A Review of the Impact of Microbiological Contaminants in Groundwater

R&D Technical Report P139

J M West[1], S Pedley[2], S J Baker[1], L Barrott[2], B Morris[1], A Storey[2], R S Ward[1] and M Barrett[2]

Research Contractor:
[1]British Geological Survey
and
[2]The University of Surrey, Guildford, Surrey

Further copies of this report are available from:
Environment Agency R&D Dissemination Centre, c/o
WRc, Frankland Road, Swindon, Wilts SN5 8YF

tel: 01793-865000 fax: 01793-514562 e-mail: publications@wrcplc.co.uk

Publishing Organisation:
Environment Agency
Rio House
Waterside Drive
Aztec West
Almondsbury
Bristol BS32 4UD

Tel: 01454 624400 Fax: 01454 624409

MD-07/98-B-BCPP

Dissemination status
Internal: Released to Regions
External: Released to the Public Domain

Statement of use
This report summaries the findings of a review of the current literature pertaining to microbial activity in subsurface environment in the UK.

Research contractor
This document was produced under R&D Project P2-089 by:

Natural Environment Research Council
British Geological Survey
Nicker Hill
Keyworth
Nottingham
NG12 5GG

Tel: 0115 936566 Fax: 0115 9363261

Environment Agency Project Leader
The Environment Agency's Project Leader for R&D Project P2-089 was:
Amanda Patterson - Environment Agency, National Groundwater & Contaminated Land Centre, Solihull

CONTENTS

EXECUTIVE SUMMARY

1.	**INTRODUCTION**	**1**
1.1	Groundwater contaminants	1
1.2	Microbiological Contamination	2
1.3	References	7
2.	**INDIGENOUS MICROBIOLOGICAL ACTIVITY IN AQUIFERS**	**8**
2.1	Introduction	8
2.2	Aquifers as environments for microbial growth	8
2.3	Microbiology and Groundwater Quality	11
2.4	Subsurface microbiology methods	13
2.5	Conclusions and Recommendations	13
2.6	References	17
3.	**ASSESSMENT OF PATHOGENS IN AQUIFERS**	**19**
3.1	Introduction	19
3.2	Classification of water-related diseases	19
3.3	Microbial agents commonly associated with waterborne disease	20
3.4	Bacteria	20
3.5	Viruses	22
3.6	Protozoa	23
3.7	Bovine Spongiform Encephalopathy	24
3.8	Sources of microbial pathogens	25
3.9	The Coliform Test	26
3.10	Conclusions	28
3.11	References	35
4.	**FACTORS AFFECTING MOVEMENT AND FATE OF MICROBIOLOGICAL CONTAMINANTS IN THE SUBSURFACE**	**38**
4.1	Introduction	38
4.2	Physical characteristics of microbes and aquifer materials	38
4.3	Inactivation (half-lives) of microbes	39
4.4	Adsorption	41
4.5	Other factors	42
4.6	Transport	42

4.7	Modelling microbial transport in groundwater	47
4.8	References	58
5.	**SEPTIC TANKS AND PACKAGE TREATMENT PLANTS (PTPs)**	**63**
5.1	Septic tanks and Package Treatment Plants (PTPs)	63
5.2	Public health problems arising from the use of septic tanks and PTPs	65
5.3	Septic tank systems	65
5.4	Chemical and microbiological removal mechanisms in the Septic Tank System	65
5.5	Package Treatment Plants (PTPs)	69
5.6	Regulations and guides covering the design, installation and operation of septic tanks and PTPs	72
5.7	Identification of design and operational factors leading to groundwater contamination by septic tanks and PTPs	72
5.8	Best Practice Guidelines for Septic Tanks and PTPs	75
5.9	Recent and future research into septic tank design	81
5.10	Summary and Suggestions for Research	85
5.11	Conclusions	86
5.12	References	95
6.	**BIOREMEDIATION**	**97**
6.1.	Introduction	97
6.2.	Drivers for the introduction of bioremediation	98
6.3.	Microorganisms and biochemical degradation of pollutants	99
6.4.	Summary of methods used for the remediation of contaminated groundwater	110
6.5.	Conclusions	112
6.6.	Recommendations for future work	114
6.7.	References	127
7.	**ANTIBIOTIC RESISTANT AND GENETICALLY MODIFIED ORGANISMS IN AQUIFERS**	**134**
7.1.	Introduction	134
7.2.	Overview of the characteristics and production of GMOs	134
7.3.	Production of Antibiotic Resistance	135
7.4.	Pathways into the environment	137
7.5.	Fate of Antibiotic Resistant Bacteria and GMOs in the environment	138
7.6.	Legislation governing the release of GMOs into the environment	139
7.7.	Risk Assessment methodologies	139
7.8.	Methods for the detection of GMOs in groundwater	142
7.9.	Assessment of the impact of GMOS and antibiotic resistant bacteria	144
7.10.	Summary	146
7.11.	Recommendations for future work	146
7.12.	References	148

8. USING THE POLICY AND PRACTICE FOR THE PROTECTION OF GROUNDWATER (PPPG) TO ASSESS MICROBIOLOGICAL RISK TO GROUNDWATER 151

8.1. PPPG principles and their relevance to risk from microbes 151
8.2. Risk to groundwater sources; assessment using source to pathway to receptor principles 151
8.3. References 160

9. CONCLUSIONS AND RECOMMENDATIONS 161

9.1. Groundwater Microbiology 161
9.2 Subsurface Microbiology Protocols 161
9.3 Septic Tanks and Package Treatment Plants (PTPs) 162
9.4 Bioremediation 162
9.5 Antibiotic resistant and genetically modified organisms 163
9.6 Microbiology and Policy and Practice for the Protection of Groundwater (PPPG) 163
9.7 Future concerns 163
9.8 Training 163
9.9 References 164

10. ACKNOWLEDGEMENTS 165

GLOSSARY & ABBREVIATIONS 166

Summary of Figures

Figure 1.1 Groundwater use for public supply in England and Wales (1992)

Figure 1.2. Frequency of various contamination sources considered to be major threats to groundwater in the USA

Figure 2.1 Redox (Eh) ranges in natural waters over which microbially mediated reduction processes occur

Figure 4.1. Pathogen diameters compared to aquifer matrix apertures

Figure 4.2 Dispersion effect of spreading

Figure 5.1. Typical two chamber septic tank system

Figure 5.2. Schematic cross section of a conventional septic system including septic tank, distribution pipe and groundwater plume

Figure 5.3. Typical layout of the drainage pipes in gravel-filled trenches

Figure 5.4. Schematic diagrams of cylindrical and 'onion-shaped' tanks

Figure 5.5 Diagram of RBC

Figure 6.1. Theoretical pathways of TCE oxidation by monooxygenases and dioxygenases (from Wackett, 1995)

Figure 6.2. Possible microbially mediated changes in the chemical species and redox conditions in the direction of groundwater flow in the presence of organic contaminants (Bouwer, 1992)

Figure 6.3. Proposed pathways for the initial anaerobic degradation of toluene (Krumholz et al, 1996)

Figure 6.4. Catabolic pathways for the aerobic degradation of toluene (Zylstra, 1994)

Figure 8.1. Relationship of vulnerability, residence time and type of abstraction to microbial risk

Figure 8.2. Adited Chalk systems can induce downward leakage from overlying river gravels, greatly reducing time of travel through the aquifer

Figure 8.3 Conceptual model for assessment of risk to groundwater source from microbiological contamination

Summary of Tables

Table 1.1. Proportion of groundwater used for Drinking water supply in selected European countries in 1988

Table 2.1. Range of Concentrations of Chemical Constituents of Groundwater in the Principal British Aquifers (summarised from data in Edmunds et al, 1989)

Table 2.2. Bacterial populations in deep groundwater environments (after West, 1995)

Table 2.3. Temperature Ranges for Microorganisms Groups (after Ehrlich, 1990)

Table 3.1. Some examples of infectious disease hazards associated with microorganisms in water (Hurst, 1996).

Table 3.2. Illnesses acquired from the ingestion of water (Moe, 1996).

Table 3.2. Illnesses acquired from the ingestion of water (continued).

Table 3.3. Recent outbreaks of HAV-associated with water (Nasser, 1994).

Table 3.4. *Cryptosporidium* outbreaks in drinking water supplies in the UK (adapted from Lisle and Rose, 1995).

Table 3.5. Occurrence of bacterial pathogens in water (Emde et al., 1992).

Table 3.6. Occurrence of viral and other waterborne pathogens in water (adapted from Emde et al., 1992)

Table 4.1. Factors affecting fate and transport

Table 4.2. Sizes of selected microbe

Table 4.3. UK aquifer properties. (The value ranges presented are for indication only)

Table 4.4. Factors affecting microbe survival and half-life

Table 4.5. Examples of half-lives of pathogenic microbes derived from experimentation. (Environmental conditions not specified)

Table 4.6. Laboratory column experiments used for investigating transport of microbiological contaminants

Table 4.7. Selected field tracer tests in which microbial tracers have been used

Table 5.1. Types and features of Packaged Treatment Plants

Table 5.2. Instruction sheet for use with septic tanks and PTPs

Table 5.3. Per capita volume of sewage generated at non-domestic premises

Table 5.4. Point scoring system for septic tank soakaway assessment

Table 6.1. Oxygenases and organisms implicated in TCE oxidation and their relative *in vivo* rates (from Wackett, 1995)

Table 6.2. Concentration of hydrocarbons in water after contact with distillate products (Chapelle, 1993)

Table 6.3. Isolates that have been documented to degrade toluene under anaerobic conditions (Krumholz et al, 1996)

Table 6.4. Representative polycyclic aromatic hydrocarbons metabolised by different species of bacteria (Mueller et al, 1996)

Table 6.5. Representative polycyclic aromatic hydrocarbons metabolised by different species of fungi (Mueller et al, 1996)

Table 6.6. Representative polycyclic aromatic hydrocarbons metabolised by different species of cyanobacteria and algae (Mueller et al, 1996)

Table 6.7. Microorganisms that metabolise aromatic hydrocarbons (Rosenberg and Ron, 1996)

Table 6.8. Advantages and disadvantages of in situ bioremediation of groundwater (from Anon, 1995)

Table 6.9. UK companies that have been approached for information

Table 7.1. Genera of bacteria capable of natural transformation (after Stewart 1989)

Table 7.2. Control of competence in Transformable bacteria (after Stewart 1989)

Table 8.1. Guide for use with GPP policy statements to assess likely microbiological significance of activities potentially contaminating groundwater

Summary of Appendices

Appendix 5.1 List of manufacturers visits.

EXECUTIVE SUMMARY

This review shows that there is a general paucity of information on groundwater microbiology (both indigenous and introduced populations) in the UK, and even less on the role of indigenous microbiology on groundwater quality and human health. In addition, it shows that there are no clear protocols for sampling subsurface materials or for examining microbial population diversity and activity. Historically, faecal and total coliform tests have been used to assess the safety of waters, but it is now generally agreed that they are of limited value as universal pathogen indicators in groundwater. New protocols are required to cover both indigenous populations and risk contaminant organisms hazardous to health which coliform tests do not guard against.

The study has shown that there is little information on the effectiveness of removal of microbiological contaminants across septic tanks and Package Treatment Plants (PTPs). There is no consideration of biological quality of effluent nor of the capacity of the subsurface to treat effluent. This is a serious omission because, unlike sewage treatment works, both septic tanks and PTPs are designed to dispose of their effluent to the subsurface, without consideration of the ability of the receiving body to attenuate/absorb/dilute the effluent loading. The only concern is if a soakaway can physically cope with the volume of effluent. Yet soakaway design and the optimisation of effluent water quality is a national issue for groundwater protection reasons especially where on-site sanitation systems discharge to formations also used locally for water-supply purposes with minimal or no disinfection facilties (eg many rural private supplies).

The publication in February 1998 of user and regulator guides to septic tank system operation by CIRIA has provided excellent good practice literature. However the paucity of information from UK field studies on the microbial and chemical nature of typical septic and PTP effluent means that the impact of these on-site sanitation systems on particularly fragile aquifer settings has not been assessed.

Much of the information from case studies on bioremediation in the UK is 'commercial in confidence' and unavailable. However, from the available work it is clear that there is a need to examine different remediation technologies and their effects on groundwater quality in terms of changes in microbiology and chemistry. For instance, oxygen and nutrient injection can change redox status, and this could lead to mobilisation of metals occuring naturally in the matrix. This process has already been observed below leaking sewers in Germany and below districts served by on-site sanitation in Bolivia and Thailand. Indeed some technologies may not be appropriate for certain sites.

No information is available on the genetic stability of organisms in the subsurface or in groundwaters.

This review has shown that the Policy and Practice for the Protection of Groundwater (PPPG) can be used as a framework to assess potential hazard from microbiological agents. The PPPG policy statements for instance, tailored to focus on microbial agents, can assist in identifying and prioritising those activities which may impact adversely on groundwater resources. However, one weakness of the PPPG is that it does not easily allow for curtailment of potentially prejudicial **existing** activities/practices on the land surface rather than proposed ones. The UK is subject to the requirements of the Urban Wastewater Treatment Directive and once the present transitional arrangements expire in 1999, it is

estimated that UK sludge disposal to agricultural land will increase from 465,000 tonnes in 1991 through 777,000 tonnes in 1999 to 926,000 tonnes in 2006, a rise of almost 100% in 15 years. The same report referred to a further 441,000 tonnes of sludge as having an 'uncertain disposal route' and it remains to be seen whether part of this extra tonnage would also be disposed to land. This report has shown that in the face of such scant knowledge of the microbiological effectiveness of on-site sanitation systems and of the high intensity sludge disposal to land, there are major uncertainties about the capability of the subsurface to accept and attenuate such waste loadings.

1. INTRODUCTION

Groundwater is an important natural resource worldwide. In Europe, and the United States greater than half the water supplied for public use is groundwater. The proportion of groundwater used in European countries is shown in Table 1.1. National figures for the UK (UNEP, 1989) shows that an average of 29% of drinking water supplies are taken from groundwater sources. This figure rises to 80% in parts of southern and eastern England (Figure 1.1). Traditionally, groundwater has been perceived as having two advantages over surface water. First, it is attractive in terms of capital investment because development can progress in stages with rising water demand. Second, it is much less vulnerable to pollution. However, there is now a significant body of evidence to suggest that the latter advantage is being eroded due to the increasing number of point and diffuse sources of pollution arising from urban, industrial and agricultural activities (Mather, 1993). Moreover, while less easily polluted, contaminated aquifers may be difficult to restore to their original status.

