<0.01
Zn	<0.015	<0.015	<0.015	<0.015	<0.015
Co	<0.02	<0.02	<0.02	<0.02	<0.02
Cd	<0.02	<0.02	<0.02	<0.02	<0.02
La	<0.02	<0.02	<0.02	<0.02	<0.02
Cr	<0.04	<0.04	<0.04	<0.04	<0.04
Mo	<0.10	<0.10	<0.10	<0.10	<0.10

Table 3. Analytical results for some trace elements by the ICP-mass spectrometry technique
 (analyst: Dr R M Brown, Chemical Analysis Group, AERE Harwell)
 (concentrations in $\text{ng ml}^{-1} = \text{ppb}$)

Site No	1 Nizwa Jill	3 Karku	4 Jebel Awq	5 Nidab	7 Bahla
Se [¶]	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
Pd [§]	(0.3)	(0.3)	(0.7)	(0.4)	(0.3)
Sn	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
Zr	< 0.1	< 0.1	< 0.1	0.2	0.1
Ni [#]	(< 0.7)	(1.2)	(< 0.7)	(2.0)	(1.6)
Ce	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04
Nd	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
Eu	< 0.06	< 0.06	< 0.06	< 0.06	< 0.06
Au	< 0.09	< 0.09	< 0.09	0.1	< 0.09
Th	< 0.04	0.04	< 0.04	< 0.04	< 0.04
U	< 0.01	< 0.01	0.01	< 0.01	< 0.01

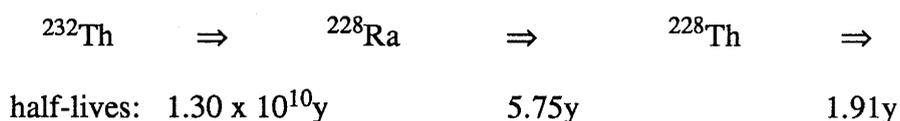
Detection limits were determined by adding 1ppb of each element to sample 3 and expressing twice the standard deviation of the noise level measured for a 1% HNO_3 blank expressed in concentration terms.

[¶]Se analysis by selenide generation and atomic absorption spectrometry.

[§]Pd analytical values are suspected too high due to analytical interference from SrOH^+ on the mass spectrum.

[#]Ni analytical values are suspected too high due to enhancement arising from the nickel inlet cone on the ICP-MS instrument.

communication). This is supported by the very high $^{228}\text{Th}/^{232}\text{Th}$ ratio in the Karku sample (Table 4) which is around 178 and therefore far from secular decay equilibrium, indicating that colloids are probably present to stabilise ^{228}Th in the groundwater. This nuclide might become mobile in the groundwater due to its origin from ^{228}Ra in the Th radioactive decay series:



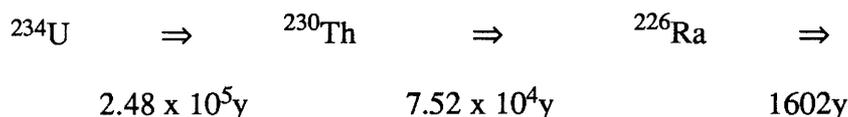
The occurrence of anomalous ^{228}Th despite its short half-life and that of its parent ^{228}Ra indicates that production from the ^{232}Th parent is continuous. A mechanism by which ^{228}Th might be enhanced involves the ^{228}Ra precursor being enhanced in the groundwater either due to its intrinsically higher solubility relative to Th or due to α -particle recoil effects accompanying ^{232}Th decay. Decay of this labile ^{228}Ra might produce ^{228}Th which is more likely to become associated with colloids than the immobile ^{232}Th nuclide. It is worth noting that preliminary evidence from studies in Australia suggests that ^{228}Th in groundwaters tends to be preferentially associated with the $<1\text{nm}$ fraction, whereas ^{232}Th tends to be associated with the $>1\mu\text{m}$ fraction (M. Ivanovich, personal communication).

Table 4. Analytical results for U and Th isotopes by α -spectrometry
(Analyst: M.A. Wilkins, Uranium Disequilibrium Section, AERE Harwell)
(Analyses carried out on $<0.45\mu\text{m}$ -filtered solutions)

Site No		1 Nizwa Jill	4 Karku
U	(ng g ⁻¹)	0.009±.002	0.23±.01
U [†]	(ng ml ⁻¹)	<0.01	<0.01
Th	(ng g ⁻¹)	0.016±.003	0.004±.001
Th [†]	(ng ml ⁻¹)	<0.04	<0.04
$^{234}\text{U}/^{238}\text{U}$		1.15±.31	1.23±.05
$^{230}\text{Th}/^{234}\text{U}$		1.45±.32	0.12±.01
$^{230}\text{Th}/^{232}\text{Th}$		3.19±.69	40.0±9.0
$^{228}\text{Th}/^{232}\text{Th}$		3.12±.88	178±10

[†]U and Th values obtained by ICP-MS on $<0.1\mu\text{m}$ filtered samples; see Table 3.

^{230}Th originates in the U radioactive decay series:



and its activity is therefore not directly related to that of ^{232}Th , and no conclusion can be drawn directly from the value of around 40 for the $^{230}\text{Th}/^{232}\text{Th}$ ratio (Table 4). However this value is high relative to that of around 3 measured on the Nizwa Jill sample. This contrast might be linked to the inferred presence of colloidal U in the Karku sample: ^{230}Th would be relatively enhanced in a colloidal phase with which ^{234}U is also associated. It is also interesting to note that the total Th concentrations shown in Table 4 are uniformly low and do not indicate a quantitative increase of Th in the coarsely-filtered Karku sample. This supports the model described here whereby Th nuclides become mobile in colloidal association only when they have as precursors nuclides which are themselves mobilised by solubility or by recoil effects.

The overall implications of these data are that some form of 'colloidal' material may be stable under these highly alkaline solution conditions and that this material may contain U and Th, depending on sources. However it must be concluded that these present data are clearly insufficient to arrive at definitive conclusions, although they indicate some interesting and potentially important aspects of U and Th behaviour. A final note of caution is necessary over analytical uncertainties and sampling problems which may also contribute to exceptional overall uncertainty on the values in Table 4, particularly for the Th isotope ratios taking account of the low abundance of Th.

3.5 Compositions of Resin-Retained Species, Filter Deposits and Natural Precipitates

3.5.1 Resin-retained species

On return to the laboratory, the mixed ion-exchange resin samples were washed with distilled water prior to adding 25ml of 4M Aristar HNO_3 . The acidified mixture was shaken and left overnight after which the supernatant solution was collected. This procedure was repeated, giving a total of 50ml of combined eluent which was filtered through a $0.2\mu\text{m}$ Acrodisc™ filter and stored in a Nalgene FEP bottle. A blank solution was prepared by the same procedure. The solutions were analysed for relevant species by the methods described above.

The analytical results for the eluent solutions are shown in Table 5 which also lists the calculated values of equivalent concentrations in the original water samples. These values can be compared to measured total solution concentrations from Table 2.

It is clear from the data in columns 'b' of Table 5 that the mixed ion-exchange resin retained only a fraction of the total ionic species in the groundwater solutions. For example, about 30-40% only of total K was retained on the resin. This behaviour is repeated by other species: Sr, Ba, Li, mostly with lower proportions being retained. However Si is very close in some cases to the total solution value, which suggests a more efficient removal process. In the high pH conditions, Si is present as both uncharged Si(OH)_4 and as anionic species and the high recovery suggests that the latter predominates (but see below, Section 3.5.2). The total sulphur analysis could include both sorbed SO_4^{2-} and S^{2-} . The comparable total solution analyses are shown in Table 1 which also indicates the relative amounts of the two species: for instance, Karku and Nidab have predominant S^{2-} whereas Jebel Awq has predominant SO_4^{2-} . The ΣS results suggest that SO_4^{2-} is retained by the resins more efficiently than S^{2-} . In summary, these experiments with mixed ion-exchange resins have had only limited success, giving only a qualitative guide to ion abundances and no indication of speciation (it is implausible that the low recoveries were due to predominance of uncharged species in solution). No further trace element analyses have been considered worthwhile on these solutions. However this type of experiment with ion exchange resins could be improved by careful selection and pretreatment of resins in the light of these results.

3.5.2 Filter deposits

The deposits on filters used in the field were dissolved by adding 20ml of 4M HNO_3 to a half of each filter in cleaned 30ml high-density polyethylene containers. The solutions were left for 2 hours in a sonic bath. After 2 days standing, the solutions were filtered through 0.2 μm . A blank solution was prepared by the same procedure. Solutions were analysed as above, and the results are shown in Table 6.

The only data worth noting are the higher Si values coming from the 1 μm filters, indicating that Si may be present in particulate form, or may be sorbing onto the first filter in the series (a tendency to sorb onto surfaces could also explain efficient resin retention of Si - see

Section 3.5.1). The higher Ca value on the 0.1 μ m Karku filter is explained by precipitation of CaCO₃ during the filtration - this is illustrated very well on scanning electron micrographs (A. E. Milodowski, personal communication; Plates 6 and 7). The SEM photographs of filters from Nizwa Jill and Karku samples otherwise show no systematic differences in particle types or abundances with filter size. Particles include possible organic fragments, spores up to 10 μ m size, possible bacterial clusters (unidentifiable due to drying), clay particles and occasional Fe-oxide and Ca-rich particles - both presumably artefacts of aeration during sampling.

3.5.3 Natural precipitates

Samples of natural precipitates associated with the alkaline springs were collected and retained in 30ml Sterilin polypropylene containers. On return to the laboratory, weighed subsamples (<1g) of a selection of these precipitates (dry or wet) were placed in cleaned Sterilin tubes and 5ml of 8M HNO₃ was added. These were then left for 2 days, after which the resulting solutions were transferred to 10ml graduated flasks and made up to volume with deionised water. These solutions were filtered through 0.2 μ m prior to analysis. A blank solution was also made up by this procedure. The analytical results as micrograms per gram of sample are listed in Table 7. In the cases of wet precipitates which were amorphous or otherwise impossible to filter and dry, these results cannot be related quantitatively to the composition of the solid.