1.1 Groundwater Contaminants

A groundwater contaminant may be defined as any dissolved solute or non-aqueous liquid that enters groundwater as a consequence of people's activities (Domenico and Schwarz, 1990). It will also include microbial contaminants which may include disease-causing pathogens. Using this definition, the potential sources of groundwater contamination are numerous. In a 1984 report, *Protecting the Nations Groundwater from Contamination*, the Office of Technology Assessment (OTA) of the U.S. Congress listed more than 30 different potential sources of ground-water contamination. The OTA report divides the contamination sources into six categories (Fetter, 1993) practically all which will contain microbial populations:

- Category I:

Sources designed to discharge substances

- Category II:

Sources designed to store, treat and dispose of substances

- Category III:

Sources designed to retain substances during transport

- Category IV:

Sources discharging substances as a consequence of other planned activities

- Category V:

Sources providing a conduit for contaminated water to enter aquifers

- Category VI:

Naturally occurring sources whose discharge is created and/or exacerbated by human activity

- Category VII:

Accidents, spillages, unplanned disposal routes

A United States, EPA survey of States and Territories (Fetter, 1993) has revealed that some sources of contamination represent a greater threat than others to groundwater quality (Fig 1.2). The greatest threat was found to be from underground storage tanks, septic tanks,

agricultural activities, municipal landfills and abandoned hazardous waste sites many of which will contain pathogens. Other sources frequently found to pose a threat were landfills, injection wells, regulated hazardous waste sites and land application.

In the UK, the National Rivers Authority (now the Environment Agency) compiled a list of potential sources of groundwater contamination (NRA, 1992) as part of its published policy and practice for the protection of groundwater. The main categories of contamination sources are similar to those published by the US EPA although there are some differences between the sources that have been listed and the categories within which they are classified. Sections C to H of the groundwater protection policy statements list the categories as:

- Waste disposal to land
  - Landfilling activities
  - Scrap yards
  - Transfer stations
  - Incinerators
  - Waste storage and treatment
- Contaminated land
  - Coal-gas manufacture
  - Landfill sites and other waste disposal activities, waste lagoons
  - Chemical manufacture
  - Heavy industry
  - Mining
  - Sewage treatment works
  - Metal refining
  - Oil refining and hydrocarbon storage
- Application of liquid effluents, sludges and slurries to land
  - Controlled wastes
  - Sewage sludges
  - Agricultural wastes
- Discharges to underground strata
  - Sewage effluent discharges
  - Trade effluent discharges
  - Surface water discharges
- Other activities of concern
  - Production storage and use of chemicals (raw and waste)
  - Storage of farm wastes and intensive livestock housing
  - Graveyards and animal burial sites
  - Sewage works foul sewers and storm overflows
  - Oil and petroleum storage and transport via pipelines
  - Major infrastructure developments

1.2 Microbiological Contamination

Microbes are organisms that are too small to be clearly perceived by the unaided human eye (usually with a diameter of less that 1mm). They include protozoans, many algae and fungi, bacteria and viruses. The role of microbiologically contaminated groundwater in the transmission of disease has been well established by epidemiological investigations of

waterborne disease outbreaks. Summary statistics from the USA show that 51% of all waterborne disease outbreaks, and 40% of all waterborne illness is caused by the consumption of contaminated groundwater Craun (1985). In Finland also, contaminated groundwater has been identified as a more significant cause of waterborne disease than contaminated surface water. Whilst few other countries have published comparable, national statistics, the available data suggest that groundwater has the potential to be a significant vehicle for the transmission of waterborne disease.

Groundwater, although of high microbiological quality in terms of faecal contamination in its natural state, can become contaminated rapidly and easily. A variety of processes may lead to contamination: inadequate sanitary completion of boreholes and wells; siting of abstraction points in close proximity to on-site sanitation; leaking sewers; groundwater recharge by wastewater; landfill leachate; and land-based disposal of sewage sludge. In the USA, septic tanks are the most frequently reported source of groundwater contamination.

Concern about the microbiological contamination of groundwater in the UK has developed along two lines. First, by recent advances in the understanding of subsurface microbiological processes as a result of improved sampling and analytical techniques, and second by the appreciation that pathogens may access groundwater and be transmitted to potable water supplies with serious consequences for public health. Thus groundwater systems are both active ecosystems, with their own indigenous populations, and effective vehicles for the transmission of microbial contaminations derived from, for example, sewers and septic tanks. Recent outbreaks of disease caused by *Cryptosporidium* attributed to groundwater contamination, and concerns about the transmissivity of the BSE prion have resulted in this report which will review and assess behaviour and fate of microbial contaminants in groundwater. The report encompasses:

1. A review and
2. Recommendations for a detailed programme of further research

It is divided into a number of sections:

- Indigenous microbiological activity in aquifers
- An assessment of pathogens in groundwater
- Factors affecting movement and fate of microbiological contaminants in the subsurface
- Septic Tanks and Package Treatment Plants
- Bioremediation
- Antibiotic resistant and genetically modified organisms in aquifers
- Use of the Policy and Practice for the Protection of Groundwater (PPPG) to assess microbiological risk to groundwater
- Conclusions and recommendations

This document is intended to be a reference for staff of the Environment Agency needing up to date information on particular aspects of microbiological contaminants of groundwater. The review assumes a basic knowledge of biological processes and microbiology. Further background information can be obtained from any good microbiology text book, for example Brock et al (1994).

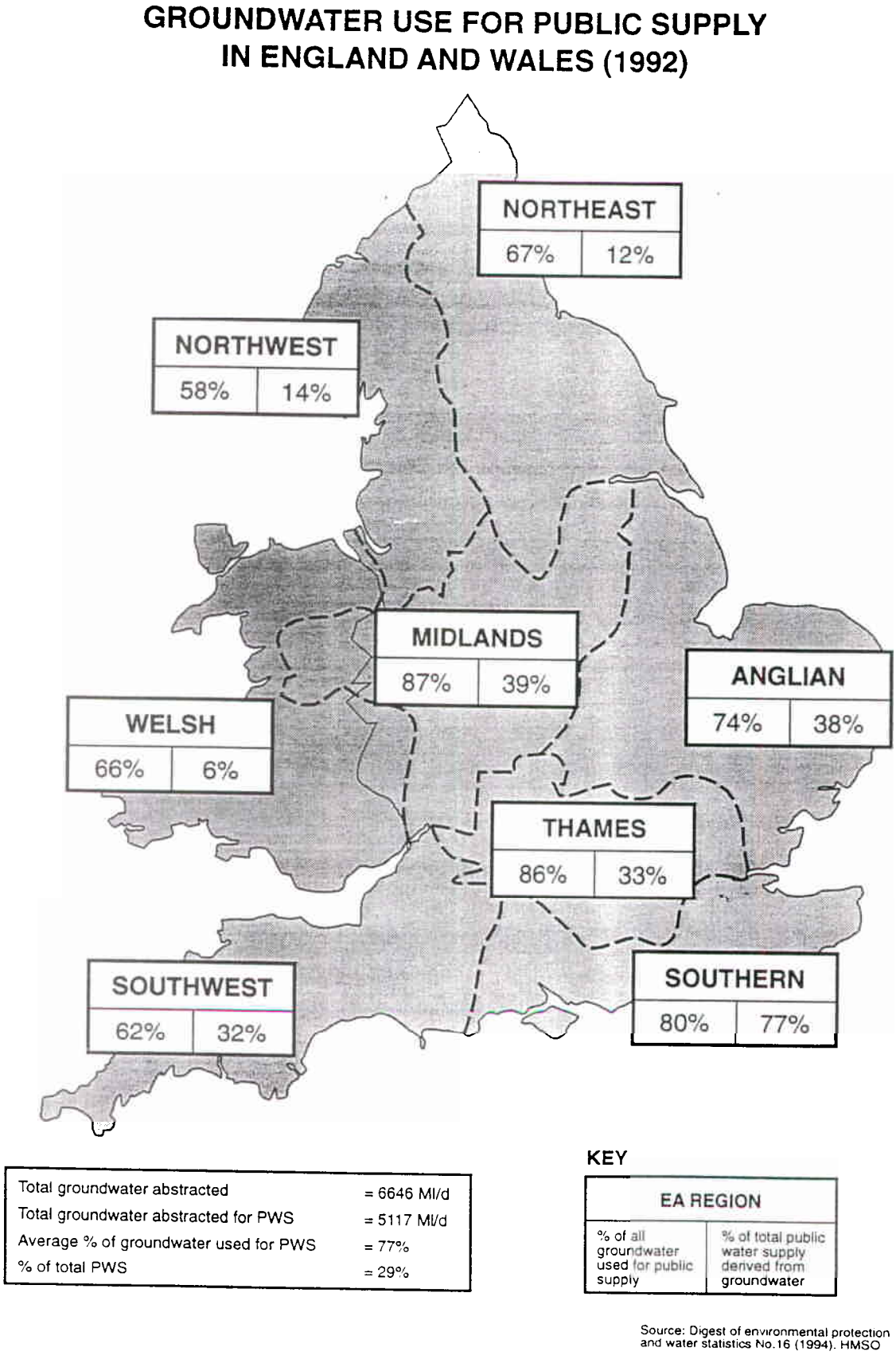


Figure 1.1. Groundwater use for public supply in England and Wales (1992)

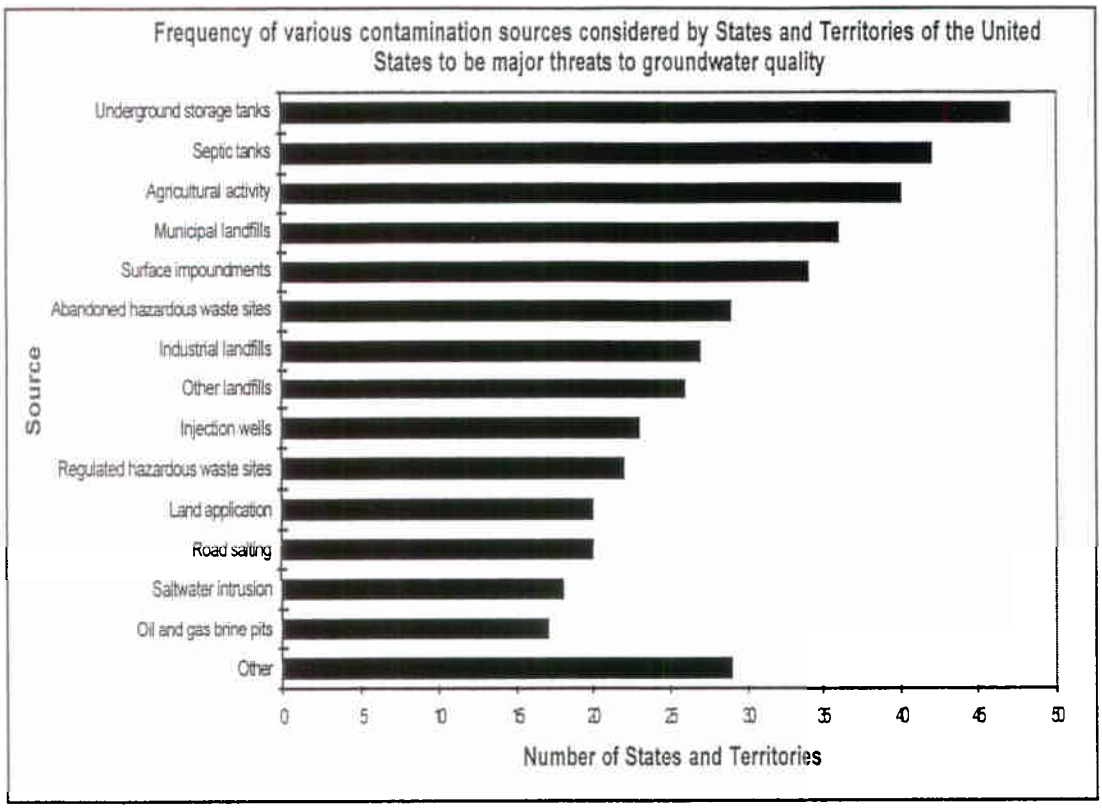


Figure 1.2. Frequency of various contamination sources considered to be major threats to groundwater in the USA (after Fetter, 1993)

Table 1.1. Proportion of groundwater used for Drinking water supply in selected European countries in 1988

Denmark	98%
Germany	89%
Italy	88%
Switzerland	75%
Belgium	67%
Netherlands	67%
Sweden	49%
United Kingdom	35% (29% in 1992)
Spain	20%
Norway	15%

(From UNEP, 1989)

1.3 References

Brock, T.D., Madigan, M.T., Martinko, J.M. and parker, J. (1994) Biology of Microorganisms, (7th Ed). Prentice-Hall. Internation, London.

Craun, C.F. (1985) A summary of waterborne illness trasmitted through contaminated groundwater. J. Environmental Health **48**, 122-127.

Dominco, P.A. and Schwarz, F.W. (1990) Physical and Chemical Hydrogeology, Wiley and Sons, New York. pp 824.

Ember L. (1992) Toxic release inventory data show steady drop in emissions. Chemical Engineering News, 70, 13-15.

Fetter, C.W. (1993) Contaminant hydrogeology, Prentice Hall, New Jersey. pp 458.

Mather, J. (1993) The impact of contaminated land on groundwater: A United Kingdom appraisal. Land Contamination and Reclamation, 1, 187-195

National Rivers Authority (1992) Policy and Practice for the protection of groundwater, NRA, Bristol pp 51

UNEP (1989) Environmental Data Report, 1st Edition, Blackwell Reference, Oxford.

UNEP (1992) Environmental Data Report, 2nd Edition 1989/90, Blackwell Reference, Oxford.

2. INDIGENOUS MICROBIOLOGICAL ACTIVITY IN AQUIFERS

2.1 Introduction

To many biologists and geologists the idea of the biosphere extending more than a few metres below the soil is a strange concept. 'Rock' is perceived as incapable of supporting life as it is thought to be dense, fairly dry and low in nutrients with a harsh environment more ideally suited to the preservation and fossilisation of biological materials than to providing a habitat for life. However, this perception can be dispelled by examining aquifers in terms of their potential to support life.

2.2 Aquifers As Environments for Microbial Growth

2.2.1 Microbial growth requirements

Suitable conditions for the synthesis of protoplasmic constituents and for the liberation of energy necessary for life processes must exist for an active microbial population to develop. Microbial growth requires a carbon source (organic and/or inorganic), nitrogen, phosphorus, sulphur, trace elements and water. The biochemical liberation of energy in the absence of light requires:

1. The presence of an electron donor such as oxidisable organic compounds or, in the case of chemolithotrophs (organisms obtaining energy from oxidation of inorganic compounds), oxidisable inorganic substances such as molecular hydrogen, ammonia, sulphide or ferrous ions;

2. The presence of an electron acceptor such as molecular oxygen, sulphate, nitrate, ferric compounds, carbon dioxide or simple organic compounds.

Qualitatively, by this approach, most aquifers have a capacity to support at least a limited microbial population.

However, the availability of nutrients and energy sources will control microbial growth and activity (McNabb and Dunlap, 1975). While aquifers might be considered as oligotrophic, supporting organisms which require only low levels of nutrients, most groups of bacteria distinguished by their nutritional requirements, may find suitable conditions. These would include denitrifiers (which use nitrate as their terminal electron acceptor), sulphate reducers (which use sulphate as their terminal electron acceptor) and 'iron' bacteria (which use oxygen as their terminal electron acceptor). Most would be attached to surfaces in a gelatinous matrix, a biofilm. Biofilms are composed monolayers or multilayers of bacterial cells that are bound to surfaces by extracellular polysaccharides (EPS). Organisms within the biofilm create microenvironments which, under low nutrient and hostile conditions, may provide some protection from environmental extremes.

2.2.2 Nutrient and energy supplies in British Aquifers

The supply of nutrient and energy sources in aquifers must be obtained from the groundwater itself or via solubilisation of compounds from the rock matrix. Table 2.1 provides some

ranges of concentrations of chemical constituents of groundwater in three prominant British aquifers (Edmunds et al, 1989). These concentrations of dissolved inorganic compounds are sufficient to provide energy for chemolithotrophic organisms. This is confirmed by the observed sulphate reduction which occurs in each of these three aquifers as they become confined beneath clay layers (Edmunds et al, 1989). Table 2.1 provides information on inorganic carbon, HCO_3, which can be used by such organisms.

It should be noted, however, that both solid and dissolved organic matter provide sources of carbon for microbial activity. Indeed the cells themselves may be used as a nutrient source during life (predated upon by other microbes such as protists) or after death. Predation of microbes in the subsurface is a very new area of study. Recent work by Kinner et al (1998) have shown that aquifer nanoflagellates (mostly *Spumella guttula*) can clear between 12 and 74% of unattached bacteria in sandy aquifer material in a day. Studies of the interactions between the various microbial population types (protozoans, bacteria, viruses etc) present in the subsurface is in its infancy but is likely to be complex. Sibille et al (1998) describe a functional ecosystem that developed in drinking water distribution systems and, although this is not analagous to an aquifer system, it does show how complex an oligotrophic system can be.

The occurrence of organic matter in these three British aquifers was reviewed by Foster et al (1991). All three have low organic carbon contents (Table 2.1). The Chalk has the greatest proportion of adsorbing material, disseminated widely in the matrix. The Jurassic Limestones contain the lowest overall proportions of organic carbon, but this appeared to be unevenly distributed. Low levels of bitumens were found in the oxidised areas close to fissures (Lawrence and Foster, 1986), and generally higher amounts in unaltered parts of the limestone. Kerogen, a stable substance formed from ancient organic material, is probably more plentiful but less active in adsorption. It was not quantified because of the difficulty of separating it from pyrite, but was thought to follow a similar distribution. The Triassic Sandstones are believed to contain only low levels of mature refractory organic matter, consistent with their generally oxidised state (Foster et al, 1991). Little information exists on the availability of organic matter for microbial activity. Whitelaw and Edwards (1980) examined the unsaturated zone of the Chalk for carbohydrates. All samples were found to contain sufficient material which, if used as an energy source, could produce significant bacterial denitrification. The origin of the carbohydrate was considered to be organic material in the overlying soil.

The general scarcity of organic carbon in aquifers is confirmed by measurements of dissolved organic carbon (DOC) in groundwater. In the absence of pollution, natural concentrations of dissolved organic carbon in groundwater are typically 1-2 mg/l and rarely exceed 5 mg/l. This compares with concentrations of 10-100mg/l for soil solutions, with highest concentrations in acid soils. Vertical profiles of DOC in the unsaturated zone have been obtained in a recent study of the Chalk and Triassic Sandstones in Britain related to vulnerability to nitrate leaching. At sites where cultivation has been supported by significant organic manure, DOC concentrations of 50 mg/l or more were measured at 2-3m depth, and at some sites remained above 20 mg/l to the base of the profiles at 10-15 m depth. Preliminary interpretation of the forms of nitrate, nitrite and DOC profiles suggest that the organic material available from the manure is enhancing denitrification. Denitrifying microorganisms are facultative anaerobes requiring, in general, an organic carbon source and nitrate as the terminal electron acceptor reducing it to nitrite ions, nitrous oxide or nitrogen. It appears that the additional carbon from the manure is enhancing the activity of denitrifying

microorganisms. The *in-situ* biodenitrification rate cannot be calculated from this limited information although is probably higher than the rates of 1.5 mg NO_3 -N m^{-2} d^{-1}observed in the confined Lincolnshire Limestone (Clark et al, 1991).

If these three lithologially distinctive formations are typical of other British aquifers, then it would seem that the prerequisites for the maintenance of microbial life will be present. The extent of activity will be dependent on nutrient and energy supplies and hence there will be a limiting growth factor. Existing data suggest that the availability of carbon may be the main control. No analysis has been made of numbers and types of bacterial populations present in UK aquifers. However, Table 2.2 shows bacterial counts in a range of deep groundwater environments which would suggest that 10^5 colony forming units (CFU)/ml is not unreasonable in fracture planes. This is likely to be a gross underestimate as studies in the USA and Canada (eg Chapelle, 1997) have shown that 80-90% are living but are unculturable and are therefore unidentifiable. They can, however, be viewed by direct microscopy but cannot be grown using conventional isolation methods. The challenge for microbiologists is to develop new methodologies for studying these novel species.

2.2.3 The role of Environmental Conditions

Environmental conditions such as pH, temperature, pressure, redox conditions all influence microbial growth and activity (West et al, 1991 and West and Chilton, 1997).

Each microorganism has an optimum pH. Most natural groundwaters have pH values in a relatively narrow range, from about 6.0 to 8.5 and microorganisms with optima in this range are common. Lower pH values (2-5) are found in humid tropical environments, mining areas, peat bogs and geothermal areas, although the latter are not usually important for water supply because of high levels of mineralisation. Acid pH is important in areas where acid mine water comes into contact with water supplies as may occur in the West Country. In contrast, high pH values (above 9) are also found in soda lakes and high carbonate environments. Some organisms can grow at these extreme pHs. Acidophilic organisms include species of *Thiobacillus, Sulfolobus and Thermoplasma.* Both *Thiobacillus* and *Sulfolobus* can oxidise sulphide minerals and produce sulphuric acid. Alkaliphilic organisms (many are *Bacillus sp)* can have pH optima as high as 10-11.

An important physical aspect of aquifer environments which controls microbial activity is temperature. Three groups of microrganisms are recognised based on their preferred temperature ranges (Table 2.3). Similarly, microbes have been shown to be tolerant of extreme pressures up to 150MPa (West et al, 1991) and hydrostatic pressure is not likely to prevent microbial activity in subsurface environments which are otherwise suitable as habitats. Microbial populations will adapt to the temperature and pressure range of a particular subsurface environment (Ehrlich, 1990).

Within the first few metres below the surface, groundwater temperatures respond to seasonal climatic fluctuations, but the insulating qualities of the ground rapidly dampen the large diurnal and seasonal variations experienced at the surface. Below about 10 m in the tropics, 15 m in temperate regions and 20m in polar regions, groundwater temperatures remain remarkably uniform and equal to the mean annual air temperature, rising from 10-15°C in Europe to 25-30°C in equatorial regions. At greater depths, beyond the range of most potable water abstraction, groundwater temperatures rise in accordance with the geothermal gradient at about 3°C for each 100 m. Likewise, interstitial hydrostatic pressure in the saturated zone increases with depth at about 1MPa for each 100m.

One of the most important features of the aquifer as an environment for microbial activity is the presence or absence of oxygen. Above the water table, conditions are largely aerobic, although moisture is not necessarily uniformly distributed, so anaerobic conditions may exist locally producing micro-environments in the smaller discontinuous pore spaces. Moreover, seasonal variations in the elevation of the water table may produce highly variable conditions in the zone of fluctuation. The concentration of dissolved oxygen in the infiltrating water will be close to saturation near the surface, i.e. about 10mg/l. Below the water table, the oxygen supplied from infiltrating recharge reacts with oxidisable material encountered along the flow path. If little such material is available, water containing measurable amounts of dissolved oxygen (2-5 mg/l) may persist well into the aquifer. If oxidisable material is plentiful, either naturally or as a result of pollution of groundwater, then anaerobic conditions may be established more quickly. Oxidation and reduction processes play an important role in controlling the chemistry of many elements including C, N, O, S, Mn and Fe. In most groundwaters, the reaction sequence follows a predicted thermodynamic sequence (Stumm and Morgan, 1981). In a closed system, such as along a flow line in a confined aquifer, dissolved oxygen will be utilised first by reaction with organic compounds. Denitrification will follow, with reduction of MnO^{2} then occurring. Reduction of FeOOH to Fe^{+2} should follow nitrate ammonification. When Eh values are sufficiently low, $CO_2$and SO_4 reduction and fermentation reactions may occur almost simultaneously. Many of these reactions are microbially mediated with energy derived for microbial useage. In most reactions, molecules of organic carbon represent the electron donor, whereas compounds containing N, S, Mn, Fe etc act in general as electron acceptors. Thus a succession of microbes will catalyse the reactions in many groundwater systems (Figure 2.1).

The assessment of nutrient and energy supplies in the three prominant British aquifers (Table 2.1) indicates that carbon is the likely control on growth. They are thus carbon limited aquifers (Chapelle and Bradley, 1997). Electron acceptor limited aquifers are those where there is an excess of electron donors (usually carbon) so microbial metabolism is limited by the availability of electron acceptors such as dissolved oxygen, sulphate and nitrate. Examples include most natural petroleum reservoirs and aquifers that have been chemically contaminated. A well documented example of this type of aquifer is given by Baedecker and co-workers for a site of a crude oil spill in Minnesota, USA (Baedecker et al. 1993). A series of zones were identified ranging from methanogenesis near the crude oil, followed by Fe (III) reduction 10 to 20m downgradient followed by oxygen reduction 100m downgradient. Sulphate reduction was not possible as sulphate was not available in sufficient quantities.

2.3 Microbiology and Groundwater Quality

As can be seen in Section 2.2. the geochemistry of the groundwater can be much influenced by microbial processes. Geochemical modelling of groundwater chemistry can be used to differentiate between inorganic and microbiological processes. Chapelle (1993) describes the Floridan aquifer where production of carbon dioxide into the system could only be explained as a result of sulphate reduction - a purely microbiological process. Other work has shown how both microbiological and abiotic processes together influence groundwater chemistry (eg Bennett et al, 1996). Work performed by the British Geological Survey (BGS) in Thailand and Bolivia also considered that microbially mediated reactions were pivotal in understanding the transformation of downward moving urban recharge (BGS, DMR and PSU University, (1997); BGS and Cooperativea de Servicios Publicos (1997)).

Table 2.1 gives the principal chemical constituents of groundwater in some British aquifers but does not include parameters such as trace metals, free hydrogen sulphide etc. Some of these can, in themselves, act as energy sources for microbial activity. As an example, arsenic is widely distributed in the upper crust of the Earth but mostly at low concentrations. Arsenopyrite is the most common form of arsenic. In nature 3 oxidation states are most common: As(+V) (arsenate), As(+III) (arsenite - the most toxic and mobile form), As(-III) (arsine). Although changes between these states can be achieved chemically, microorganisms can mediate the reactions such as oxidation, reduction and methylation (Cullen and Reimer, 1989). Studies by BGS in gold-mining areas in West Africa have shown that microbes can mobilise arsenic in groundwaters from arsenopyrite (West et al, 1996); from the rock matrix in Thailand (BGS, DMR and PSU University, (1997)). Mn was also mobilised from the matrix in studies in Bolivia (BGS and Cooperativea de Servicios Publicos (1997)). Consideration of such effects should be included where water is being abstracted from areas where the mineralogy is of concern such as Cornwall.

Recently demands on groundwater have increased and concerns about unforeseen contamination have heightened. Awareness of a pollutant in an aquifer may come only when large amounts are already present. These worries are particularly illustrated by nitrate pollution in certain aquifers in the UK. Nitrate from intensively cultivated land is entering recharge water. It is stored in both the unsaturated and saturated zones and may take decades to pass through the system.

The EC water quality directive (EC 1982) states that nitrate levels in potable water should not exceed 50mg NO_3/l (11.3mg-N/l). Some groundwaters exceed this value already and others are likely to do so before the end of the century. Such levels are of concern to the water suppliers who can tackle the problem either by looking for alternative low nitrate sources for direct supply, by blending, or by installing nitrate removal plants. These alternatives all involve large investment. The existence and efficacy of *in-situ* microbial denitrification has therefore been studied for some years in order to assess how far it can contribute to nitrate reduction in affected aquifers.