The compositions of natural precipitates in all cases, except the Jebel Awq salt crust, are dominated by Ca \pm Mg. It is concluded that these are mixtures of Ca(OH)₂ and CaCO₃, depending on the extent of reaction with atmospheric CO₂; it is probable that complete conversion to CaCO₃ has occurred during storage of samples prior to analysis. The Mg content of the Karku precipitate is higher in equivalent terms than the Ca, indicating the presence of magnesite, dolomite or huntite (see Section 2.2). It is interesting that this Karku precipitate also has the highest Ni value; Ni and Mg have the same source - the alteration of olivine in the primary rock. The Ni could be precipitating as a carbonate or could be co-precipitating with Fe as oxide or carbonate (compare with Section 4.3). The Nizwa Jill concretion, which is predominantly CaCO₃, shows a similar Ca-Mg-Fe-Ni relationship. It also contains substantial S (probably as SO₄²⁻) which is interesting in view of the very low

dissolved S in the corresponding groundwater (Table 1) and the confirmed presence of sulphate-reducing bacteria (Section 5.2). This precipitate contains notably high contents of K, Ba, Li, B and Si which are attributed to the 'scavenging' effect of very slow concretionary growth in this case.

4 TRACE ELEMENT MODELLING

4.1 Background

The background to the trace element analyses and modelling has been described in Section 1.5. Trace elements selected for analysis and thermodynamic modelling were some of those identified as key components of the L/ILW inventory in the Swiss Project Gewähr 1985 safety analysis (Nagra, 1985).

As already stated, uncertainty over the source mineral phases and abundances of relevant trace elements is a major limitation in the attempt to obtain quantitative validation from the natural analogue. In the present case, there is substantial host rock geochemical data for only Ni and Zr of the modelled trace elements (this is because these are used as important indicator elements in petrogenetic studies; Lippard et al., 1986).

4.2 Thermodynamic Models and Databases

The calculations reported here were carried out using two well-known thermodynamic equilibrium codes, MINEQL and PHREEQE, which have been described elsewhere (Westall et al., 1976; Parkhurst et al., 1985). Both codes were run at EIR, Switzerland, where databases for both codes are undergoing continual revision and expansion. An extensive actinide database for MINEQL (Schweingruber, 1983) was used for modelling U and Th. The speciation and solubility of these species were also evaluated using a database from NEA (NEA, 1986). PHREEQE was used to model Se, Ni, Zr, Pd and Sn using a database compiled predominantly from the NEA, Benson and Teague (1980), and Smith and Martell (1976). Although this database has not been fully reviewed, this work is currently in progress. Due to uncertainties in thermodynamic data for sulphide solid phases and the lack of equilibrium within the natural S system, these species were not considered for the base calculations but are discussed qualitatively where relevant. Calculations of speciations and solubilities for trace elements in hypothetical and real solutions, such as the present case, are

Table 5. Analyses by ICP-OES of eluent solutions from ion exchange resins

500ml groundwater sample → resin → 50ml 4M nitric acid. The calculated equivalent concentrations in the original water samples are also shown, for comparison with total solution analyses. Concentrations in mg l⁻¹, blank corrected.

Site No	1		3		4		5		7	
	Nizwa Jill		Karku		Jebel Awq		Nidab		Bahla	
	a.	b.	a.	b.	a.	b.	a.	b.	a.	b.
K	30.2	3.0 ⁹	37.4	3.7 ¹¹	91.8	9.2 ²⁸	16.6	1.7 ⁴	34.9	3.5 ⁸
Mg	<0.14	-	<0.14	-	<0.14	-	<0.14	-	<0.14	-
ΣSi	2.69	0.3 ^{0.3}	1.82	0.2 ^{0.4}	2.72	0.3 ^{0.8}	4.24	0.4 ^{1.3}	1.20	0.1 ^{0.1}
Sr	0.35	0.04 ^{0.3}	0.40	0.04 ^{0.3}	1.19	0.1 ^{0.6}	0.68	0.07 ^{0.3}	0.19	0.02 ^{0.1}
Ba	.008	.0008 ^{.02}	.013	.001 ^{.003}	.004	.0004 ^{.01}	<.002	-	<.002	-
Li	.034	.003 ^{.02}	.035	.004 ^{.02}	.045	.005 ^{.02}	<.005	-	.046	.005 ^{.02}
B	.021	.002 ^{<.02}	<0.01	-	<0.01	-	<0.01	-	<0.01	-
Fe	0.24	0.02 ^{<0.01}	0.22	0.02 ^{<0.01}	0.18	0.02 ^{<0.01}	0.23	0.02 ^{<0.01}	0.17	0.02 ^{<0.01}
Mn	<.004	-	<.004	-	<.004	-	<.004	-	<.004	-
Ni	<.085	-	<.085	-	<.085	-	<.085	-	<.085	-
Zr	<.045	-	<.045	-	<.045	-	<.045	-	<.045	-
ΣS	0.8	0.08 ^{0.4}	1.4	0.14 ²⁷	31.6	3.2 ¹¹	10.0	1.0 ¹⁹	1.2	0.12 ^{<0.2}

- a. Eluent concentrations (Ca, Na and Cl were not measured because resin was pretreated with Ca-Na-Cl-OH.
b. Calculated equivalent concentrations in original water, compare with total solution analysis shown in italics in superscript (from Table 2).

Table 6. Analyses of solutions derived by acid washing (20ml 4M HNO₃) of filters used for groundwater sampling at Nizwa Jill and Karku sites.
(Concentrations in mg l⁻¹, blank corrected).

Filter size	----- Nizwa Jill -----			----- Karku -----		
	1μ	0.45μ	0.1μ	1μ	0.45μ	0.1μ
Na	1.66	2.34	2.86	1.38	2.78	2.62
K	<2	<2	<2	<2	<2	<2
Ca	.96	1.34	1.34	3.32	1.26	5.62
Mg	<.28	<.28	<.28	<.28	<.28	<.28
ΣSi	.40	<.16	.22	.76	<.16	<.16
Sr	<.004	<.004	.004	.010	.004	.008
Ba	<.004	.014	<.004	<.004	<.004	<.004
Li	<.010	<.010	<.010	<.010	<.010	<.010
B	<.02	<.02	<.02	<.02	<.02	<.02
Fe	.12	<.04	<.04	.05	<.04	.062
Mn	<.008	<.008	<.008	<.008	<.008	<.008
Ni	<.17	<.17	<.17	<.17	<.17	<.17
Zr	<.9	<.9	<.9	<.9	<.9	<.9
ΣS	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5

Table 7. Analysis of acid-soluble (8M HNO₃) fractions of natural precipitates associated with hyperalkaline springs.

µg g⁻¹ of sample, blank corrected.

Site	1	2	3	4	4	7
	Nizwa Jill concretions	Nizwa Jill - Birkat settling tank sediment	Karku platelets (wet)	Jebel Awq bottom sediment (wet)	Jebel Awq salt crust	Bahla sediment (wet)
Na	27,100	507	33.1	584	323,000	114
Ni	145	30.0	554	36.0	<22	61.3
K	4090	<171	<131	<112	1630	<163
S	25,900	1,609	431	436	384	1,130
Zr	<70	<7.7	<5.9	<50	<11.5	<7.3
B	164	1.88	43.5	<1.1	<2.6	7.01
Si	125	<13.7	<10.5	<9.0	<20.5	<13.1
Mg	42,700	5,360	73,200	4770	1490	11,100
Mn	320	29.8	230	14.8	8.45	129
Ca	412,000	330,000	80,900	103,000	2650	233,000
Fe	9640	1580	13,900	806	415	2990
Sr	4602	197	413	591	10.8	700
Ba	956	10.1	14.5	10.1	1.28	9.95
Li	84.2	<0.86	1.83	<0.6	<1.28	<0.81

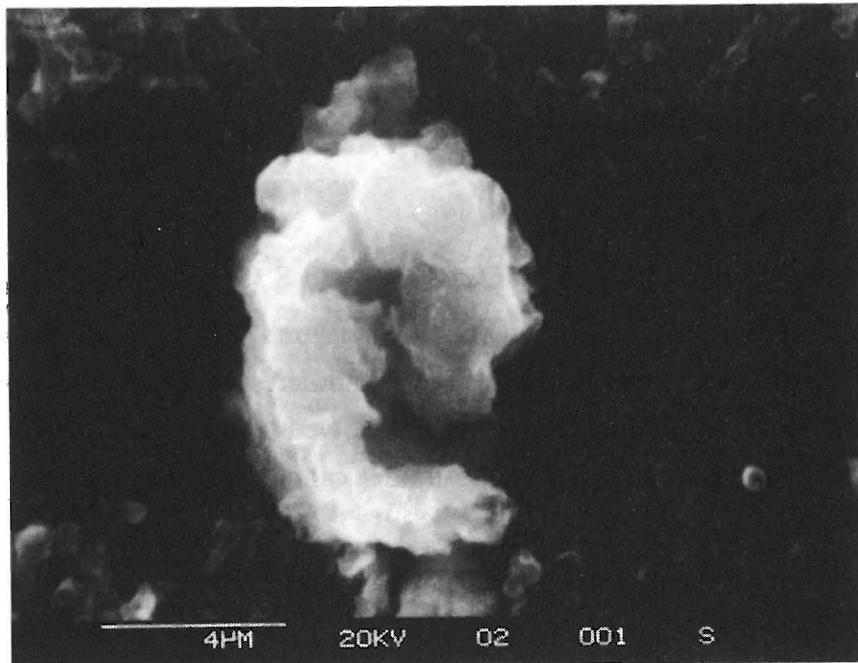


Plate 6. Scanning electron micrograph of probable clay particle retained on 0.45µm filter during membrane filtration of Karku alkaline groundwater.



Plate 7. Scanning electron micrograph of 0.1µm filter used for Karku groundwater sample, showing a rhombic crystal of calcite grown partly within the membrane itself. It is suggested that the precipitation of calcite in this case has been induced by the filtration process introducing CO₂ into the Ca-OH groundwater.

an important basis for review and comparison with other databases and are currently planned within the CEC ChemVal project.

Both codes clearly show that the N ($\text{NO}_3^-/\text{NH}_4^+$) and S ($\text{SO}_4^{2-}/\text{S}^{2-}$) systems are not in equilibrium with each other or with the field measured 'Eh'. This is not unusual for groundwaters (e.g. Lindberg and Runnells, 1984) and here is assumed not to compromise the basic assumption of equilibrium between the dissolved species. The N and S couples are, however, not allowed to equilibrate in the model calculations in order to preserve the chemistry as measured.