Denitrifying microbes are anaerobic heterotrophs and autotrophs which use nitrate as their terminal electron acceptor. Nitrogen gas is the final end product with the intermediate formation (and sometimes accumulation) of nitrite and nitrous oxide. There is considerable indirect evidence, such as decreasing nitrate levels, to indicate that microbiological denitrification is occurring in groundwaters under various conditions but until recently little direct work had been directed at this phenomenon. A long term project on microbial denitrification processes in UK aquifers carried out by the British Geological Survey has examined the three major British aquifers, (Lincolnshire Limestone aquifer between Grantham and Aslackby; Permo-Triassic Sandstone aquifer at Carlton in Yorkshire; Chalk aquifer at Mattishall and Lyng, Norfolk) for the presence of denitrifiers and the capacity of the confined parts of the aquifer systems to support denitrification processes (eg Clark et al, 1991; Chilton and Shearer 1993; Kinniburgh et al, 1996). In all cases denitrifiers were present and the capacity to denitrify demonstrated if suitable conditions were provided. The extent to which denitrification can proceed again appears to be controlled by amounts of available carbon present in the subsurface.

Groundwater supplies also face a threat of pollution from a range of synthetic organic chemicals. These chemicals include pesticides and herbicides used for agricultural and horticultural purposes; and chlorinated organic solvents arising from industrial use which have been disposed, leaked or spilled onto land. Little is known about the movement of such

chemicals through the unsaturated and saturated zones. However, once in the saturated zone there is significant risk of long distance movement and great difficulty in estimating and managing contaminant behaviour. These chemicals are potential carbon sources for microbial growth although many of the contaminants may prove recalcitrant and may themselves be toxic to microbial populations. The bioremediation of a contaminated aquifer is a possibility if the potential for microbial degradation is established. Treatment could be achieved by enhancing the natural *in-situ* processes (Chapelle, 1993). This sort of work is already being carried out in the United States where spills are of a localised nature and is described in more detail in Section 6. Care needs to be taken to avoid the introduction of potential pathogens into the system.

2.4 Subsurface Microbiology Methods

There are considerable technical difficulties in sampling subsurface environments. Groundwater sampling is usually undertaken using conventional techniques. Water is collected in sterilised, sealable systems during conventional geochemical sampling (eg Chapelle, 1993). Sampling of rock material is considerably more difficult as contamination is almost inevitable during drilling (eg Bitton and Gerba, 1984; Stroes-Gascoyne and West, 1997). Complex methodology development is being carried out in the USA (eg Chapelle, 1993) but most sampling of rock is carried out in an ad hoc manner. Methods for examining indigenous populations are diverse, ranging from classic microbiological techniques to modern molecular biological analysis of these organisms (West, 1995). No one standard technique emerges for use in the subsurface. When concern arises about contamination of a groundwater with organisms hazardous to health (see Section 3) then the coliform test is used (eg Price, 1996).

2.5 Conclusions and Recommendations

Most aquifers have the capacity to support an indigenous microbial population and their activity can influence the quality of groundwaters. A survey of deep groundwaters indicates that a population of 10^5 CFU/ml might be typically present along fracture systems although this is probably a gross underestimate due to the difficulty in culturing oligotrophe microorganisms. Techniques for sampling of groundwater and subsurface material are being developed and many methods are being used to examine the range and activity of microbial populations. The literature suggests that scope exists for a microbial contribution to the degradation of organic compounds in aquifers but little research has been carried out to quantify this potential. Subsurface environments with low groundwater flow are also capable of sustaining microbial populations. The potential for microbial activity in aquifers is dependent on the availability of nutrients and energy sources, the physical characteristics of the aquifer and the environmental conditions which will be experienced by the organisms. However, there is little information on the microbiological compositions and activities in UK aquifers, apart from indirectly, based on hydrogeochemical pointers only.

It is recommended that a baseline pilot-scale microbiological study of indigenous populations be undertaken of one UK aquifer as part of existing groundwater sampling programmes. A pilot study to help strengthen the current limited understanding of groundwater pathogens in the UK and also from hydrochemical changes mediated by microbial activity is also recommended.

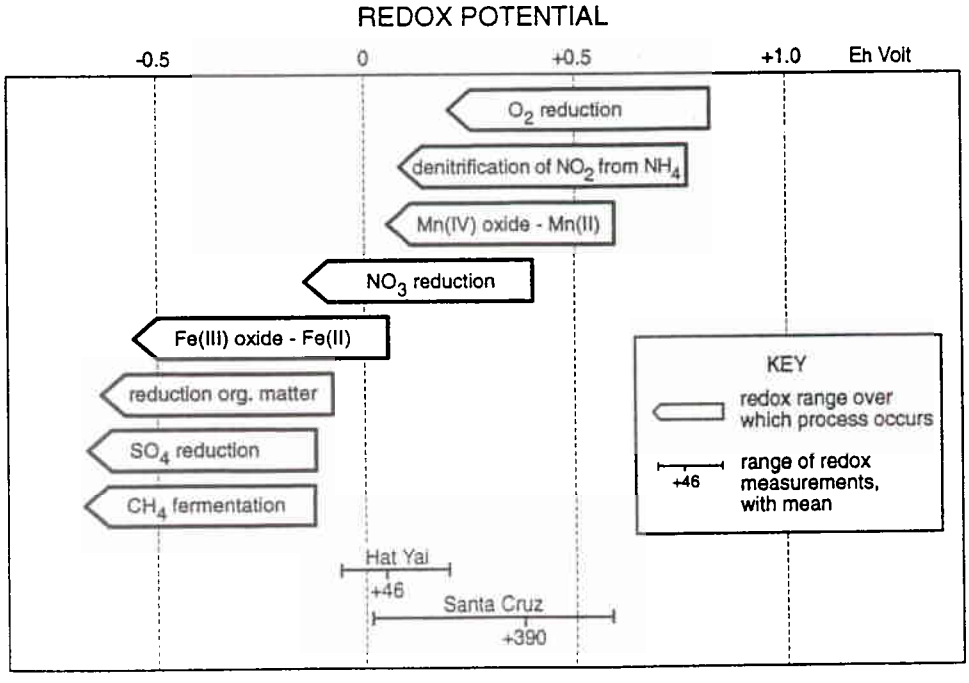


Figure 2.1. Redox (Eh) ranges in natural waters over which microbially mediated reduction processes occure

Table 2.1 Range of Concentrations of Chemical Constituents of Groundwater in the Principal British Aquifers (summarised from data in Edmunds et al, 1989)

Parameter	Cretaceous Chalk (Berkshire)			Jurassic Limestone (Lincolnshire)			Triassic Sandstone (Shropshire)		
	Low	Average	High	Low	Average	High	Low	Average	High
Temp ^{O}C	10.1	11.0	12.5	9.0	10.5	12.0	8.5	10.3	11.5
pH	6.8	7.2	7.5	7.0	8.0	9.5	6.5	7.0	7.3
Eh (mV)	-50	+120	+400	-300		+400	+250	+350	+500
Na	7	16	110	12	30	800	8	12	50
K	0.8	2	10	2	3	4	105	205	405
Ca	40	85	140	3	30	750	45	75	110
Mg	1.5	9	20	3	7	16	2.5	13	23
SO_4	6	18	55	7	65	150	14	24	65
Cl	12	17	95	22	35	950	16	35	50
NO_3-N	<0.5	<1	7	<0.5	<1	5	1.0	7	9
HCO_3	250	300	350	260	320	520	150	210	320
Fe	<0.001	0.1	0.7	0.01	0.1	1.0	0.0005	0.001	0.06
TOC	0.05 - 0.2			0.02 - 0.05			0.2 - 2		

All concentrations in mg/l
Low = ten percentile, Average = fifty percentile and High = ninety percentile
TOC = Total organic carbon % weight

Table 2.2. Bacterial populations in deep fracture environments **(after West, 1995)**

Location	**Geology**	**Depth (mbgl)**	**Bacterial count ($CFUml^{-1}$)***
Harwell, UK	Oxford Clay	165-331	$8.6x10^{3}$ - $3.5x10^{5}$
Altnabreac, UK	Granite	10-281	$9.4x10^{5}$
Stripa, Sweden	Granite	340	$3x10^{1}$ - $1.3x10^{5}$
Cornwall, UK	Granite	80-800	$2.5x10^{2}$ - $2.5x10^{4}$
Mol, Belgium	Boom Clay	190-223	$1.2x10^{3}$
Asse, Germany	Salt	750	ND
Grimsel, Switzerland	Granite	approx 350	$9.5x10^{1}$ - $9x10^{4}$
Felsenau, Switzerland	Gypsum/anhydrite	Not known+	$1x10^{1}$ - $1.6x10^{4}$

CFU = Colony forming unit ND = Not detected + = beneath mountains mbgl = m below ground level * = aerobic heterotrophs

Table 2.3. Temperature Ranges for Microorganisms Groups (after Ehrlich, 1990)

Group	**Temperature range (^{o}C)**	**Optimum temperature (^{o}C)**
Psychrophiles	<10 to 20	15
Mesophiles	10 to 45	25 to 30
Thermophiles	42 to 99 (or higher)	Depends on organisms and normal habitat

2.6 References

Alexander, R. (1993) A natural analogue study of the Maqarin hyperalkaline groundwaters. I. source term description and thermodynamic database testing. NAGRA Technical Report 91-10, Wettingen, Switzerland.

Baas Becking, L. G. M., Kaplan, I. R. and Moore, D. (1960) Limits of the natural environment in terms of pH and oxidation-reduction potentials. Journal of Geology. **68** 243-284

Barker, W. W., Welch, S. A. And Banfield. J. F. (1997) Biogeochemical weathering of silicateminerals. In: Geomicrobiology: Interactions between Microbes and Minerals. Reviews in Mineralogy Vol. 35. (Eds. J. F. Banfield and K. H. Nealson) Mineralogical Soc of America, Washington DC. USA

Baedecker, M J., Cozzarelli, I. M., Siegel, D. I., Bennett, P. C. and Eganhouse, R. P. (1993) Crude oil in a shallow sand and gravel aquifer. 3. Biogeochemical reactions and mass balance modelling in anoxic groundwater. Appl. Geochem. **8**: 569-586.

Bennett, P. C., Hiebert, F. K. And Choi, W. J. (1996) Microbial colonisation and weathering of silicates in a petroleum-contaminated groundwater. Chem. Geol. **132**: 45-53

Bitton, G. and Gerba, C. P. (1984) Groundwater Pollution Microbiology. John Wiley, New York.

British Geological Survey and Cooperativa de Servicios Publicos 'Santa Cruz' Ltda. (1997) Assessment of pollution risk to deep aquifers from urban wastewaters: Santa Cruz city report. BGS Technical Report WC/97/11.

British Geological Survey, Dept of Mineral Resources, Thailand and Prince of Songkhla University, Thailand. (1997) Assessment of pollution risk to deep groundwaters from urban wastewaters: Hat Yai city report. BGS Technical Report WC/97/16.

Chapelle, F. H. (1993) Groundwater Microbiology and Geochemistry. John Wiley, New York.

Chapelle, F. H. and Bradley, P. M. (1997) Alteration of aquifer geochemistry by microorganisms. In: Manual of Environmental Microbiology (Editors C. J. Hurst, G. R.Knudsen, M. J. McInerney, L. D. Stetzenbach and M. V Walter). American Society for Microbiology Press. Washington DC, USA. 558-564

Chilton, P. J. Amd Shearer, T. (1993) Nitrate in groundwater in the central part of the Lincolnshire. Limestone: Results of recent monitorings and modelling. BGS Technical Report WE/93/5C.

Clark, L., Blakey, N. C., Foster, S. S. D. and West, J. M. (1991) Microbiology of Aquifers. In Applied Groundwater Hydrology: A British Perspective (RADowning and W B Wilkinson, Editors), Clarendon Press, Oxford, 164-176.

Cullen, W. R. And Reimer, K. J. (1989) Arsenic speciation in the environment. Chemical Review. **89**: 713 - 764.

Edmunds, W. M., Cook, J. M., Kinniburgh, D. G., Miles, D. L. and Trafford, J. M. (1989) Trace-element Occurrence in British Groundwaters. British Geological Survey Research Report SD/89/3.

Ehrlich, H. L. (1990) Geomicrobiology. Marcel Dekker, New York.

Foster, S. S. D., Chilton, P. J. and Stuart, M. E. (1991) Mechanisms of groundwater pollution by pesticides. Journal of Institute of Water and Environmental Management **5**: 186-193.

Kinner, N. E., Harvey, R. W., Blakeslee, K., Novarino, G. and Meeker, L. D. 1998 Size-selective predation on groundwater bacteria by nanoflagellates in an organic-contaminated aquifer. Appl. Environ. Microbiol. **64**: 618 - 625.

Kinniburgh, D., Morris, B., Coleby, L. Bird, M., West, J. M. And Bennett, J. (1996) Denitrification in the unsaturated zone. BGS Technical Report WD/96/63

Lawrence, A, R. and Foster, S. S. D. (1986) Denitrification in a limestone aquifer in relation to the security of low-nitrate groundwater supplies. Journal of Institution of Water Engineers. **40**: 159-172.

Lewis, W. J., Foster, S. S. D. and Drasar, B. S. (1982) The Risk of Groundwater Pollution by On-Site Sanitation in Developing Countries. Report 01/82, International Reference Centre for Wastes Disposal, Duebendorf, Switzerland.

McNabb, J. F. and Dunlap, W. J. (1975) Subsurface biological activity in relation to groundwater pollution. Groundwater **13**: 33-44.

Price, M., Bird, M. J. and Foster, S. S. D. (1976) Chalk pore-size measurements and their significance. Water Services, Oct: 596-600.

Price, M. 1996. Introducing Groundwater. Chapman & Hall.

Savage, D. C. and Fletcher, M. (Eds) (1985) Bacterial Adhesion. Plenum Press, New York.

Sibille, I., Sime-Ngando, T., Mathieu, L. and Block. J. C. 1998 Protozoan bacterivory and *Escherichia coli* survival in drinking water distribution systems. Appl. Environ. Microbiol. **64**: 197- 202.

Stroes-Gascoyne, S. And West, J. M. 1997 Microbial studies in the Canadian nuclear fuel waste management programme. FEMS Microbiology Reviews. **20**: 573-590.

Stumm, W., and Morgan, J. J. (1981) Aquatic Chemistry (2nd Ed). Wiley-Interscience, New York

West J M, Grogan H A and McKinley I G (1991) The role of microbiology in the geological containment of radioactive wastes. In: Diversity of Environmental Biogeochemistry (J Berthelin, Editor), Elsevier, Amsterdam, 205-216.

West, J. M. (1995) A review of progress in the Geomicrobiology of Radioactive Waste Disposal. Radioac. Waste Manag. & Env. Rest. **19** 263-283.

West, J. M., Smedley, P. L. and Paintsil, A. (1996) Arsenic in drinking waters in the Obuasi area, Ghana - The role of microbiology. In: 4th International Symposium on the Geochemistry of the Earth's surface. (22 - 28 July 1996) (Ed. S E Bottrell). University of Leeds. 699 - 702.

West, J. M. and Chilton, P. J. (1997) Aquifers as environments for microbiological activity. Q. J. Eng. Geol. **30**: 147-154.

Whitelaw, K. and Edwards, R. A. (1980) Carbohydrates in the unsaturated zone of the Chalk, England. Chemical Geology, **29**: 281-291.

3. ASSESSMENT OF PATHOGENS IN AQUIFERS

3.1 Introduction

Pathogens are organisms that are capable of causing an infectious disease although not all isolates of a pathogen will cause disease. The degree to which a pathogen causes disease is termed virulence. It would therefore require few cells of a highly virulent strain to cause disease in a susceptible host, whilst many cells of a low virulence stain would be required to have a similar impact on its host. Examples of waterborne disease agents are (caused by indigenous aquatic organisms), *Legionella* sp., *Vibrio* sp., *Aeromonas hydrophilia*. Control of these infections may depend on controlling exposure to water containing such organisms or where possible, treating the water to remove or inactivate the infectious agent. However, most waterborne infectious agents are derived from the enteric tracts of humans and animals, and enter the aquatic system via contamination by faeces or urine. Control of these diseases relies on preventing contamination of the water supply, by adequate sanitation and by treatment of waste waters.

The sources for pathogenic organisms found in groundwater and surface water may be either humans, animals or the environment itself (soil, water, and air) (Grimes, 1991). Table 3.1 provides examples of infectious diseases and the range of microorganism which are derived from each source. The human-related contamination can occur during either defecation in water or recreational activities carried out in water. Additionally, domestic wastewater appears to have particular importance as a contributor of the pathogenic contaminants found in aquatic environments (Straub et al., 1993). Wastewater treatment efforts may help to reduce the incidence of problems which can follow the discharge of contaminated wastewaters into the environment but does not prevent contamination from leaking sewers or on-site sanitation. These processes often result in reductions in pathogen loads but such reductions are not legislated for (and are thus not measured). They may also reduce the contamination of aquifers, which can result indirectly from the percolation of surface-applied wastewaters and sludges into the subsurface.

3.2 Classification of Water-Related Diseases

Water related infections can be grouped into four main categories:

- Waterborne infections
- Water-washed infections
- Water-based infections
- Infections with a water based insect vector.

Waterborne infections are those classically recognised as waterborne disease, such as typhoid and cholera, whereby an enteric microorganism enters the water source through faecal contamination and transmission occurs by ingestion of contaminated water. Transmission by this route depends upon (1) the concentration of pathogens in water, which is determined by the number of infected persons in the community, the amount of faecal contamination in the water, and the survival of the microorganism in water, (2) the infective dose of the microorganism, and (3) individual exposure (ingestion) to contaminated water. Control of

such infections is mainly through improvements in water quality and control of possible routes of contamination.

Water-washed infections are diseases due to poor personal and domestic hygiene. These diseases are not due to the presence of pathogens in water but due to a lack of readily available water for the washing of utensils and hands with resulting ingestion of faecal material. This allows the transmission of pathogenic organisms, such as *Shigella* sp.,. This type of infection will not be discussed further, but it should be noted that a lack of adequate water supply will increase the incidence of these types of infections.

Water-based infections are those in which the pathogen must spend a part of its life-cycle in the aquatic environment. This type of infection can be divided further into illnesses acquired by contact with infected water, or ingestion of infected water. Again, no further discussion of this type of infection is thought to be necessary, as it is thought to be unlikely that this type of infection will pose a major risk to subsurface water supplies in the UK.

Water-vectored infections will pose very little risk to subsurface water supplies, as they are transmitted by insects which breed in surface waters, such as mosquito vectors of malaria.

Two additional modes of transmission which are water related are the transmission of infectious agents via contaminated shellfish or fish and aerosols. The major pathogens associated with aerosol transmission are *Legionella* sp., particularly *Legionella pneumophilia.* (the etiologic agent of Legionnaires' disease) and Pontiac fever and will be discussed later. Infection via consumption of contaminated shellfish and fish is associated with surface waters and will not be discussed further.

3.3 Microbial Agents Commonly Associated with Waterborne Disease

The commonly recognised waterborne pathogens consist of several groups of enteric and aquatic bacteria, enteric viruses, and enteric protozoa (Table 3.2). The microbes of principal concern in the UK are the protozoans *Cryptosporidium parvum* and *Giardia lamblia*, enteric viruses, and to a lesser extent, bacteria such as *Escherichia coli* .

3.4 Bacteria

Bacteria are single-cell organisms which utilise soluble food and may operate either as autotrophs or heterotrophs. They range in size from 0.5 to 5 μm diameter, and reproduction is by binary fission with generation times as short as 20 minutes. Some bacteria can form resistant spores which remain dormant for considerable periods of time but which can be reactivated on a return to favourable conditions. Most bacteria prefer near neutral pH environments, although some bacteria can exist in extremely acidic environments (*Thiobacillus ferrooxidans* is routinely isolated from acid mine drainage, and grows optimally at pH 3 (Leduc & Ferroni, 1994)).

The first waterborne pathogens to be recognised were *Vibrio cholera* and *Salmonella typhi.* Identified in the 19th century, they have been responsible for tremendous morbidity and mortality world-wide. In 1854 a cholera epidemic struck Soho, in central London, causing an estimated five hundred deaths in ten days. At the time of the outbreak the cause of the disease was thought to be due to 'mists' or 'vapours' associated with the low-lying areas near the river Thames, where the incidence of cholera was greatest (Price, 1996). Dr John Snow

noticed that the low-lying areas in which cholera was most prevalent, were all served by two water companies which drew water from the Thames; the higher healthier areas were all served by other sources of water. Further detailed study showed that the incidences of cholera were centred around a well with a hand-pump, which drew water from the terrace gravels of the Thames. The water being drawn up by the hand-pump had become contaminated from human excrement. Snow was able to show how diseases like cholera are transmitted, long before the microorganisms causing the diseases were identified (Price, 1996).

Vibrio species are aquatic bacteria that are well adapted to both the aqueous environment (including estuaries) and the intestinal tract. Waterborne enteric bacteria include both human-associated and animal-associated species.

Campylobacter and *Salmonella* species are found in the intestinal tracts of numerous domestic and wild animals. Therefore, contamination of water from animal faeces also poses a significant human health risk. For other enteric bacteria, such as *S. typhi* and *Shigella* species, infections are generally limited to humans. The infective dose (the minimum number of organisms required to cause infection) for enteric bacteria depends upon several host factors, including host gastric acidity and the mode of transmission. Studies have shown that when the bacteria are ingested with milk, the median infectious doses are approximately 10^2 colony forming units (CFU) for *Shigella* species, approximately 10^7 CFU for *S. typhi*, and approximately 10^8 CFU for enterotoxigenic *E. coli* (Nataro & Levin, 1994). As such data are derived from studies of healthy, adult human volunteers under controlled conditions, care must be taken when these findings are extrapolated to the general population. For example, studies of *Vibrio cholera* indicated that 10^6 organisms when ingested with water by fasting volunteers caused no illness. However, the same innoculum ingested with food or sodium bicarbonate caused illness in 90-100% of volunteers, because these vehicles decreased the protective gastric acidity (Nataro & Levin, 1994).

The persistence of enteric bacteria in aquatic environments depends on the species and on a variety of environmental factors (temperature, pH, predation or competition by indigenous aquatic microorganisms, dissolved organics, attachment to particulates, presence of salts and other solutes). These are discussed further in Section 4.

A more recently identified health risk has been associated with *Aeromonas* sp whose primary habitat is the aquatic environment. Their reported populations in water range from 10^2 to 10^3 ml^{-1} in river waters to 1 to 100 l^{-1} in groundwater. These organisms have frequently been observed in fresh surface water, groundwater, estuarine and sea water, sewage and waste water and chlorinated water supplies. The wide distribution of *Aeromonas* sp. is probably a consequence of its high capacity to adapt to different environments. High population densities appear to be related to faecal contamination and temperature, and aeromonads proliferate in domestic and industrial wastewaters (Schubert, 1991). There is some evidence to suggest that a high proportion of environmental isolates have the capacity to produce enterotoxins (chemicals which act on the small intestine, generally causing massive secretion of fluid into the intestinal lumen, leading to symptoms of diarrhoea), and several reports have suggested an association between *Aeromonas* sp. in drinking water and gastroenteritis (Burke et al., 1984; Schubert, 1991). Concern about the possible health effects of these organisms in the Netherlands has led to the establishment of drinking water guidelines requiring concentration to be <20 CFU/100 ml for drinking water leaving the treatment plant and <200 CFU/100 ml for drinking water in the distribution system (van der Kooij, 1993).

Outbreaks of Legionnaires' disease have lately come to some prominence and there is strong evidence to link the incidence of this sometimes fatal type of pneumonia with the presence of *Legionella pneumophilia* in hot water supplies, shower heads, cooling waters and other aquatic systems which produce droplets or fine sprays. The disease is not caused by ingesting contaminated water supplies but by breathing in an aerosol. *Legionella pneumophilia* does occur in the natural environment and has been isolated from the subsurface. Since it is resistant to normal treatment processes it can colonise water service systems in buildings, particularly those with warm surroundings (Wadawsky et al., 1982). The implications for public health are significant, and routine monitoring of water supplies are required to ensure that colonisation of water service systems does not occur. To date, the transmission of *E.coli* 0157, as well as other stain of *E. coli*, have rarely been associated with drinking water supplies. Information presently available would suggest that infection with pathogenic strains of *E. coli* are predominantly asociated with contaminated food.(Draft Report - Rolling Revision of the Guidelines for Drinking Water Quality, Aspects of Protection and Control, and of Microbiological Quality).

3.5 Viruses

Viruses are the simplest form of oranism. A virus is essentially a package of genetic material and protein. They can only reproduce within a living organism by infecting (invading) the cells of that organism. Viruses range in size from approximately 0.01 to 0.3μm diameter/size and many kinds exist which can cause illness and disease in man. They are all parasitic and cannot grow outside another living organism. All are highly specific both in regards to the host organism and the disease they can produce. Sewage effluents normally contain large numbers of viruses (up to 1000 viruses per litre - Straub et al, 1993) and they are also present in most surface waters subjected to pollution. Due to their small size, their removal by conventional water-treatment systems cannot be guaranteed, although they are generally inactivated by normal disinfection procedures.

The enteric viruses are more recently recognised waterborne pathogens. Human rotaviruses, human astroviruses, and human calciviruses were first described in the early to mid-1970s. Unlike enteric bacteria, concern about waterborne transmission of enteric viruses is generally limited to the strains that have humans as their natural reservoir. Although there are animal strains of many of these enteric viruses, animal-to-human transmission is believed to be uncommon. The infectious dose of these agents is extremely low, typically in the range 1 to 10 infectious units. Human volunteer studies with Group A human rotavirus indicates the median infectious dose to be between 5 and 6 focus-forming units (Ward, et al., 1986).

Enteric viruses tend to be more persistent in the aquatic environment than most enteric bacteria. However, their survival depends on numerous physical, chemical and microbiological characteristics of the water, as well as the virus type. Their prolonged survival time and smaller size enable viruses to move greater distances in soil, sediments and water. As they cannot reproduce in the subsurface, their impact and migration is limited by survival time (Moe, 1996).

Hepatitis A virus (HAV) is one of the most prevalent enteric viral diseases (Craun, 1985; Szumuness et al., 1976). HAV is transmitted by direct contact (person to person), but outbreaks associated with the consumption and usage of contaminated water have also been identified (Craun 1986; Moore et al., 1994; Mosely, 1967). Such outbreaks are usually traced to faults in the distribution system, and to the use of contaminated untreated water supplies

(Lippy and Waltrip, 1984). Some examples of HAV outbreaks associated with the consumption or usage of contaminated water are shown in Table 3.3. Of the nine outbreaks shown in Table 3.3, three were associated with groundwater sources. A water borne HAV outbreak involving 73 cases in Meade County, Kentucky, was reported by Bergeisen et al. (1985). The most probable cause was identified as consumption of untreated spring water. Water samples taken at the time of the outbreak were shown to contain faecal coliforms. Vogt (1961) reported on occurrence of a waterborne HA outbreak in Posen, Michigan. The HAV appeared to have been introduced into the groundwater through septic tank effluent.

Few studies have been undertaken to determine the prevalence and concentration of HAV in wastewater and natural waters (Nasser and Metcalf, 1987; Jaing et al., 1987). However, data on the prevalence of antibodies of HAV in various parts of the world reveal high percentages of the population are exposed to HAV (Dienstag et al., 1978).

3.6 Protozoa

Protozoa are unicellular organisms 1-100 μm in length which reproduce by binary fission. Most are aerobic heterotrophs and often utilise bacteria as their main food source. Free-living protozoa are ubiquitous in natural waters and moist soils. They are often abundant in surface water and some groundwater supplies as part of the natural aquatic community.

Most protozoa have no impact on human health. However, there are a number of pathogenic protozoa associated with the enteric tract of humans and animals which may enter surface and groundwaters. Since 1981 enteric protozoa have become the leading cause of waterborne disease outbreaks for which an aetiologic agent was determined.

Most enteric protozoa have two stages in their life cycles. The trophozoite is an actively feeding, growing, and reproducing stage. Trophozoites generally do not survive outside the host unless they are propagated in a specific growth medium (Radulescu & Meyer, 1990; Upton et al., 1994). The other stage results in a dormant transmission form caused by stimuli in the host's intestinal tract which induce the organisms to encyst or produce spores. In this form protozoa are known to survive for long periods of time outside a host, especially under moist, cool conditions.

Human cryptosporidiosis, resulting from infection by *Cryptosporidium parvum*, was first described in 1976. The first reported waterborne outbreak is believed to have occurred in Texas in 1984, where a public groundwater source was contaminated with sewage by an unknown mechanism (Lisle & Rose, 1995). The first confirmed waterborne outbreak in the UK was traced to contamination of a water tank from a cross-connection with a land drain receiving water runoff from an agricultural field (Lisle & Rose, 1995). Recent evidence suggests that Cryptosporidium species is the third most common waterborne pathogen world-wide (Lisle & Rose, 1995).