4.3 Model Predictions

4.3.1 Fission / activation products

The behaviours of the elements Se, Pd, Sn, Zr and Ni were modelled using the PHREEQE code. Calculated solubility limit concentrations, dominant species in solution, and the solid phases inferred to control solubilities in the model are presented in Table 8. Several points should be noted:

- The database used for these elements is rather small and there is a risk that important species are missing from the calculations. In general, neglecting an important solution-phase species will result in under-estimation of solubility whilst missing an important solid phase could only result in a 'conservative' overestimate.
- The quality of the thermodynamic data is very variable. The importance of apparently small uncertainties in the source data (standard free energies and enthalpies of formation) cannot be overemphasised. Typically such errors would be in the range 1-10 kJ/mol for the species involved which translates to an uncertainty in the calculated solubility of up to *3 orders of magnitude*.
- Se shows a marked difference in solubility between the oxidising and reducing waters due to the selenite-selenide transition. It should be noted, however, that the closely analogous sulphur system does not appear to be in equilibrium with the measured Eh while recent experimental work shows similar non-equilibrium for the selenate-selenite system (Runnells et al., 1987). Therefore the overall applicability of, and species relevant to, an equilibrium approach to this element are difficult to assess.

- The solubility of Pd is very low for the most oxidising case and negligible for all other waters. No real significance can be attributed to differences between concentrations at the 10^{-20} M level but it may be noted, in any case, that consideration of the PdS solid phase would lower further the predicted solubilities for the Karku and Nidab conditions by 9-10 orders of magnitude.
- The solubility of Sn is calculated to be negligible in all cases but predicted values must be considered with caution as no data for stannates (e.g. $\text{Sn}(\text{OH})_6^{2-}$) or organo-tin aqueous complexes were included in the database.
- The solubility of Zr is calculated to be relatively high in all cases while that of Ni is relatively low. In the latter case, consideration of NiS as a solid phase would lower solubility further for the Karku and Nidab cases by 5-6 orders of magnitude (no data were found for an equivalent ZrS_2).

4.3.2 The actinides

Th and U were modelled using MINEQL and two different databases - one compiled at EIR for safety assessment modelling and one from the NEA. The results of these calculations are shown in Table 9.

- The picture for Th is remarkably consistent for the two databases. To some extent this can be explained by the relatively straightforward chemistry of this element. However the consistency between the two databases is not necessarily an indication of correctness since data for Th are relatively scarce and, to a large extent, the same sources are used for both databases.
- The solution species and solid phase model for U is much more complicated. There is a general trend of decreasing solubility with increasingly reducing conditions but otherwise there are considerable differences between the two databases: up to three orders of magnitude between predictions. The relation between solubilities predicted by the two databases varies between springs (e.g. NEA>EIR for Nizwa and Jebel whereas EIR>NEA for the three more reducing waters). Note that the EIR database assumes the existence of the somewhat 'dubious' $\text{U}(\text{OH})_5^-$ species (e.g. Bruno et al., 1987) which is excluded from the NEA database.

Table 8. Calculated solubilities and predominant solution species for Se, Pd, Sn, Zr and Ni

Site No	1	3	4	5	7
	Nizwa Jill	Karku	Jebel	Nidab	Bahla
pH	11.2	11.4	11.4	11.2	11.4
pe	-2.9	-5.9	0.6	-6.2	-6.1
T°C	33	36	31	35	34
Se					
conc (molar) [§]	5x10 ⁻³	5x10 ⁻⁷	5x10 ⁻³	5x10 ⁻⁷	5x10 ⁻⁷
aqueous species [¶]	SeO ₃ ²⁻	Se ²⁻ / HSe ⁻	SeO ₃ ²⁻	Se ²⁻ / HSe ⁻	
solid phase [#]	CaSeO ₃	Se	CaSeO ₃	Se	
Pd					
conc (molar)	10 ⁻¹⁶	10 ⁻²²	10 ⁻¹⁰	10 ⁻²³	10 ⁻²²
aqueous species			Pd(OH) ₂		
solid phase			Pd		
Sn					
conc (molar)	10 ⁻¹⁹	10 ⁻¹⁸	10 ⁻¹⁹	10 ⁻¹⁸	10 ⁻¹⁸
aqueous species	SnO(OH) ⁺	Sn(OH) ₃ ⁻	SnO(OH) ⁺	Sn(OH) ₃ ⁻	
solid phase			SnO ₂		
Zr					
conc (molar)	5x10 ⁻⁴	2x10 ⁻³	5x10 ⁻⁴	1x10 ⁻⁴	5x10 ⁻³
aqueous species			Zr(OH) ₅ ⁻		
solid phase			ZrSiO ₄		ZrO ₂
Ni					
conc (molar)	3x10 ⁻⁷	3x10 ⁻⁷	6x10 ⁻⁷	3x10 ⁻⁷	3x10 ⁻⁷
aqueous species			Ni(OH) ₃ ⁻		
solid phase			NiO		

§ conc = saturation concentration (molar); ¶ aqueous species = dominant solution-phase species; # solid phase = inferred solubility-controlling solid phase.

Table 9. Calculated solubilities and dominant solution species for U and Th, using both NEA and EIR databases on MINEQL code at EIR.

Site No	1		3		4		5		7	
	Nizwa Jill		Karku		Jebel		Nidab		Bahla	
pH	11.2		11.4		11.4		11.2		11.4	
pe	-2.9		-5.9		0.6		-6.2		-6.1	
T°C	33		36		31		35		34	
	NEA	EIR	NEA	EIR	NEA	EIR	NEA	EIR	NEA	EIR
Th										
conc (molar) [∞]	1x10 ⁻¹⁰	5x10 ⁻¹⁰	1x10 ⁻¹⁰	5x10 ⁻¹⁰	1x10 ⁻¹⁰	5x10 ⁻¹⁰	1x10 ⁻¹⁰	5x10 ⁻¹⁰	1x10 ⁻¹⁰	5x10 ⁻¹⁰
soln. species [#]	-----Th(OH) ₄ ^o -----									
solid phase [¶]	-----ThO ₂ -----									
U										
conc (molar)	2x10 ⁻⁴	2x10 ⁻⁷	3x10 ⁻⁹	1x10 ⁻⁶	5x10 ⁻³	4x10 ⁻⁴	2x10 ⁻⁹	6x10 ⁻⁷	3x10 ⁻⁹	7x10 ⁻⁷
soln. species [§]	D	B	A+D	B	D+C	C	A	B	A	B
solid phase	-----U ₄ O ₉ -----		-----UO ₂ -----		- see footnote [†] -		-----UO ₂ -----			

[∞] conc. = saturation concentration (molar); # soln. species = dominant solution-phase species;

[¶] solid phase = inferred solubility-controlling solid phase.

[§] soln. species A = U(OH)₄^o

B = U(OH)₅⁻ (note that the existence of this species is doubted)

C = (UO₂)₃(OH)₇⁻

D = UO₂(OH)₃⁻

[†] In this case the predicted solid phase differed: U₃O₈ by the NEA database and Ca(OH)₂(UO₂H₂SiO₄)₂ by the EIR database.

- The variation in U-bearing solid phases which are inferred to be controlling the concentration of aqueous U is reflecting the varying redox conditions at the springs. The same controlling solid is inferred by both databases for Nizwa (U_4O_9) and the most reducing springs (UO_2) but they disagree for the most oxidising case, Jebel (EIR: $Ca(OH)_2(UO_2H_2SiO_4)_2$; NEA: U_3O_8). The EIR database solid phase may seem somewhat anomalous but, in fact, is very similar in form to many cement minerals; the importance of such species has been suggested elsewhere (F. Glasser, personal communication).
- It is notable that calculations of maximum U solubilities using the PHREEQE code and the AERE Harwell database give significantly lower values than those in Table 9 (F. Ewart, personal communication). For example, 2×10^{-10} M total dissolved U is predicted for Nizwa, which is 3 and 6 orders of magnitude lower respectively than the EIR and NEA databases suggest although the controlling solid phase selected in each case is the same (U_4O_9). Clearly there is a need for detailed comparison of the databases and the particular set of species modelled in each case, both for reducing and oxidising conditions, in order to ascertain the sources of these discrepancies.

4.4 Discussion

The measured concentrations of the modelled trace elements, already presented in Table 3, are shown in comparison with the predicted equilibrium solubility concentrations in Table 10. It should be noted that in all cases concentrations are extremely low and mostly at the "state of the art" detection limits.

Comments on the comparisons between measured and predicted concentrations for the individual elements are as follows:

Se In all cases the model predicts concentrations higher than those observed. No obvious sink for Se is expected in the presence of relatively high S concentrations (Karku, Nidab and Jebel Awq; Table 1) hence it is expected that this simply reflects a source limitation.

Pd The measured values are suspected to be too high due to Sr interference on the ICP-MS peak for Pd. Pd is identified as an element for which experimental work is required to confirm the low model solubility.

- Sn** In all cases a very low concentration of Sn is observed which is, at least, consistent with the model.
- Zr** The predicted relatively high solubility of Zr is not reflected in the measured concentrations. However, Zr is at extremely low abundance in the host rock (Lippard et al., 1986), reflecting depletion of elements which are 'incompatible' with the major mineral phases during the original crystallisation process. Either the model database may omit a solid phase which controls the solubility at a lower level than predicted here, or restricted source is the constraint on dissolved concentrations.
- Ni** The analytical data are suspect due to the probability of peak enhancement by dissolution of the nickel inlet cone on the ICP-MS. However the analytical data as such are consistent with the model predictions. This would not be so if NiS was considered as a solid phase and hence there is still a certain amount of uncertainty for this element. Ni-As and Ni-silicate secondary minerals are observed in the Oman rocks, but not NiS (Lippard et al., 1986; p63). Ni is relatively abundant in the primary mineralogy principally as a minor element in olivine, and is present in significant amounts in some of the natural precipitates at the springs (Section 3.5.3 and Table 7).
- Th** Considering the errors involved, the Th analytical data are consistent with either database.
- U** In all cases, the U concentration is below that predicted by either database. In particular, the concentration for oxidising conditions is especially low. Although this could reflect low abundance in the host rock or could be an artefact due to input of air into a previously reducing water, it might indicate that suggested species such as $\text{Ca}(\text{OH})_2 \cdot \text{UO}_2(\text{OH})_2$, which would lower solubility by about 10 orders of magnitude for oxidising conditions, actually exist. If the latter is the case, both databases could be drastically overconservative for such conditions. The much lower concentrations predicted with the AERE database (Section 4.3.2) should also be noted in this respect.

Table 10. Comparison of predicted and observed molar concentrations of trace elements.