There have been a number of outbreaks of cryptosporidiosis associated with drinking water supplies in the UK (Table 3.4). However, most outbreaks of the disease have been associated with spring or surface water sources. In an outbreak of cryptosporidiosis in Warrington in 1992, 47 cases were recorded. Of the known modes of transmission, the only one implicated in this outbreak was unboiled tap-water, supplied from a well. Contamination of the groundwater was linked to heavy rainfall and surface run off from agricultural land, which contained livestock. Another possible explanation was that contamination occurred due to seepage of foul sewage from a cross connection (Lisle & Rose, 1995). However, it appears

that in certain settings crytopsporidiosis is a concern which is not recognised in recent reviews (eg Badenoch 1 and 2).

In common with other waterborne parasitic protozoa *Cryptosporidium* sp. complete their life cycle within a single host (monoxenous). *Cryptosporidium* sp. has a complex monoxenous life cycle, involving both sexual and asexual reproduction, and transmission is via an environmentally robust oocyst excreted in the faeces of the infected host. *Cryptosporidium parvum* is an obligate parasite which develops only within a living host cell. Unlike other protozoa transmitted by drinking water, but in common with other coccidia, it has several characteristic development stages. Infection is initiated by ingestion of the 4-6 μm sized oocyst, which contains four naked, motile sporozoites. The banana-shaped sporozoites, released through the suture in the oocyst wall following exposure to acid, trypsin and bile salts, attach themselves intimately to the surface of adjacent enterocytes (the epithelial cell which lines the gastro-intestinal tract) and develop through asexual and sexual stages finally to become cysts. The zygote formed during the fusion of two gametes, develops into the oocyst which becomes detached from the enterocyte and sporulates during passage through the gut. About 80 % of zygotes, develop into the environmentally resistant thick-walled oocysts, which are infective when excreted. Up to 20% of oocysts are thin-walled and their sporozoites are released and infect additional enterocytes during passage through the intestine, which further magnifies the infection.

3.7 Bovine Spongiform Encephalopathy

Bovine Spongiform Encephalopathy (BSE) is one of a class of diseases called transmissible spongiform encephalopathies (TSEs) which occur in several species. TSEs are so named because the disease leads to a spongy appearance in the brain when examined under the light microscope. The disease can be transmitted, at least experimentally, to other animals of the same or different species. With all spongiform encephalopathies there is a species barrier. This means that the disease is more likely to transmit to a member of the same species than to other species.

There is still considerable scientific uncertainty about the precise causative agent of BSE and other TSEs. The issue is the subject of continuing scientific debate. The prion protein, PrP, is very important in the development of TSEs. Modified forms of this protein are associated with infectivity and also accumulate in the brain in the diseased state. Put simply, the prion hypothesis says that infectivity is caused by a modified form of the PrP protein which converts other PrP molecules into the same form, and these accumulate to produce the disease. However, whether the prion (the PrP protein alone, with no associated nucleic acid) is the cause of BSE is not certain. Other theories suggest that the causative agent might be a "virino", an infectious pathogen containing a core of nucleic acid associated with lost derived cellular proteins. Alternatively, some scientists have argued that a filamentous virus is the cause of TSE. There is also no known minimum dose for infectitivity.

Since 1996, new control measures introduced into the UK to eradicate BSE have resulted in the production of large amounts of cattle-derived waste material. The major contributor to the waste arising is from the Governments scheme for culling and disposing of all cattle over the age of 30 months.

As part of its responsibility for the regulation and management of waste disposal in England and Wales, the Environment Agency (EA) has carried out several assessments to quantify the risks of BSE infection being transmitted to humans as a result of disposal of the cattle-derived

waste material. The broad conclusions which the EA has drawn from its assessments is that, for the disposal options considered (incineration, and landfilling of carcasses and rendered products), the risk of human infection by the BSE agent is extremely small (Environment Agency, 1997). In all cases, the results show that in one year the most exposed individual would be unlikely to consume from environmental sources more than a minute fraction (significantly less than one millionth part) of the dose of BSE needed to cause infection in humans. Assumptions about the does needed to cause infection in humans are based on the advice of SEAC (Spongiform Encephalopathy Advisory Committee). This is equivalent to a risk of less than one in one million, a level the Chief Medical Officer has suggested may be neglected. For comparison, the risk of dying from cancer is approximately one in three hundred per year, and the risk of being killed in a road accident is one in ten thousand per year.

3.8 Sources of Microbial Pathogens

Sources of contamination can be classified as point or non-point. A point source is characterised by the presence of an identifiable, small scale source, such as a leaking storage tank, one or more disposal ponds, or a landfill. Usually, this source produces a well defined plume. A non-point source refers to larger scale, relatively diffuse contamination originating from many smaller sources, whose locations are often poorly defined (Domenico & Schwaetz, 1990). They include, but are not solely limited to, agricultural runoff, livestock, urban runoff, land development, recreational activities, inadequate soakaways, and illegal dumping or discharges. Non-point sources of contamination are much more difficult to control than point sources, and in some respects pose a much greater risk to groundwater quality and public health. (Margolin, 1996). For both of these types of sources, controlling the introduction of faecal pathogens into source waters relies on stringent effluent management, strict adherence to disinfection procedures and efficient maintenance of treatment facilities (Margolin, 1996).

Table 3.5 and Table 3.6 summarise the occurrence, and the reported environment of bacterial, viral and other water borne pathogens. Potentially pathogenic bacteria, such as *E. coli*, are known to occur in shallow aquifers near bacterial sources such as lakes, rivers, septic tanks, livestock feeding areas, leaky sewerage pipes, and outdoor latrines (Robertson & Edberg, 1997). The UK is subject to the requirements of the Urban Wastewater Treatment Directive (EC 91/271) and once the present transitional arrangements expire in 1999, it is estimated that UK sludge disposal to agricultural land will increase from 465 000 tonnes in 1991 through 777 000 tonnes in 1999 to 926 000 tonnes in 2006, a rise of almost 100% in 15 years (Anon, 1993). The same report referred to a further 441 000 tonnes of sludge as having an 'uncertain disposal route' and it remains to be seen whether part of this extra tonnage would also be disposed to land.

The recent House of Commons Select Committee Report (1998) recommended that by 2002 all sewage sludge recycled to land should be subjected to stabilisation and pasturisation recognising that 'a number of substances find their way into sewage sludge which present techniques have varying success in eliminating'. The Committee was unhappy with the current Code of Practice wanting the scientific basis to 'be made clear' pointing out that some of the guidelines were voluntary. Clearly changes will be made in this method of sewage sludge disposal in the near future.

Potable water supplies can become contaminated with pathogenic microorganisms from normal, diseased, or carrier human and animal excrement. This can occur by cross connections between a water main and a sewer, or from the entry of sewage water through leaks in damaged pipes. Also treatment deficiencies may allow the passage of organisms, especially when the source water contains high densities of pathogenic organisms. Furthermore, microorganisms can gain access to water from air-water interface in the distribution system and may remain unaffected by standard disinfection processes such as chlorination or ultra-violet irradiation (Singh & McFeters, 1992).

3.9 The Coliform Test

The ideal manner of determining the presence of waterborne pathogens would be to analyse the water for the presence of specific pathogens of concern. However, many types of microorganisms have been shown to be involved in waterborne disease outbreaks. It is therefore impractical to look for every potentially pathogenic microorganism. Culture methods are usually used for bacteria and cell culture techniques for the detection of viruses, while microscopic methods are used for protozoa. However, the target bacteria may not grow in nutrient rich culture media (being adapted to oligotrophic conditions) and because they are frequently injured as a result of exposure to environmental stresses. Additionally, several enteric viruses cannot be cultured in the laboratory, and methods for the detection of protozoan pathogens are notoriously inaccurate. As a result, analysing water for the presence of pathogenic microorganisms becomes extremely difficult and does not ensure complete safety to the consumer. Thus, indicator organisms (coliforms) are used as a warning of possible contamination and as an index of water quality deterioration. Heavy reliance has been placed on the coliform group of bacteria to determine the safety of potable water supplies. Although this practice is not perfect, with considerable variety in the ways the different indicator organisms are applied in different geographical areas and situations, public health concerns have been generally well served. The presence of indicator organisms will continue to be used as a criterion of water quality that will be of value if attention is given to the development and use of optimal methods for the recovery of these organisms.

3.9.1 Coliform bacteria

The traditional definition of the coliform group of bacteria specifies that they are aerobic and facultatively anaerobic, gram-negative, nonsporeforming, rod-shaped bacteria that ferment lactose with gas and acid production in 24 to 48 hours at 35 deg C (Dutka *et al.*, 1974). Hence these criteria are not strictly taxonomic, although coliform bacteria belong to the family *Enterobacteriaceae* and usually include *E. coli* as well as various members of the genera *Enterobacter*, *Klebsiella*, and *Citrobacter*. These bacteria are classically used as indicators of faecal contamination or water pollution from sewage and thus are of sanitary significance, although it should be noted that while these organisms can originate from the intestinal tracts of homeothermic animals, other bacteria numerically dominate that type of microbial community (Toranzos & McFeters, 1996). In addition, it has been demonstrated that some members of the coliform group can originate from non-enteric environments such as wastes from the wood industry, biofilms within drinking water distribution systems, and epilithic algal mat communities in pristine streams (Toranzos & McFeters, 1996).

Analysis for the presence of indicator organisms is a shortcut attempt to determine the microbiological quality and safety of water. Very few natural, pristine, waters are completely

free of indicator organisms. High concentrations of coliforms can be associated with plant material. Thus high levels of coliforms in water may not be cause for concern.

The subset of the total coliform group, that is more definitive as an indicator of homeothermic faecal contamination of water consists of what are termed as the faecal coliforms, also now described as the thermotolerant coliforms. These bacteria conform to all the requirements used to describe total coliforms plus the requirement that they grow and ferment lactose with the production of acid and gas at 44.5±0.2 deg C. Bacteria in this subgroup have been found to have an excellent positive correlation with faecal contamination from warm-blooded animals. However, faecal coliform bacteria which conform to this definition have been isolated from environmental samples in the apparent absence of faecal contamination (Toranzos & McFeters, 1996). Thus caution needs to be exercised when trying to decide whether the presence of indicator organisms does indeed represent a threat to human health due to the presence of faecal material. Toranzos and NcFeters (1996) describe a number of rapid methods to assess the potential contamination of waters. The methods described still rely on coliforms as an indicator and require a higher amount of technical ability than conventional methods.

3.10 Conclusions

The potential for contamination of groundwater supplies with microbial pathogens is significant. In the last 10 years the reported cases in the UK have been limited to contamination by *Cryptosporidium parvum*. The cause of the contamination is usually associated with a physical breakdown of the treatment processes used or due to contamination of untreated groundwater supplies with animal or human waste.

Table 3.1. Some examples of infectious disease hazards associated with microorganisms in water (Hurst, 1996).

Sources of microorganisms	Disease	Causative genus or genera
Human	Cholera	*Vibrio*
	Encephalitis	*Enterovirus*
	Entamoebiasis	*Entamoeba*
	Gastroenteritis	*Astrovirus, Calicivirus, Coronavirus, Rotavirus*
	Hepatitis	*Calicivirus, Hepatovirus Meningitis, Enterovirus*
Animal	Campylobacteriosis	*Campylobacter*
	Cryptosporidiosis	*Cryptosporidium*
	Giardiasis	*Giardia*
	Leptospirosis	*Leptospira*
Environmental	Encephalitis	*Naegleria*
	Cholera	*Vibrio*
	Legionellosis	*Legionella*

Table 3.2. Illnesses acquired from the ingestion of water (Moe, 1996).

Agent	Source	Incubation Period	Clinical Syndrome	Duration
Viruses				
Astrovirus	Human faeces	1-4 days	Acute gastroenteritis	2-3 days, occasionally 1-14 days
Calicivirus	Human faeces	1-3 days	Acute gastroenteritis	1-3 days
Enteroviruses (polioviruses, coxsackieviruses, echoviruses)	Human faeces	3-14 days	Febrile illness, respiratory illness, meningitis, herpangina, pleurodynia, conjunctivitis, myocardiopathy, diarrhoea, paralytic disease, encephalitis	Variable
Hepatitis A virus	Human faeces	15-50 days	Fever, malaise, virus abdominal pain, jaundice, anorexia, nausea	1-2 weeks to several months
Hepatitis E virus	Human faeces	16-65 day	Fever, malaise, jaundice, abdominal pain, nausea, anorexia	1-2 wks to several months
Group A rotavirus	Human faeces	1-3 days	Acute gastroenteritis with predominant nausea and vomiting	5-7 days
Group B rotavirus	Human faeces	2-3 days	Acute gastroenteritis	3-7 days
Bacteria				
Aeromonas hydrophilia	Freshwater		Watery diarrhoea	Avg. 42 days
Campylobacter jejuni	Human and animal faeces	3-5 days	Acute gastroenteritis, possibly bloody and	1-14 days occas<10 days
Enterohemorrhagic *Escherichia coli*	Human and animal faeces	3-8 days	Watery then grossly bloody diarrhoea, vomiting, possible haemolytic uremic syndrome	1-12 days (usually 0157:H7 7-10 days)

Table 3.2. Illnesses acquired from the ingestion of water (continued).

Agent	Source	Incubation Period	Clinical syndrome	Duration
Enteroinvasive *E. Coli*	Human faeces	1-3 days	Possible dysentery with fever	1-2 wk
Enteropathogenic *E. Coli*	Human faeces	1-6 days	Watery to profuse watery diarrhoea	1-3 wk
Enterotoxigenic *E. Coli*	Human faeces	12-72 h	Watery to profuse watery diarrhoea	3-5 days
Plesiomonas shigelloides	Fresh surface water fish and crustaceans	1-2 days	Bloody and mucoid diarrhoea, abdominal pain, nausea, vomiting	Avg. 11 days
Salmonellae	Human and animal faeces	8-48 h	Loose, watery, occasionally bloody diarrhoea	3-5 days
Salmonella typhi	Human and animal faeces	7-28 days (avg. 14 days)	Fever, malaise, headache, abdominal pain, vomiting, nausea	Weeks to months
Shigellae	Human faeces	1-7 days	Possible dysentery with fever	4-7 days
Vibrio cholerae 01	Human faeces	9-72h	Profuse, watery diarrhoea, vomiting, rapid dehydration	3-4 days
Non-01 *V. cholerae*	Human faeces	1-5 days	Watery diarrhoea	3-4 days
Yersinia enterocolitica	Animal faeces and urine	2-7 days	Abdominal pain, mucoid occasionally bloody diarrhoea, fever	1-21days
Protozoans				
Balantidium coli	Human and animal faeces	Unknown	Abdominal pain, occasionally mucoid or bloody diarrhoea	Unknown
Cryptosporidium species	Human and animal faeces	1-2 wk	Profuse, watery diarrhoea	4-21 days
Entamoeba histolytia	Human faeces	2-4 wk	Abdominal pain, occasionally mucoid, bloody diarrhoea	Weeks to months

Table 3.2. Illnesses acquired from the ingestion of water (continued).

Agent	Source	Incubation Period	Clinical syndrome	Duration
Giardia lamblia	Human and animal faeces	5-25 days	Abdominal pain, bloating, flatulence, loose, pale, greasy stools	1-2 wk to months and years
Algae				
Cyanobacteria (*Anabaena*, *Aphanizomenon*, and *Microsystis* species)	Cyanobacteria blooms in marine water freshwater	Few hours	Toxin poisoning (blistering of mouth, or gastroenteritis, pneumonia)	Variable

Table 3.3. Recent outbreaks of HAV-associated with water (Nasser, 1994).

Location	Number of cases	Water source	Transmission mode
Georgia	16	Private well	Drinking
Kentucky	73	Spring	Drinking
Michigan	89	Groundwater	Drinking
Louisiana	20	Pool	Swimming
South Carolina	14	Lake	Swimming
Texas	263	Ocean	Oysters
Kentucky	202	Unknown	Lettuce
Spain	21	River	Drinking

Table 3.4. *Cryptosporidium* outbreaks in drinking water supplies in the UK (adapted from Lisle and Rose, 1995).

Year	Location	Source water	Water treatment	Suspected cause
1983	Cobham, Surrey	Spring	Chlorination, softening, filtration	NA
1985	Cobham, Surrey	Spring	Chlorination, softening, filtration	NA
1986	Sheffield, S. Yorks.	Surface	NA	Cattle faeces in storm run-off
1988	Ayrshire	NA filtration	Chlorination, cattle slurry	Cross connection/
1989	Swindon/Oxon.	Surface	Conventional	Recycled water backwash/animal faeces
1989-90	Humberside	NA	NA	NA
1990	Loch Lomond	Surface	NA	NA
1990-91	Isle of Thanet	Surface	Conventional	Treatment deficiencies
1992	Warrington	Subsurface	Chlorination	Cattle faeces in storm run-off/ raw sewage cross-connection

NA = information not available

Conventional treatment = coagulation, flocculation, filtration and disinfection

Table 3.5. Occurrence of bacterial pathogens in water (Emde et al., 1992).

Organism	Drinking	Ground	Surface	Waste
Acinetobacter sp.	X		X	
Aeromonas sp.	X		X	X
Azotobacter sp.	X			
Campylobacter sp.	X	X	X	X
Clostridium perfringens	X		X	
Coliform bacteria	X		X	X
Cyanobacteria			X	
Enterobacter sp.	X			
Enterococci sp.	X		X	
Escherichia coli	X		X	
Helicobacter sp.	X			
Heterotrophic plate count bacteria	X			
Klebsiella sp.	X			
Legionella sp.	X		X	X
Pseudomonas sp.	X		X	
Salmonella sp.	X			
Shigella sp.		X	X	
Staphylococci sp.			X	X
Vibrio sp.	X			X
Vibrio cholera	X			X
Yersinia enterocolitica		X		

Table 3.6. Occurrence of viral and other waterborne pathogens in water (adapted from Emde et al., 1992).

Organism	Drinking	Ground	Surface	Waste
Adenovirus			X	
Astrovirus	X		X	
Calicivirus	X		X	
Coxsackievirus			X	
Echovirus	X		X	X
Enterovirus	X		X	X
Hepatitis	X		X	
Norwalk agent	X	X	X	
Poliovirus			X	
Reovirus			X	
Potavirus	X		X	X
Acanthamoeba	X			
Cryptosporidium spp.	X	X	X	X
Giardia lamblia	X		X	X

3.11 References

Anon(1993) Review of the rules for sewage sludge application to agricultural land . Report of the Independant Scientific Committee Ministry of Agriculture, Fisheries and Food and Department of the Environment. MAFF London.

Bergeisen, G. H., Hinds, W. M., and Skaggs, J. W. (1985). A waterborne outbreak of hepatitis A in Meade County, Kentucky. American Journal of Public Health 75:161-164.

Burke, V., Robinson, J., Gracey, M., Peterson, D., Meyer, N. and Haley, V. (1984). Isolation of *Aeromonas* spp. from an unchlorinated domestic water supply. Applied and Environmental Microbiology. 48: 367-370.

Craun, G. F. (1985). A summary of waterborne illness transmitted through contaminated groundwater. Journal of Environmental Health 48:122-127.

Craun, G. F. (1986). Waterborne diseases in the United States. CRC Press, Boca Ratton, FL.

Dienstag, J. L., Szmuness, W., Stevens, C. E., and Purcell, R. H. (1978). Hepatitis A virus infection: new insights from seroepidemiologic studies. Journal of Infectious Diseases 137 328-340.

Dutka, B. J., Chau, A. S. Y., Coburn, J. (1974). Relationship between bacterial indicators of water pollution and fecal sterols. Water Research 8: 1047-1055.

Domenico, P. A., and Schwartz, F. W. (1990). Physical and chemical hydrogeology. John Wiley & Sons.

Emde, K. M. E., Mao, H. And Finch, G. R. (1992) Detection and occurrence of waterborne bacterial and viral pathogens. Water Env. Research. 64 641 - 647.

Environment Agency Summary (1997). Risks from BSE via environmental pathways.

Grimes, D. J. 1991. Ecology of estuarine bacteria capable of causing human disease: a review. Estuaries 14: 345-360.

House of Commons Environment, Transport and Regional Affairs Committee (1998). Sewage Treatment and Disposal (2nd Report). The Stationaery Office, London.

Hurst, C. J. (1996). Overview of water microbiology as it relates to public health. In Manual of environmental microbiolgy Edited by C. J. Hurst, G. R. Knudsen, M. J. McInerney, L. D. Stezenbach, M. V. Walter. American Society for Microbiology.

Jaing, Xi, Estes, M. K., and Metcalf, T. G. (1987). Detection of hepatitis A virus by hybridisation with single-stranded RNA. Applied and Environmental Microbiology 53 2487-2495.

Leduc, L. G. & Ferroni, G. D. (1994). The chemolithotrophic bacterium *Thiobacillus ferrooxidans*. FEMS Microbiology Reviews, 14, 103-120.

Lippy, E. C. and Waltrip, S. C. (1984). Waterborne disease outbreaks, 1946-1980: a thirty-five-year perspective. Journal of American Water Works Association

Lisle, J. T. & Rose, J. B. (1995). *Cryptosporidium* contamination of water in the USA and UK: a mini-review. Journal Water SRT- Aqua 44, 103-117.

Margolin, A. B. (1996). Control of micro-organisms in source water and drinking water. In Manual of environmental microbiology Edited by C. J. Hurst, G. R. Knudsen, M. J. McInerney, L. D. Stezenbach, M. V. Walter. American Society for Microbiology.

Moe, C. L. (1996). Waterborne transmission of infectious agents. In Manual of environmental microbiology Edited by C. J. Hurst, G. R. Knudsen, M. J. McInerney, L. D. Stezenbach, M. V. Walter. American Society for Microbiology.

Moore, A. C., Herwaldt, B. L., Craun, G. F., Calderon, R. L., Highsmith, A. K. & Juranek, D. D. (1994). Waterborne disease in the United States, 1991 and 1992. Journal of American Waterworks Association 86, 87-100.

Mosely, J. W. (1967). Transmission of viral hepatitis by drinking water, in Transmission of viruses by water route, Berg, G., Ed., Interscience, New York.

Nasser, A. M. (1994). Prevalence and fate of hepatitis virus in water. Critical Reviews in Environmental Science and Technology 24, 281-323.

Nasser, A. M., Metcalf, T. G. (1987). An A-ELISA to detect hepatitis A virus in estuarine samples. Applied and Environmental Microbiology 53 1192-1195.

Nataro, J. P., and Levin, M. M. (1994). Bacterial diarroheas, p.697-752. In A.Z. Kapikian (ed.), Viral infections of the gastrointestinal tract. Marcel Dekker, New York.

Price, M. (1996). Introducing groundwater. Chapman & Hall.

Radulescu, S., Meyer, E. A. (1990). In vitro cultivation of *Giardia* trophozoites, p. 99-110. In E. A. Meyer (ed.), Giardiasis. Elsevier-Science Publishers, New York.

Robertson, J. B. & Sedberg, S. C. (1997). Natural protection of spring and well drinking water against surface microbial contamination. I. Hydrogeological parameters. Critical Reviews in Microbiology 23, 143-178.

Schubert, R. H. W. (1991). Aeromonads and their significance as potential pathogens in water Journal of Applied Bacteriology 70: 131S-135S.

Singh. A. & McFeters, G. A. (1992). Detection methods for waterborne pathogens. In Environmental microbiology, pp. 125-156. Edited by R. Mitchell: Wiley-Liss, John Wiley & Sons.

Straub, T. M., Pepper, I. L., and Gerba, C. P. (1993). Hazards from pathogenic microorganisms in land-disposed sewage sludge. Rev. Environmental Contamination and Toxicology 132: 55-91.

Szumuness, W., Dienstag, J. L., Purcell, R. H., Harley, E. J., Stevens, C. L. and Wong, D. C. (1976). Distribution of the antibody to hepatitis A antigen in urban populations. New England Journal of Medicine 295: 755-759.

Toranzos, G. A., and McFeters, G. A. (1996). Detection of indicator microorganisms in environmental freshwaters and drinking waters. In Manual of environmental microbiology Edited by C. J. Hurst, G. R. Knudsen, M. J. McInerney, L. D. Stezenbach, M. V. Walter. American Society for Microbiology.

Upton, S. J., Tilley, M., Brillhart, D. B. (1994). Comparative development of *Cryptosporidium parvum* (Apicomplexa) in 11 continuous host cell lines. FEMS Microbiology Letters 118 233-236.

Van der Kooij, D. (1993). Importance and assessment of the biological stability of drinking water in the Netherlands, p. 165-179. In C.F. Craun (ed.) Safety of water disinfection: balancing the chemical and microbial risks. ILSI Press, Washington D.C.

Vogt, J. E. (1961). Infectious hepatitis epidemic at Posen, Michigan. Journal of American Water Works Association 53 1238-1242.

Wadowsky, R. M., Yee, R. B., Mezmar, L., Wing, E. J. and Dowling J. N. (1982). Hot water systems as sources of *Legionella pneumophilia* in hospital and non-hospital plumbing fixtures. Applied and Environmental Microbiology 43: 1104-1110.

Ward, R. L., Bernstein, D. I. And Young, E. C. (1986). Human rotavirus studies in volunteers: determination of infectious dose and serological response to infection. Journal of Infectious diseases. 154: 871-880.

4. FACTORS AFFECTING MOVEMENT AND FATE OF MICROBIOLOGICAL CONTAMINANTS IN THE SUBSURFACE

4.1 Introduction

This section aims to consider the factors which control the fate of microbiological contaminants in the subsurface. It will identify and describe the physico-chemical properties of the microbes and the subsurface which control the survival, distribution and movement of microbes.

Within the subsurface, water is the primary solvent. Water infiltrating the surface from rainfall rivers or lakes moves through the soil, generally downward, to the water table. Between the surface and the water table is a zone known of variable thickness called the unsaturated (or vadose) zone. Microbiological contaminants, where they enter the subsurface, are generally transported along with natural or artificial recharge, e.g. rainfall or waste water discharge or leakage.

The transport of microbes within the subsurface by groundwater is affected by a number of processes. Studies have examined these processes (Gerba et al, 1991 and Yates et al, 1985) which can be divided into two types: those that are related to the characteristics of the organism and those which are related to the environment in which they are located (groundwater, aquifer and soil). In many cases there is interrelation between the two types and the processes cannot be treated in isolation. Table 4.1 lists the most significant characteristics (identified by Robertson and Edberg, 1997) of the microbe and the aquifer/soil environment which affect mobility and fate.

4.2 Physical Characteristics of Microbes and Aquifer Materials

The extreme size variability of microbial contaminants (Table 4.2) controls their mobility in the subsurface. Soil/rock pore sizes are also variable and the two size ranges overlap. The subsurface can therefore filter out microbes in some situations. Where filtration is effective, it can be very rapid and self enhancing - a build up of filtered material reduces effective pore size further by clogging. The flow mechanisms and summary of properties of the major UK aquifer types is shown in Table 4.3. Large interconnected voids, be they pore necks or microfissures, provide pathways with least resistance and higher hydraulic conductivity and so have the highest potential for transmitting microbes. Figure 4.1 shows a comparison between microbial size and fissure aperture/pore sizes. As can be seen the potential for microbes to enter fissures and/or pores inside the rock exists. As sub-surface soils and rocks are rarely homogeneous, preferential flow paths may exist. Additionally as sub-surface colloids are defined as particles which remain in suspension by Brownian motion and have a linear dimension in the range of approximately 0.001 - 1.0 μm and so are similar to microbes.

Very little research has been undertaken to study movement of microbes through consolidated rock which typically comprises UK major aquifers. On the other hand considerable research on colloids has been performed (Harrison, 1996, Grolimundm et al, 1996 and Harvey and Garabedian, 1991) Research has focussed on colloids since the recognition that colloid flow dynamics differ from those of clear groundwater and that they are important in transporting contaminants, e.g. radionuclides. In the absence of research on microbes, colloid behaviour is

a useful analogue of microbe movement and the processes which affect colloids can be directly extended to microbes.