Site No	1	3	4	5	7
	Nizwa Jill	Karku	Jebel	Nidab	Bahla
pH	11.2	11.4	11.4	11.2	11.4
pe	-2.9	-5.9	0.6	-6.2	-6.1
T°C	33	36	31	35	34
Se					
predicted	5×10^{-3}	5×10^{-7}	5×10^{-3}	----- 5×10^{-7} -----	
observed	----- $< 3 \times 10^{-9}$ -----				
Pd					
predicted	10^{-16}	10^{-22}	10^{-10}	10^{-23}	10^{-22}
observed	----- 2.8×10^{-9} ----- ----- 6.6×10^{-9} ----- 3.8×10^{-9} ----- 2.8×10^{-9} -----				
	----- (suspect values due to analytical interference) -----				
Sn					
predicted	10^{-19}	10^{-18}	10^{-19}	----- 10^{-18} -----	
observed	----- $< 2 \times 10^{-9}$ -----				
Zr					
predicted	5×10^{-4}	2×10^{-3}	5×10^{-4}	1×10^{-4}	5×10^{-3}
observed	----- $< 1 \times 10^{-9}$ ----- ----- 2.2×10^{-9} ----- 1.1×10^{-9} -----				
Ni					
predicted	----- 3×10^{-7} ----- ----- 6×10^{-7} ----- ----- 3×10^{-7} -----				
observed	$< 1 \times 10^{-8}$ 2.0×10^{-8} $< 1 \times 10^{-8}$ 3.4×10^{-8} 2.7×10^{-8}				
	----- (suspected too high due to analytical interference) -----				
Th					
predicted (EIR)	----- 5×10^{-10} -----				
predicted (NEA)	----- 1×10^{-10} -----				
observed	----- $< 2 \times 10^{-10}$ -----				
U					
predicted (EIR)	2×10^{-7}	1×10^{-6}	8×10^{-4}	6×10^{-7}	1×10^{-7}
predicted (NEA)	2×10^{-4}	3×10^{-9}	5×10^{-3}	2×10^{-9}	3×10^{-9}
observed	----- $< 4 \times 10^{-11}$ ----- ----- 4.2×10^{-11} ----- ----- $< 4 \times 10^{-11}$ -----				

5 MICROBIOLOGICAL SAMPLING AND ANALYSES

5.1 Materials and Methods

5.1.1 Sampling Methods

Where possible, media were prepared and sterilised at tenfold concentration. These were reconstituted to normal strength by addition of water or water/sediment inocula (see Appendix A). Where both water and sediment samples were required, water samples were taken first to prevent microbial addition from the sediment. Water samples were taken directly into sterile 60ml syringes, or, where deeper water samples were required the sampling device (Appendix B) was constructed. Samples of water were then injected into the sterile media. Sediments were disturbed by expelling a syringe filled with interface water. Water/ sediment mixtures were used as sediment inocula for the media. An on-site test for aerobic heterotrophs was performed using dip-slides (Millipore SPC tester) at Sites 1, 2 and 3.

5.1.2 Microbial Enrichments

Enrichments were carried out for the following groups of microorganisms:

- Aerobic and anaerobic heterotrophic and oligotrophic bacteria
- Nitrifying bacteria
- Denitrifying bacteria
- Sulphur-oxidising bacteria
- Alkalophilic bacteria (photo- and non photo-synthetic)
- Methanogenic bacteria
- Sulphate-reducing bacteria (SRB)

In addition, enrichments for eukaryotic (i.e. non-bacterial) microorganisms were made.

All the media used are listed in Appendix A. The major aerobic and anaerobic heterotrophic bacteria were isolated from enrichment cultures for identification and pH tolerance tests.

5.1.3 pH Gradient Tests

The pH range for growth of each heterotrophic isolate was determined using pH gradient plates (Grant and Tindall, 1980) although there is difficulty in maintaining high pH values.

70µl of 1-2 day old liquid culture of each isolate was inoculated at 90° to the trough along the pH gradient of between 11.1-6.9, and the limits of subsequent growth determined. The pH values of the gradient plates were determined using spade and flat-head electrodes (Russell pH, Auchtermuchty, Scotland) attached to an Orion Model 611 pH/millivolt meter. Each isolate was classified as alkalotolerant or alkalophilic according to its growth range. Anaerobic isolates were incubated on gradients in an atmosphere of 90% N₂:10% H₂.

5.1.4 Identification of Isolates

Various physical and biochemical tests were used to identify the isolates. Ultimately a genus or group was assigned to each isolate where possible.

The oxidation-fermentation (OF) test was carried out using Difco O-F Basal Medium (see Collins and Lyne, 1979) with the addition of 10% glucose. Young cultures were stab inoculated and a 1cm glycerol plug introduced to maintain anaerobiosis in the medium. A positive result was indicated by a colour change from dark blue-green to yellow.

The cytochrome oxidase test was performed using filter paper soaked in 1% aqueous solution of tetramethyl-p-phenylene diamine dihydrochloride. Young colonies were smeared onto the filter paper using a glass rod. A positive result was indicated by a deep blue colour on the filter.

Catalase activity was indicated by gas production after addition of 1.5% hydrogen peroxide to bacterial colonies.

A further range of biochemical tests present on API-20B Identification Strips (API Systems SA, France) were done (see Table 13). The strict anaerobic isolates were identified using methods in Collins and Lyne (1979) and Bergey's Manual of Determinative Bacteriology (1974).

5.1.5 Heterotrophic Activity

Heterotrophic activity in natural springwater samples was tested by examining carbon dioxide production. It was appreciated that degradation of organic carbon with the concomitant release of CO₂ could not be assessed by looking for gaseous products in the

headspace of an incubation system because $\text{Ca}(\text{OH})_2$ would react with the CO_2 formed. Natural springwaters with or without organic carbon additions, were placed in sealed systems and incubated for a period of up to 4 weeks. After this period, HCl was added to release CO_2 formed through biological activity and precipitated as CaCO_3 . Released CO_2 was analysed using a Gow-Mac Model 562 gas chromatograph fitted with a thermal conductivity detector. Organic carbon addition (yeast extract) to natural springwater did not exceed 10 mg l^{-1} to encourage activity of oligotrophic bacteria in these nutrient-poor waters. Oligotrophic bacteria require a minimum content of organic carbon between $1\text{-}15 \text{ mg l}^{-1}$ (Kuznetsov et al., 1979).

5.2 Results

The enrichments for nitrifying, denitrifying, sulphur-oxidising and methanogenic bacteria were all negative. The on-site test for aerobic heterotrophs using Millipore dip-slides gave low counts for Sites 1 and 3 with significantly higher counts at Site 2 (Table 11). Table 12 shows the counts for aerobic and anaerobic heterotrophic bacteria on a variety of media. Oligotrophic conditions were simulated using 1% CPS medium. The population trend is similar to that obtained with dip-slides, with Site 2 giving a higher and more diverse bacterial content. An unexplained observation was the absence of colonies on full strength and 1% CPS media incubated under anaerobic conditions. It may be that the obligate anaerobic species present are unable to grow at neutral pH, and are alkalophilic. A wide range of colony types were isolated from aerobic and anaerobic heterotrophic plates incubated at 30°C . Table 13 summarises many of the investigated characteristics of these bacteria including biochemical tests, pH range for growth and microscopic details. Most of the isolates were alkalotolerant, with very few, under strict definition, being alkalophilic. Two of the isolates judged to be alkalophilic (JA3 and NS1) are strict aerobes and are of less interest to the present work than anaerobes. They have been identified as *Caulobacter* and *Flavobacterium* spp. (Table 13). The alkalotrophic facultative anaerobe NS4 is a *Bacillus* sp.

Sulphate-reducing bacteria produced positive enrichments from 5 samples (sediments from Sites 1 and 2; water from Sites 3,4 and 5) of which three were isolated in pure culture for further studies. Positive enrichments were only encountered with Postgate Medium B (Appendix A), and no enrichments were obtained using acetate, propionate or butyrate media. It is probable that the isolates are lactate-oxidising SRB of the genera *Desulfovibrio* or *Desulphotomaculum*. These have proven difficult to obtain in axenic (i.e. pure) culture and no identification tests have been done as yet.

Examination of the photosynthetic enrichment cultures showed the presence of a wide variety of organisms including rod, coccoid and vibroid bacteria, most of which were Gram negative. Virtually the whole length of Site 1 displayed algal growth, usually associated with the walls of the channel at the air/water interface. Unicellular algae were cultured from Site 1. Indeed a whole diversity of photosynthetic microorganisms both filamentous and unicellular were observed (Plate 8). The major genera resembled *Synechocystis*, *Arthrospira* and *Plectonema*. These are all members of the cyanobacteria (cyanophyta or blue-green algae).

Table 13 also gives the tentative identification of the bacterial isolates to at best the genus level according to the API, Bergey's Manual (1974) and Starr et al. (1986) procedures. The results in general show the microbial flora of the alkaline springwaters to be similar to those of less extreme environments, with *Bacillus* and *Clostridium* spp. predominating. True oligotrophic bacteria such as *Caulobacter* and *Hyphomicrobium* (Dow and Lawrence, 1980) were also found in these nutrient-poor environments containing little nitrogen and negligible phosphorus (Table 1). It must be appreciated, however, that most heterotrophic microorganisms may be able to adapt to growth in low organic carbon waters, including the majority of species delineated in this study. Some of the isolates were identified as *Actinobacillus* sp. and some were obvious members of the *Enterobacteriaceae*. The presence of these microorganisms which inhabit animals implies that the waters are being contaminated by animal excreta. The presence of domestic animals was noted during sampling of these waters. Plates 9 and 10 show two of the bacterial isolates.

Table 13 includes the pH ranges for growth of the isolates from aerobic and anaerobic heterotrophic enrichments. Most have a relatively wide range, from at least 6.9 to beyond 10, and in some cases beyond 11. Tests for growth at the pH of the spring-waters were not possible due to difficulties in maintaining a high pH on the gradient plate. Because of difficulties in obtaining single colony cultures of SRB, the upper pH limit for growth of SRB could only be tested with other organisms present in the enrichments prepared using both spot and spread inoculation methods (Philp et al., 1987). Distinctive bands of blackening due to sulphide production were seen on these pH gradients. The contaminant organisms produced predominantly small cream-coloured colonies and gas characteristic of butyrate-producing clostridia. They grew overnight, often swamping plates. The formation of a pH gradient was often restricted to a narrow range on agar (Fig. 2). Possible reasons for this are zero exposure to CO₂ in the N₂:H₂ atmosphere used to grow the organisms, prolonged incubation and growth of contaminants. The blackening front was at about pH 10.2 indicating that growth of SRB can take place up to at least this pH value. At this stage it

is not known whether the growth and blackening fronts are coincident. There was no evidence for growth of SRB above pH 10.2 in enrichments. Experiments with single colony cultures indicate that maximum growth occurs between pH 8.5 and 9.5.

Table 11. Total aerobic heterotrophic bacteria determined with Millipore (SPC tester) dip slides on site in Oman.

Site	Springwater	Counts (CFU ml ⁻¹)
1	Nizwa Jill 1 (1F)	3.8 x 10 ¹
2	Nizwa Jill 2 - Birkat	>3 x 10 ²
3	Karku	3.4 x 10 ¹

Table 12. Plate counts of heterotrophic bacteria from three springwaters in Oman.
(Analyses carried out on return to U.K.)

Site	Springwater	Alkalophilic agar		CPS		1% CPS		Fungi
		Aerobes	Anaerobes	Aerobes	Anaerobes	Aerobes	Anaerobes	
1	Nizwa Jill 1 (1F)	ND	ND	5x10 ¹	ND	ND	ND	ND
2	Nizwa Jill 2 - Birkat	1.05x10 ³	1x10 ⁴	6.5x10 ²	ND	5.4x10 ²	ND	ND
3	Karku	6x10 ²	ND	ND	ND	ND	ND	ND

Table 13. Morphological and biochemical characteristics of heterotrophic bacteria isolated from the various springwaters in Oman.

Isolate	Origin	Colony Morphology				Microscopic Characteristics	
		Diameter	Perimeter	Colour	Elevation	Form	Gram reaction
5S	Nizwa Birkat	2mm	entire	σW	convex	short rods	-
5L	Nizwa Birkat	2.5	entire	σW	convex	short rods	-
6S	Nizwa Birkat	1.2	entire	σW	umborated	dumbbell rods	+
6L	Nizwa Birkat	2.0	entire	σW	convex	short rods	-
7	Nizwa Spring 1F	1.5	lobulate	σW	flat	rods	+
8	Nizwa 1F	1.5	entire	σW	umborated	short rods	-
9	Nizwa 1F	0.8	entire	σW	flat	short rods	+
11	Nizwa 1F	0.8	entire	σW	convex	short rods	+
12	Nizwa 1F	0.7	entire	σW	convex	short rods	variable
13S	Nizwa 1F	1.4	entire	σW	convex	short rods	-
13L	Nizwa 1F	1.8	entire	σW	convex	short rods	-
16S	Nizwa Birkat	1.0	entire	σW	flat	short rods	-
16L	Nizwa Birkat	1.2	entire	σW	flat	short rods	-
17	Nizwa 1F	0.1	entire	σW	convex	short rods	+
18	Nizwa 1F	0.2	entire	σW	convex	short rods	+
19S	Nizwa Birkat	0.1	entire	σW	convex	short rods	-
19L	Nizwa Birkat	0.9	entire	σW	convex	rods	+
25	Nizwa Birkat	1.2	entire	σW	convex	short rods	-
JA1	Jebel Awq	0.8	entire	orange/flat		filamentous	-
JA2	Jebel Awq	1.0	lobulate	W	convex	short rods	-
JA3	Jebel Awq	1.2	lobulate	red	flat	filamentous	-
NS1	Nizwa Spring	3.5	entire	orange/convex		short rods	-
NS2	Nizwa Spring	1.2	entire	pale orange/convex		short rods	variable
NS3	Nizwa Spring	2.0	entire	σW	flat	rods	+
NS4	Nizwa Spring	0.9	entire	σW	flat	long rods	+(poor)
NS5	Nizwa Spring	2.5	lobulate	σW	flat	rods	+
NS/An1	Nizwa Spring	2.1	lobulate	σW	flat	rods	+
NS/An2	Nizwa Spring	1.0	entire	orange/convex		rods	-

σW = whitish colonies

Table 13 (continued). Morphological and biochemical characteristics of heterotrophic bacteria isolated from the various springwaters in Oman.

Isolate	Microscopic characteristics			Growth characteristics		O-F medium
	Size (l x b)	Spores	Motility	Growth conditions	Growth pH range	
5S	0.8µm x 0.5µm	-	-	facultative; alkalotolerant	pH 6.9 - 10.3	+
5L	1.0 x 0.7	-	-	"	6.9 - 10.3	+
6S	2.7 x 0.5	-	-	"	6.9 - 10.8	+
6L	1.0 x 0.5	-	-	"	6.9 - 10.3	+
7	1.8 x 0.5	+	+	"	6.9 - 10.2	-
8	1.0 x 0.8	-	+	"	6.9 - 11.1	+
9	1.1 x 0.6	-	-	"	6.9 - 10.2	+
11	1.8 x 0.5	-	σ+	"	6.9 - 10.2	-
12	1.1 x 0.5	-	σ+	"	6.9 - 10.2	+
13S	1.0 x 0.6	-	-	"	6.9 - 10.8	+
13L	1.2 x 0.7	-	-	"	6.9 - 10.3	+
16S	0.8 x 0.4	-	σ+	"	6.9 - 10.6	+
16L	0.7 x 0.4	-	-	"	6.9 - 10.2	+
17	1.0 x 0.6	-	-	"	6.9 - 10.2	-
18	0.8 x 0.5	-	-	"	6.9 - 10.2	-
19S	1.0 x 0.6	-	-	"	6.9 - 11.1	+
19L	3.1 x 0.4	+	+	"	6.9 - 10.1	-
25	0.6 x 0.4	-	+	"	6.9 - 10.2	+
JA1	? x 0.4	-	σ-	strict aerobe; alkalotolerant	6.9 - 10.1	-
JA2	1.8 x 0.5	-	++	"	6.9 - 11.1	-
JA3	? x 0.4	-	-	strict aerobe; alkalophilic	8.5 - 11.1	-
NS1	1.0 X 0.5	-	-	"	8.9 - 10.3	-
NS2	0.8 X 0.6	-	σ+	facultative; alkalotolerant	6.9/9.2 - 10.9	-
NS3	3.0 x 0.9	+	-	strict aerobe; alkalotolerant	6.9/9.7 - 10.5	-
NS4	3.2 x 0.3	+	-	facultative; alkalophilic	9.4 - 10.6	-
NS5	3.8 x 0.6	+	+	strict anaerobe; alkalotolerant	6.9 - 10.4	-
NS/An1	3.0 x 0.6	+	+	"	6.9 - 10.4	-
NS/An2	2.0 x 0.7	-	-	facultative; alkalotolerant	6.9 - 10.5	-

? = length varied greatly

Table 13 (continued). Morphological and biochemical characteristics of heterotrophic bacteria isolated from the various springwaters in Oman.

Isolate	Gelatin liquifac'n	Nitrate reduction	Galacto -sidase	Carbohydrate metabolism with acid production							
				Saccharose L(+)	Mannitol arabinose	Fructose	Glucose	Maltose	Starch	Rhamnose	
5S	-	-	+	+	+	+	+	+	+	-	+
5L	-	-	+	+	+	+	+	+	+	σ+	+
6S	-	+	σ+/-	-	+	+	+	+	+	+	+
6L	-	-	+	+	+	+	+	+	+	+	+
7	+	++	+	-	-	-	-	+	+	-	-
8	-	+	+	+	+	+	+	+	+	+	+
9	-	σ+	-	-	-	σ+	+	+	+	-	σ+
11	-	+	-	-	+	+	+	+	+	-	+
12	-	σ+	-	-	+	+	+	+	+	-	+
13S	-	+	-	-	+	+	+	+	+	+	+
13L	-	σ+	+	+	+	+	+	+	+	+	+
16S	+	+	-	+	+	+	+	+	+	+	+
16L	-	σ+	+	+	+	+	+	+	+	+	+
17	-	+	-	+	-	-	+	+	+	-	-
18	-	+	-	+	-	-	+	+	+	-	-
19S	-	+	-	+	+	+	+	+	+	+	+
19L	-	-	+	+	+	+	+	+	+	+	+
25	-	σ+	+	+	+	+	+	+	+	+	+
JA1	+	+	-	-	-	-	σ+	+	-	-	-
JA2	σ+	+	-	-	-	-	-	-	-	-	-
JA3	σ+	+	-	-	-	-	-	-	-	-	-
NS1	-	+	+	+	+	+	+	+	+	+	+
NS2	+	-	σ+	+	+	+	+	+	+	+	σ+
NS3	σ+	-	+	+	+	+	+	+	+	σ+	σ+
NS4	σ+	+	+	+	-	+	+	+	+	+	+
NS5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
NS/An1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
NS/An2	+	+	+	+	-	+	+	+	+	+	+

nd = not done

Table 13 (continued). Morphological and biochemical characteristics of heterotrophic bacteria isolated from the various springwaters in Oman.

Isolate	Carbohydrate metabolism (cont'd)				Urease	Trypto- phanase	H ₂ S formation	Acetoin production	Citrate metabolism	Cytochrome oxidase
	Galactose	Mannose	Sorbitol	Glycerol						
5S	+	+	+	+	+	-	-	+	+	+
5L	+	+	+	+	+	-	-	-	+	+
6S	+	+	+	+	-	-	-	+	-	-
6L	+	+	+	+	+	-	-	-	+	+
7	+	+	-	+	+	-	-	+	+	σ+
8	+	+	+	+	-	-	-	+	-	-
9	+	+	+	+	-	-	-	+	-	-
11	+	+	+	+	-	-	-	+	-	-
12	+	+	+	+	-	-	-	+	-	+
13S	+	+	+	+	-	-	-	+	-	-
13L	+	+	+	+	+	-	-	-	+	+
16S	+	+	+	+	-	-	-	+	-	+
16L	+	+	+	+	+	-	-	+	+	-
17	+	+	-	-	-	-	-	+	+	-
18	+	+	-	-	-	-	-	+	σ+	-
19S	+	+	+	+	-	-/ σ+	-	+	-	+
19L	+	+	-	-	-	-	-	+	-	-
25	+	+	+	+	+	-	-	+	+	-
JA1	-	-	-	σ+	-	-	-	-	+	+
JA2	-	-	-	-	-	-	-	+	+	+
JA3	-	-	-	-	-	-	-	-	+	-
NS1	+	+	+	+	-	-	-	+	-	+
NS2	+	+	σ+	+	-	-	-	+	-	-
NS3	+	+	+	+	+	-	-	+	-	+
NS4	+	+	+	+	+	-	-	+	-	-
NS5	nd	nd	nd	nd	nd	nd	nd	nd	nd	-
NS/An1	nd	nd	nd	nd	nd	nd	nd	nd	nd	-
NS/An2	+	+	+	+	-	-	-	+	-	+

nd = not done

Table 13 (continued). Morphological and biochemical characteristics of heterotrophic bacteria isolated from the various springwaters in Oman.

Isolate	Catalase	Possible identification (Genus / family / group)
5S	+	<i>Actinobacillus</i> sp
5L	+	<i>Actinobacillus</i> sp
6S	-	Coryneform bacterium
6L	+	<i>Actinobacillus</i> sp
7	+	<i>Bacillus</i> sp
8	+	<i>Hafnia</i> sp
9	-	Unknown
11	-	Unknown
12	+	<i>Vibrio</i> sp
13S	+	<i>Enterobacteriaceae</i>
13L	+	<i>Actinobacillus</i> sp
16S	+	<i>Vibrio</i> sp
16L	+	<i>Hafnia / Serratia</i> sp
17	-	Unknown
18	-	Unknown
19S	+	<i>Enterobacteriaceae</i>
19L	-	<i>Bacillus</i> sp
25	+	<i>Hafnia / Serratia</i> sp
JA1	-	<i>Hyphomicrobium</i> sp
JA2	-	<i>Pseudomonas / Alkaligenes</i> sp
JA3	+	<i>Caulobacter</i> sp
NS1	+	<i>Flavobacterium</i> sp
NS2	+	<i>Serratia</i> sp
NS3	+	<i>Bacillus</i> sp
NS4	+	<i>Bacillus</i> sp
NS5	-	<i>Clostridium</i> sp
NS/An1	-	<i>Clostridium</i> sp
NS/An2	+	<i>Flavobacterium</i> sp

Data on heterotrophic activity (CO_2 production from organic carbon oxidation) measured for samples from Sites 1, 2 and 3 are presented in Table 14. It is evident for Sites 1 and 2 that exogenous supply of organic carbon did not significantly increase microbial activity. Activity was stimulated in sample 3 by the addition of yeast extract. In experiments such as these, the quality and quantity of organic carbon have a critical role in heterotrophic activity and because of the limited volume of springwater transported to the UK, experimentation was restricted. Another important point was that there was a delay between collection and analysis of samples. In the case of Site 3, available organic carbon may have been utilised by the microorganisms during transportation, and activity could only be shown by addition of organic carbon substrate. Fig. 3 shows the separation of CO_2 from air during GC analysis for Site 1 samples.

Field chemistry revealed the waters to be predominantly reducing (Table 1). Although this was not the case for the water at Site 2 (Nizwa Jill - Birkat), the Eh value measured in the sediment was -580 mV, and disturbance of the sediment gave a smell of H_2S . Site 2 water was unusual in another respect that it contained carbonate (66 mg l^{-1}) as the result of interaction of the spring-water with atmospheric CO_2 . In nutrient status terms the waters are oligotrophic, containing little nitrogen and negligible phosphorus (Table 1). They are also low in sulphate (except Site 4) upon which the SRB are dependent for their activity. However, only small amounts may be necessary, and other more reduced forms of sulphur may be available (eg. thiosulphate).

5.3 Discussion

The environment of a radioactive waste disposal repository exerts pressure on the survival and growth of microorganisms, whereupon they may or may not affect the integrity of the system (West and McKinley, 1984; West et al., 1985; Christofi et al., 1985; Grogan, 1987). The most strikingly obvious barrier to microbial activity in a low-/intermediate-level waste repository is the high pH of the pore water of concrete which could be maintained between pH 13 and 11 for a period of thousands of years after which it would gradually decrease to 10.5 or lower due to leaching of calcium hydroxide (Atkinson, 1985; Berner, 1986). This work has specifically investigated the presence of physiological groups of bacteria which might be important in radioactive waste disposal (i.e. in the high-pH, strongly reducing environment), but has additionally taken the opportunity of the natural high pH conditions to examine heterotrophic bacteria more generally.



Plate 8. Rhodomicrobium? A photosynthetic bacterium isolated from the spring-waters.

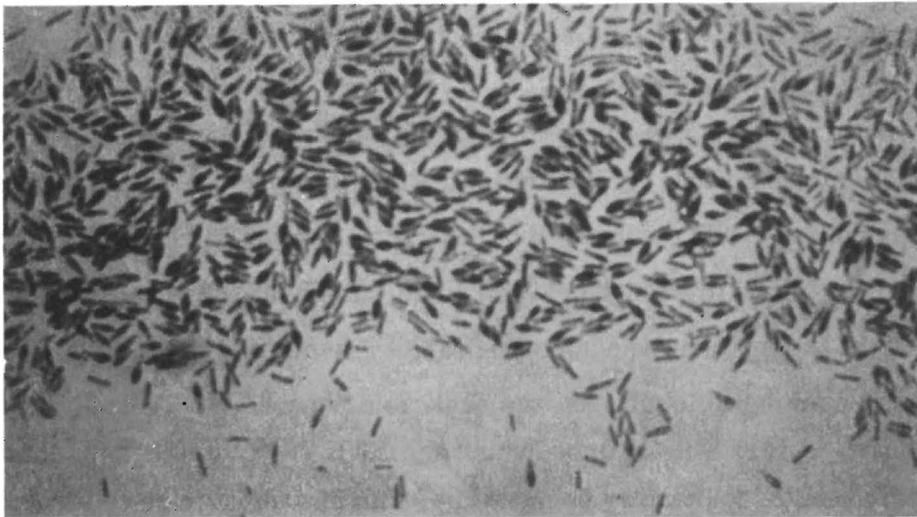


Plate 9. Alkalophilic Bacillus sp. isolated from the spring-waters. The plate shows the presence of spores in this Gram positive facultative anaerobic bacillus.

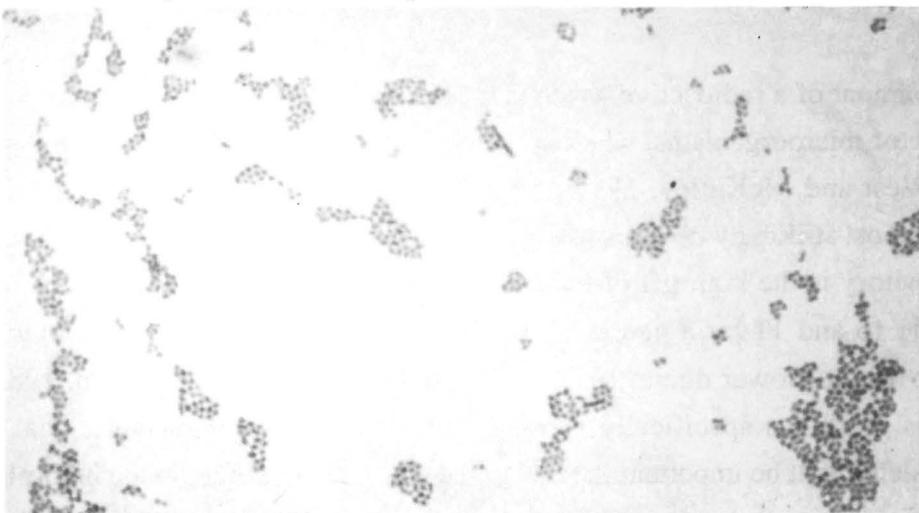


Plate 10. Unidentified alkalotolerant coccobacillus isolated from the spring-waters.

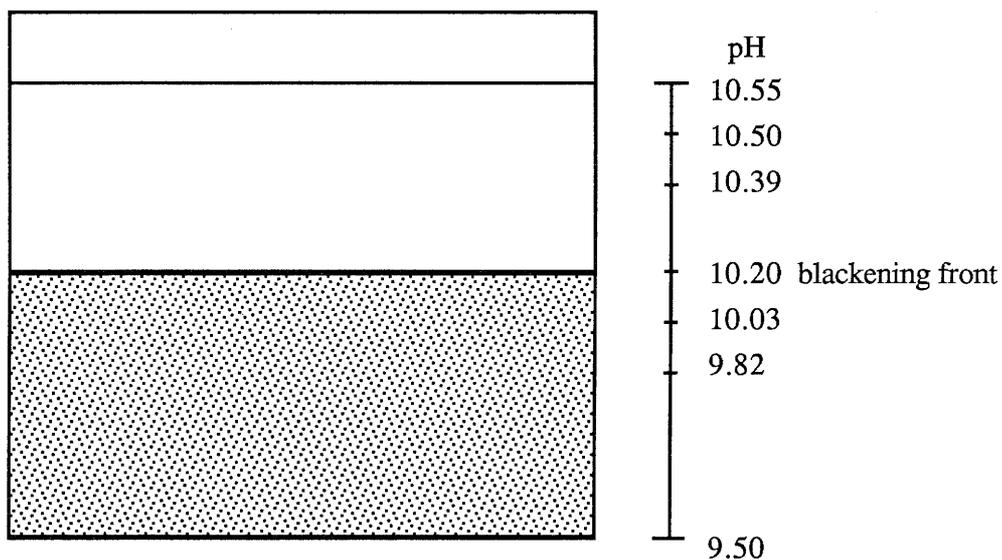


Figure 2. A typical pH gradient after inoculation with sulphate-reducing bacteria isolated from the alkaline spring-waters, and incubated in an atmosphere of 90%N₂:10%H₂.

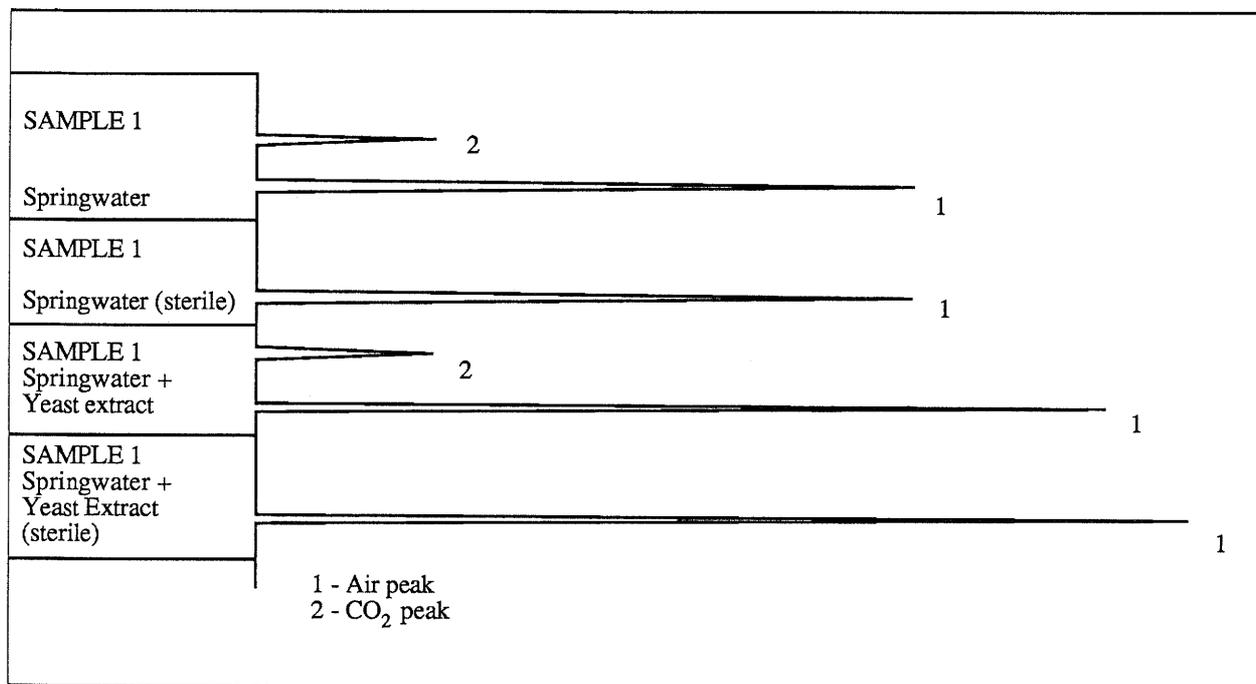


Figure 3. Gas chromatographic separation of gases generated after addition of HCl; samples with or without organic carbon addition were incubated for 4 weeks prior to analyses.

Table 14. CO₂ evolution (heterotrophic activity) in Oman springwater samples.

Springwater	CO ₂ peak height (arbitrary units)
Nizwa Jill 1 (1F)	
(a) Unamended springwater	109.6
(b) Sterile unamended springwater	ND
(c) Springwater amended with yeast extract	107.2
(d) Sterile springwater amended with yeast extract	ND
Nizwa Jill 2 - Birkat	
(a) Unamended springwater	130.4
(b) Sterile unamended springwater	ND
(c) Springwater amended with yeast extract	148
(d) Sterile springwater amended with yeast extract	ND
Karku	
(a) Unamended springwater	ND
(b) Sterile unamended springwater	ND
(c) Springwater amended with yeast extract	130.4
(d) Sterile springwater amended with yeast extract	ND

ND = not detected

It is of significance to note that the counts of bacteria present were low. According to Brock (1966), 1×10^6 bacteria ml^{-1} would be required for any significant environmental effect to be seen as a result of microbial activity. Furthermore, with expected anaerobic conditions developing rapidly in a repository, the influence of strict aerobes would be negligible, which discounts the two alkalophiles isolated (*Caulobacter* and *Flavobacterium* sp). On the other hand, it must be remembered that the long timescale of repository safety analysis requires the consideration of processes which may be minor in the short term but which may predominate over geological timescales.

The genera found in the springwaters are similar to those in less extreme soil and water

environments. However the numbers of bacteria isolated and the chemical compositions suggest that the waters are oligotrophic. Moreover the activity measurement data indicate that the high pH is not the growth limiting factor. The populations in the waters may be nutrient-limited and this is inferred from the chemical data available, especially with regard to carbon, nitrogen and phosphorus. By the Vollenweider (1968) classification system the springwaters are ultra-oligotrophic. Restricted soluble nutrient supply could be compensated for by rapid turnover, but this is not thought to be the case here.

Phosphorus limitation has a very profound effect on ecology, principally because of its function as an agent of energy transfer (Hutchinson, 1970). A deficiency of available phosphorus is more likely to limit productivity than any other material except water (Stewart and McKercher, 1982). It should be borne in mind that the phosphorus content of most Portland cements is much higher than was encountered in the springwaters (Lea, 1970).

The TOC levels are similar to those in oligotrophic lakes, clean rivers and the open ocean (Rheinheimer, 1985). This low level is at the lower limit for uptake and utilisation by most bacteria and fungi; a further growth-limiting factor is thus the availability of organic carbon, although a range of oligotrophic bacteria can thrive in these environments (Mallory et al., 1977).

The most interesting finds with relevance to the cementitious repository environment were clostridia and sulphate-reducing bacteria. Strains of SRB have been isolated from three springwater sites and there are enrichments from a further two. The SRB enrichments grow well at pH 10.2 whereas pure cultures grow much more slowly at pH 10.2 with growth being better between 8.5 and 9.5. The reason for this may be that SRB are essentially community organisms (Postgate, 1984), gaining nutritional requirements from the products of other bacteria. The identification of clostridia is consistent with the common association between clostridia and SRB in less extreme environments; some *Clostridium* species can convert carbon dioxide to acetate which may act as a substrate for SRB.

Despite the discovery of SRB at relatively high pH it is another matter entirely if they can survive the higher pH of concrete pore water and derive energy from the corrosion of steel canisters (cf. Grogan, 1987). The effect of SRB on concretes has not been widely studied. An analogous problem, however, has been researched by Moosavi et al. (1985). Reinforced concrete was exposed to various environments including cultures of SRB. The biogenic sulphide produced by sulphate reduction was capable of permeation through concrete, albeit

slowly, and subsequent pitting corrosion of the reinforcement occurred. This process also increases the risk of spalling of reinforcement cover because the volume of the sulphide film is much greater than that of the protective oxide film. The strain of SRB used in this study was not selected for any ability to withstand high pH. If strains exist which can, then the possibility of spalling and/or corrosion over a long period is increased, provided the environment can support sufficient numbers of SRB. It should be noted that autotrophic strains have been discovered (Jansen et al., 1984; Widdel et al., 1983) which would not be limited by organic carbon supply.

Heterotrophic bacteria, which predominate in the springwaters and which may be present in a waste repository, can lead to radionuclide solubilisation by production of organic acids. The extent of organic acid production relies on the availability of organic carbon substrates. An important source of organic carbon in LLW and ILW repositories is disposed plastics, ion exchange resins, bitumen and cellulosic waste. For example, alkaline hydrolysis of cellulose may lead to release of high concentrations of glucose and if microbial activity can take place within the repository, this can lead to dissolution of minerals and the formation of organic chelating/complexing agents which may solubilise radionuclides. It must be remembered, however, that any organic microbial products may act as further substrates for microbes and ultimately may not be available for radionuclide solubilisation.

6 CONCLUSIONS

In summary, the findings in the two separate aspects of this investigation, and their relevance to the microbiology and hydrochemistry of a cementitious repository, are:

Microbiology

The counts of bacteria present in the natural alkaline (pH 11.2-11.4) groundwater were low, in the order of 10^1 to 10^3 ml⁻¹, but a wide range of organisms appear to have adapted successfully to this environment. The possible factors responsible for the limitation on viability are discussed below. Furthermore, with anaerobic conditions expected to develop rapidly in a repository, the influence of strict aerobes such as the two alkalophiles (*Caulobacter* and *Flavobacterium* spp) isolated in the present study, along with all photosynthetic microorganisms, could be discounted for the cementitious repository.

The genera found in the spring-waters are similar to those in less extreme soil and water environments. The wide range of both aerobic and anaerobic heterotrophic bacteria isolated was enhanced by the local variations in conditions at specific sampling points, particularly with respect to redox conditions and nutrient availability. Indeed, at least some of the heterotrophic microorganisms could be attributed to contamination of the spring-waters by animals.

Activity measurement data suggest that the high pH of this natural environment is not the principal constraint on microbial growth. The hydrochemical data on the spring-waters indicate that populations may be limited by supply of nutrients, especially carbon, nitrogen and phosphorus. Phosphorus limitation has a very profound effect on ecology, principally because of its function as an agent of energy transfer. A deficiency of available phosphorus is more likely to limit productivity than any other material except water. It should be borne in mind that the phosphorus content of most Portland cements is much higher than was encountered in the spring-waters. The low levels of organic carbon present in the spring-waters are similar to those in many natural environments which, although at the lower limit for uptake and utilisation by most bacteria and fungi, can support oligotrophic activity.

The most relevant observations were of clostridia and sulphate-reducing bacteria, which are commonly associated in less extreme environments. SRB are of particular interest to waste disposal because of their participation in reactions which corrode steel and which may affect concrete integrity. There is already considerable documentation of their ability to withstand extremes of temperature, pH, radiation, etc and these observations further illustrate their tolerance of alkaline conditions. Isolates containing SRB were obtained from three of the springs, although positive identifications have not been possible. The enrichments grow well at pH 10.2, although pure cultures grow more slowly at pH 10.2 with better growth between 8.5 and 9.5. The reason for this may be that SRB are essentially community organisms, gaining nutritional requirements from the products of other bacteria. Despite the discovery of SRB at relatively high pH, this still has not demonstrated that they can survive the higher pH values (12.5-13) possible in concrete pore-water.

Heterotrophic bacteria, which predominate in the alkaline spring-waters and which could be present in a waste repository, can lead to production of organic acids. The extent of organic acid production depends on the availability of organic carbon substrates. An important source of organic carbon in low-level and intermediate-level radioactive waste is cellulosic materials. Alkaline hydrolysis of this waste may lead to release of high concentrations of glucose which

will stimulate microbial activity leading to the production of organic acids. These in turn could speed up neutralisation of the high pH and the organic chelating/complexing species formed may solubilise radionuclides or may act as further substrates for microbial growth.

Trace element hydrochemistry

The total solution concentrations of the trace elements of interest for thermodynamic model validation, Se, Pd, Sn, Zr, Ni, U and Th, were generally below state-of-the-art detection limits. This analytical problem in approaching real abundances or solubility limits is, of course, compounded by the effect of the high pH conditions in depressing predicted solubilities. However Se, Zr, Ni, Th and U have predicted solubilities in these conditions that are within or reasonably close to analytical limits. In these cases the significance of data is dependent on there being natural sources of the elements which buffer concentrations at solubility limits; for example Zr is considerably lower than the expected equilibrium concentration. Ni is perhaps the most interesting in this respect since the known geochemistry of the source rocks suggests a reasonable supply of Ni; the analytical data, although probably erroneous due to instrumental contamination, are close to or lower than predicted values. Therefore, although positive quantitative or semi-quantitative 'validation' of the predictive models has not been achieved, the attempt has highlighted some of the challenging aspects of the search for model validation and some of the sensitive areas in the modelling of these particular elements.

There is a powerful logic in the approach to validate the predictive model initially by means of comparing with measured natural trace element concentrations and further by attempting to identify the ionic species distributions and any colloidal or similarly mobile material. This study has illustrated the practical problems of analytical sensitivity and uncertain elemental sources in natural systems. Further investigations along these lines could be usefully carried out in normal groundwater systems, preferably with better knowledge of trace element sources and buffering reaction rates, in addition to the extreme, though directly relevant, conditions of this study.

One very interesting outcome of the analytical work is the suggestion that significant ^{238}U and daughters may be associated with colloidal material at the Karku site (Section 3.4). Colloids are acknowledged to be of potential importance in repository safety analysis (e.g. Nagra, 1985) but, in general, tend not to be stable at high pH or high salinity. The indication

here that U is present in colloidal form along with daughter Th implies that relatively long-lived colloids exist in this environment. Such discussion is inherently constrained by the limited data available but this factor may be worthy of future investigation.

The sampling and measurement of the Oman alkaline springs has been a valuable and cost-effective reconnaissance investigation, particularly when taking account of the logistical problems which have been overcome for obtaining both microbiological and trace element samples. The experience of the sampling, analytical and interpretative aspects indicate where future natural analogue investigations should make further progress. Slight improvements in analytical sensitivity, and the elimination of analytical interference problems such as occurred with Ni and Pd, will permit further valuable comparisons between predicted and observed concentrations. Preconcentration methods for trace elements which include specific ion exchange retention for charged species offer the best potential, combined with ICP-MS analysis. Further comparisons of this type which indicate the validity of solution speciation models and solid phase controls for trace elements are desirable for a wide range of chemical environments in addition to the specific hyperalkaline condition. Knowledge of mineralogical distributions of the trace elements and the reactivity of these mineral phases would also greatly improve the interpretation of data. The small amount of U-Th natural series isotope data measured in this study have suggested that further measurements including Ra nuclides might prove very valuable in clarifying the behaviour of the nuclides in a solid-colloid-solution system. However, as with all other aspects of this type of study, it will be essential to monitor the precision of both the sampling and the analytical stages of the data collection.

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APPENDIX A

Media compositions

The following media were used for enrichment of different groups of microorganisms. For "on-site" inoculation, each medium was prepared 10 times concentrated and distributed in 3ml amounts (except where stated) into acid-washed screw-cap glass bottles (30ml capacity). The media were reconstituted to normal concentration on filling the bottles to capacity with spring water samples. No adjustment to the pH of the concentrated media was made, the final pH being determined by the natural alkalinity of the water samples on inoculation. The media were sterilised by autoclaving at 15 psi, 121°C for 15 mins.

1. *Aerobic and anaerobic heterotrophic bacteria (CPS medium)*

peptone	0.5g
casein hydrolysate	0.5g
soluble starch	0.5g
K ₂ HPO ₄	0.02g
MgSO ₄ .7H ₂ O	0.05g
Glycerol	1ml
FeCl ₃ .6H ₂ O (0.01% w/v)	4 drops
Distilled water	to 100ml

2. *Oligotrophic bacteria*

1% CPS medium (1ml of the above CPS medium in 100mls distilled water).

3. *Nitrifying bacteria*

(i) Ammonia-oxidising bacteria

K ₂ HPO ₄	0.05g
FeSO ₄ .7H ₂ O	0.05g
MgSO ₄ .7H ₂ O	0.1g
CaCO ₃	1.0g
(NH ₄) ₂ SO ₄	0.26g
Distilled water	to 100ml

(ii) Nitrite-oxidising bacteria

K_2HPO_4	0.05g
$FeSO_4 \cdot 7H_2O$	0.05g
$MgSO_4 \cdot 7H_2O$	0.1g
$CaCO_3$	1.0g
$NaNO_3$	0.1g
Distilled water	to 100ml

4. *Denitrifying bacteria.*

CPS medium as for 1 with the addition of KNO_3 (2.0g)

A Durham tube was present in each enrichment bottle for gas collection.

5. *Sulphur-oxidising bacteria.*

(i) Obligate Chemolithotrophs

$(NH_4)_2SO_4$	0.1g
K_2HPO_4	0.4g
KH_2PO_4	0.4g
$MgSO_4 \cdot 7H_2O$	0.1g
$CaCl_2$	0.1g
$FeCl_3 \cdot 6H_2O$	0.02g
$MnSO_4 \cdot 4H_2O$	0.02g
$Na_2S_2O_3 \cdot 5H_2O$	10.0g
Distilled water	to 100ml

(ii) Facultative Chemolithotrophs (Starkey 1935)

K_2HPO_4	0.4g
KH_2PO_4	0.15g
$(NH_4)_2SO_4$	0.3g
$MgSO_4 \cdot 7H_2O$	0.5g
Yeast extract	0.3g
$Na_2S_2O_3 \cdot 5H_2O$	10g
Trace minerals soln.	10ml
Distilled water	to 100ml

Trace minerals soln.

$FeCl_2 \cdot 4H_2O$	1.5g
H_3BO_3	60mg
$MnCl_2 \cdot 4H_2O$	100mg
$CoCl_2 \cdot 6H_2O$	120mg
$ZnCl_2$	70mg
$NiCl_2 \cdot 6H_2O$	25mg
$CuCl_2 \cdot 2H_2O$	15mg

$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	25mg
HCl (25%)	6.5ml
Distilled water	993ml

6. *Alkalophilic photosynthetic bacteria (Grant & Tindall, 1980)*

NH_4Cl	1.0g
NaCl	0.4g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.4g
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.5g
KH_2PO_4	0.1g
Sodium acetate	1.0g
Yeast extract	1.0g
Trace element soln.	1ml
Distilled water	100ml

Trace element soln.	
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	5.5g
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	5.0g
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	1.0g
$\text{MnCl}_2 \cdot 2\text{H}_2\text{O}$	5.5g
CuSO_4	1.5g
CoCl_2	1.5g
Distilled water	to 1 litre

7. *Methanogenic Bacteria*

KH_2PO_4	0.08g
K_2HPO_4	0.15g
NH_4Cl	0.9g
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	0.2g
Na_2CO_3	2.0g
$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$	0.5g
L-cysteine hydrochloride	0.5g
Tryptone	2.0g
Yeast extract	2.0g
Sodium formate	2.0g
Sodium acetate	2.0g
Trace minerals soln.	5ml
Distilled water	to 333ml

Trace minerals soln. (Ferguson & Mah, 1983)

$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	0.1g
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.1g
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.15g
ZnCl_2	0.1g
H_3BO_3	0.01g

Na ₂ MoO ₄ .2H ₂ O	0.01g
CuCl ₂ .2H ₂ O	0.02g
NiCl ₂ .6H ₂ O	0.04g
AlCl ₃ .6H ₂ O	0.04g
Na ₂ EDTA dihydrate	0.5g
Distilled water	to 1 litre

This medium dissolved in 333ml of distilled water (i.e. 3 times concentrated) by boiling and distributed in 10ml amounts into 30ml capacity glass bottles with screw-tops, with butyl rubber septum lids. After autoclaving, 1ml sterile Na₂S.9H₂O solution (1.5% w/v) was added aseptically and mixed. The bottles were left overnight in an anaerobic glove box containing an atmosphere of 5% CO₂, 15% H₂, 85% N₂.

'On-site' inoculation was by means of injection through butyl rubber septum lids.

8. Sulphate-reducing bacteria.

(a) Postgate Medium B (Postgate, 1984)

This medium was used for the enrichment of lactate-utilising SRB.

Postgate's agar medium E was used for isolation. Medium B was made up 10 times concentrated and later made up to normal concentration using water from the appropriate site.

(b) Enrichment medium for fatty acid-utilising SRB

Solution 1

Distilled water	970ml
Na ₂ SO ₄	3g
NaCl	1.2g
KCl	0.3g
NH ₄ Cl	0.3g
MgCl ₂ .6H ₂ O	0.4g
KH ₂ PO ₄	0.2g
CaCl ₂ .2H ₂ O	0.15g

Solution 2

Distilled water	993ml
HCl (25%)	6.5ml
FeCl ₂ .4H ₂ O	1.5g
H ₃ BO ₃	60mg
MnCl ₂ .4H ₂ O	100mg
CoCl ₂ .6H ₂ O	120mg
ZnCl ₂	70mg
NiCl ₂ .6H ₂ O	25mg
CuCl ₂ .2H ₂ O	15mg
Na ₂ MoO ₄ .2H ₂ O	25mg

Solution 3
 Distilled water 100ml
 NaHCO₃ 8.5g

Solution 4
 Distilled water 100ml
 Na₂S.9H₂O 12g

Solution 5 (carbon sources)
 Each was prepared with 100ml distilled water
 (a) Na-acetate.3H₂O 20g
 (b) Propionic acid 7g
 Adjust to pH9 with NaOH
 (c) n-Butyric acid 8g
 Adjust to pH9 with NaOH

Solution 6 (vitamin solutions)
 Stock solutions of each were prepared with 100ml distilled water:
 (a) biotin 1mg
 (b) o-aminobenzoic 5mg
 (c) vitamin B₁₂ 5mg
 (d) thiamine 10mg

Solution 7
 Oxygen-free distilled water 100ml
 Na₂SO₄ 3g

Medium preparation:

Solution 1 was autoclaved for 20 min. at 121°C together with a magnetic bar.
 Solutions 2,4,5 were autoclaved as above.
 Solution 3 was CO₂ saturated and filter-sterilised into a gas-tight sterile screw-cap bottle.
 Solutions 6 (a-d) were filter sterilised.
 Solution 7 was filter-sterilised in an anaerobic cabinet into sterile, screw-cap bottles.

Proportions:
 Solution 1 970ml
 " 2 1ml
 " 3 30ml
 " 4 3ml

After mixing, the pH was adjusted upwards with sterile 2M Na₂CO₃.
 Then the following were added:

Solution 5 10ml of appropriate carbon source
 Solution 6 1ml of each vitamin solution
 Solution 7 1ml added water anaerobiosis.

The complete medium was dispensed in 25ml amounts to sterile Universal bottles.

9. *Artificial medium for algal growth (Chu. No.10)*

Ca(NO ₃) ₂	0.04g
K ₂ HPO ₄	0.01g
MgSO ₄ ·7H ₂ O	0.025g
Na ₂ CO ₃	0.02g
Na ₂ S ₂ O ₃	0.025g
FeSO ₄ ·7H ₂ O	13.6mg
Distilled water	to 100ml

10. *Fungi (Sabouraud's Dextrose Agar)*

Mycological peptone	10g
Dextrose	10g
Agar No.1 (Oxoid)	15g
Distilled water	to 1 litre

This agar medium was inoculated in the laboratory.

APPENDIX B

Sampling Device for Collecting Sterile Water Samples from Depth