4.2.1 Colloid Transport and Filtering

The physico-chemical behaviour of colloids differs from that of solute contaminated water. The differences between colloid migration and solute migration are seen in the different breakthrough patterns in column studies or aquifer tracer tests. The first arrival times of a solute and a colloid at a sampling point down gradient (assuming it is not filtered or sorbed) should be the same but the mass distribution is different, over time. At a sampling point, the breakthrough curve (concentration distribution over time) is the statistical distribution of arrivals. In an aquifer where intergranular flow dominates, the time of peak concentration of colloids will be earlier due to preferential flow along pathways with larger aperture. Solutes will migrate through the whole porous medium including the smaller pores which will have lower flow velocities. Therefore the velocity profile will be different and lead to a peak breakthrough concentration later than that for the colloids. Colloids can therefore migrate at a faster average velocity than solutes and groundwater (Harrison,1996). Similar observations apply where fissure flow dominates if the size of the colloid falls within the aperture range of the fissures through which the groundwater is flowing. Filtering and sorption effects occur and result in only a proportion of the colloids getting through the medium and extended tailing of the breakthrough curve. Filtering effects are only significant when the average size of the particle exceeds approximately 5% of the average pore size (Harvey and Garabedian, 1991). Bacteria and protozoa are most likely to be affected by filtering, but viruses are typically much smaller (<0.25 m) and so are less likely to be affected by filtering.

4.2.2 Microbe Shape

Microbe shape can also be significant in the mobility of microbes. Bacteria especially are very variable in their shape and can range from spherical to rod-shaped to filamentous. Their movement will therefore be influenced by this geometric shape. Protozoa such as *Cryptosporidium* and *Giardia* demonstrate less variability being closer to spherical or ellipsoidal in shape. The small size of viruses makes their shape less critical in transport terms.

4.2.3 Settling

The larger microbes (bacteria and protozoa) are also subject to settling out if their densities are greater than water. Their greater size precludes suspension in water as a result of Brownian motion. However, microbes are generally neutrally buoyant and so will remain in suspension. Only a small increase in relative density is necessary to settle out the larger microbes due to gravity.

4.3 Inactivation (half-lives) of Microbes

Natural groundwater systems are not the most conducive environments for long term survival and/or reproduction of non-indigenous microbes due to the lack of nutrients and other 'hostile' factors. Parasites and enteric viruses, for instance, will not reproduce because they require a suitable live host organism such as vertebrate animals/humans. Therefore pathogens that are introduced to the subsurface will be transported in a viable form only as far as their

life span will allow. The maximum distances that they will move will be determined by the groundwater velocity and their survival time which can be expressed in terms of their half life (the time taken for a 50% reduction in proportion):

$$C_t = C_o e\ (-(\lambda x/v))$$

where *Ct* is the concentration at time *t*, *Co* the initial concentration at source, λ the half life of the microbe, *x* the distance travelled and *v* the average groundwater velocity.

The half life of the microbes can be influenced by a large number of factors including, temperature, type of organism, water chemistry, organic content of soil and other predatory microbes. An indication of how these affect microbe survival is shown in Table 4.4. The most critical factors are usually temperature and moisture content.

No general rules can be applied to survival rates of non-indigenous microbes in the subsurface because there is considerable variation in both microbe type and environmental conditions. Typically they can range from a few hours to weeks. Some results from field and laboratory studies are shown in Table 4.5. For draft guidance on location of septic tanks, the US EPA assumed virus half-lives of between 2.4 and 7 days (US EPA, 1992. *Draft groundwater disinfection rule, EPA 811/P-92-001, Office of Water*).

4.3.1 Temperature

The survival rate of microbes is generally inversely proportional to temperature with colder temperatures favouring survival. Viruses have been observed to survive for up to 170 days in soil at temperatures of up to 10°C (Bitton and Gerba, 1984) and below 4°C survival of micro-organisms can extend to years (Gerba et al, 1985). As temperature increases, inactivation is rapid with half-lives halved for every 10 deg C rise in temperature between 5 and 30°C (Reddy et al, 1981). Where the microbes have been adsorbed however, there is less temperature sensitivity (Liew and Gerba, 1980). Indeed Hurst et al, 1980 suggested that soils and rocks with a low temperature, moist conditions and high adsorption capacity may favour virus survival.

4.3.2 Moisture content

Moisture content is another major factor in determining the survival and transport potential of microbes in the sub-surface. It can play two roles;

(i) A reduction in moisture content adversely affects the survival rate. Viruses have been found to persist for no more than 15-25 days in air dried soils compared to 60-90 days in the same soil with a moisture content of 10% (Bitton and Gerba, 1984).

(ii) Secondly, and importantly, the presence of moisture provides the fluid necessary for transporting the microbes such that at high moisture contents and ultimately saturated conditions, the microbes will be effected by diffusion and advection. Transport within the unsaturated and saturated zones is discussed in detail later.

4.3.3 Nutrients

Viable indigenous microbes are adapted to the oligotrophic nature of aquifers and they have developed various survival mechanisms (eg adhesion to surfaces, biofilm etc) that enables them to tolerate extremely low nutrient conditions. In contrast, many microbial contaminants such as enteric pathogens will be used to a rich supply of nutrients at warm temperatures of 37°C. When they are present within other environments such as aquifers they will starve and die unless they can adapt to the changing conditions.

4.4 Adsorption

As colloids or particulates, microbes have electrostatic potentials associated with them. Complex physiochemical interactions occur between the microbe, the surrounding solution (water) and/or the rock/soil matrix. Three types of adsorption mechanism exist: physical, chemical and ion exchange (Yavuz Corapcioglu, 1984). Viruses are most likely to be affected by adsorption because of their size. Soils/rocks which contain clays are more likely to have a higher sorption capacity due to the shape of the clay platelets and their large surface area (Savage and Fletcher, 1985).

Under most natural pH conditions, microbes suspended in water have a net negative charge as do most mineral surfaces in the subsurface. Therefore there is a tendency for the microbes to be repelled by the rock matrix and remain mobile. However, under certain conditions, where pH, mineral characteristics and groundwater chemistries are extreme the predominance of negatively charged surfaces may change and adsorption may occur. An example was given by Dowd and Pillai (1997) where experiments with viruses and bacteria showed their (reversible) adsorption in a sandy soil with a high acidic groundwater. Similar effects were also found by Goldschmid et al, 1973.

Changes in pH will affect the electrostatic surface property of the microbe and hence its potential for sorbing. The point at which the surface charge of the microbe changes from positive to negative (and vice versa) is called the isoelectric point. This property, which can be measured in the laboratory, may be a very important parameter in controlling adsorption. Dowd and Pillai (1997) and Gerba et al (1981) found that bacteria and viruses with different isoelectric points displayed different sorption rates under the same environmental conditions. However, Sobsey et al (1980) found that *Poliovirus* and *Reovirus,* exhibited very similar sorptive behaviour in a range of soil types even though they have different isoelectric points. A possible reason for the discrepancy in findings may be the size of microbe population. The charge strength (potential for sorbing) was found by Lance and Gerba (1980) and Jansons et al (1989) to be related to population size. So it is possible that different populations of the same microbe may display different sorption characteristics. In most (chemically) uncontaminated UK aquifers, the pH of groundwaters is generally very stable due to the buffering capability of the rock matrix and so significant changes in pH are unlikely.

Hydrophobicity is also important in adsorption. Hydrophobic materials (materials with a low solubility in water relative to organic solvents) tend to associate with organic matter more than with water. Many microbes, including some viruses, parasites and bacteria are hydrophobic to varying degrees. This causes them to dissociate within the groundwater and chemically sorb to the organic material and coatings in the soil/rock matrix. This process has been investigated by Bales et al (1993) and hydrophobic adsorption effects on viruses were found to be greater than electrostatic adsorption. Total organic Carbon (TOC) contents as

low as 0.0005-0.001% were found to retard virus migration rates by factors of between 15 and 150. Increased TOC can therefore adversely affect microbe mobility and reduce migration in aquifers. In UK aquifers, TOC contents range from approximately 0.02 - 2 % (Edmunds et al, 1989) (see Chapter 2, Table 2.1) providing significant potential for retardation where contact between microbes and aquifer materials occurs.

The influence of the soil gas-water interface in unsaturated porous media has also been linked to hydrophobicity and sorption of microbes in the unsaturated zone (Wan et al, 1994). Experiments have indicated that retention of microorganisms by porous media is in part a function of gas saturation due to preferential sorption on to the gas-water interface. Even relatively hydrophilic microbes, which have a lower potential for sorbing at the solid-water interface are sorbed by the gas-water interface. Sorption also appears to increase with increasing hydrophobicity and this sorption is essentially irreversible due to capillary forces.

It should be noted however that adsorption should not always be considered a one-way process and/or a de-activating mechanism. Changes in groundwater chemistry, organic degradation and other factors can act to reverse sorption and so release the microbes. Heavy rainfall leading to recharge of water of different chemical composition (lower ionic strength or pH) can flush out microbes previously sorbed (Fourie and van Ryneveld, 1995 and McCaulou et al, 1994).

In terms of the relative importance of the various types of adsorption, it was found that in a study of poliovirus adsorption to 34 different materials and soils (Moore et al, 1981) the two factors of greatest importance in virus adsorption, in normal environments, were TOC content and surface charge. Surface area of the media and pH were not as important and unlikely to be the limiting factors in adsorption.

4.5 Other Factors

Other factors which potentially affect microbe fate and transport such as pressure, and presence of nutrients are generally not significant compared to the previously described factors for non-indigenous microbes in aquifers. For the latter, the subsurface acts as a pathway for transmission from source to receptor. However, for indigenous populations nutrient availability may be important. Indigenous microbial activity is discussed in Section 2.

Predation may also have an impact on the survival of non-indigenous microbes. It has been demonstrated that the presence of indigenous populations (which would include predators) can lead to increasing rates of non-indigenous population (probably caused by predation) decline but evidence is not conclusive (Bitton and Gerba, 1984).

4.6 Transport

4.6.1 Unsaturated zone

The subsurface can be effectively divided into two parts: the saturated zone and the unsaturated zone. The unsaturated zone is that, from the surface down, in which a variable proportion of the pores are water-filled. The saturated zone is that part of the subsurface in which the pores are completely water-filled. Hydrogeological processes in the unsaturated zone are extremely complex and the behaviour of contaminants difficult to predict. The soil

zone and unsaturated zone are, however, probably the most important barriers to microbial contaminants entering the saturated zone and aquifer. The unsaturated zone can play an important role in retarding (and in some cases eliminating) contaminants and so must be considered when assessing aquifer vulnerability (Adams and Foster, 1992; Foster and Hirata, 1988). Maximisation of residence times in the unsaturated zone has been proposed as the key mechanism for removal and elimination of bacteria and viruses (Lewis et al, 1982). However, the variability in the nature and thickness of the unsaturated zones overlying UK aquifers means that adequate residence times may not be achievable.

The flow velocity in the unsaturated zone can be approximated by a modified form of Darcy's law (which describes flow in the saturated zone). The following equation describes flow velocity, v_x, in the vertical direction:

$$v_x = (K(\theta)(x/\theta).\delta h/\delta x$$

where $(K(\theta))x$, the unsaturated vertical hydraulic conductivity is a function of moisture content (or matrix potential), θ, and $\delta h/\delta x$ the hydraulic gradient.

In fractured aquifers, the variation in vertical hydraulic conductivity with saturation can be dramatic, since the permeability of the fractures (especially in aquifers such as the Chalk) is orders of magnitude greater than that of the rock matrix (Foster, 1993). The fractures only retain and conduct water during, and for limited periods after major infiltration events.

Whereas the downward migration of contaminants through the unsaturated zone of porous media (e.g. soils, unconsolidated sands and gravels) is relatively uniform, in fissured/fractured aquifers, the downward migration can be complex. Where the fractures intersect a rock matrix which has very low saturated hydraulic conductivity and low kinematic effective porosity, then recharge downward will be predominantly through the fractures. To be an effective aquifer, the fractures must be frequent and/or have large apertures otherwise aquifer yield would be minimal.

The Carboniferous and Magnesian Limestone aquifers are typical examples of where low matrix porosity and hydraulic conductivity exist and the groundwater flow is effectively confined to the fractures. In many places these fractures are solution widened and so fracture flow is even more important, such that very little interaction with the matrix may occur.

The Chalk and Permo-Triasic Sandstone aquifers, on the other hand are fractured aquifers (the Chalk more so) with a porous matrix and are called dual-porosity systems. In the Chalk, this matrix has a relatively low hydraulic conductivity and so advective flow through the matrix is negligible, whereas in the Permo-Triassic Sandstone, the matrix has a significant hydraulic conductivity and advective flow through the matrix can be important. Interchange between fissure water and matrix water is therefore possible in both the Permo-Triassic Sandstone and Chalk aquifers. In the unsaturated zone, especially in the Chalk, this is a very important mechanism in contaminant transport. Where downward movement of water and contaminants is slow (0.5-1.0 m/year) diffusion into the matrix will result in equilibration of concentrations in the fracture and the matrix. At higher rates of recharge and where the contaminant cannot physically diffuse into the matrix, movement will be restricted to the fractures and so migration may be more rapid and concentrations of contaminants reaching the water table much higher. For microbiological contaminants, both fracture flow and matrix flow are important. In the Magnesian and Carboniferous limestone aquifers the matrix pore apertures are typically less than 0.5µm and for the Chalk less than 1.0 µm. Microbes,

especially bacteria and protozoa, are therefore effectively precluded from diffusing into the matrix and so will be confined to the fractures. In the Permo-Triassic Sandstone and unconsolidated material, intergranular movement is possible for bacteria and viruses because of the much larger pore sizes (>5 μm) and the relatively high hydraulic conductivity.

Where flow is intergranular within the unsaturated zone, there is greater potential for contact with the soil/rock particles and hence greater retention potential, both sorptive and filtering. On the other hand, where excessive loading occurs, the filtering effect may lead to a blocking of the pores. The resultant reduction in hydraulic conductivity may reduce the effectiveness of the unsaturated zone to retard contaminants if the clogging forces recharge into vertical fissures where rapid downward movement can occur.

Heterogeneity and anisotropy within the subsurface also contributes to the complexity of the soil/rock system. Most formations display heterogeneity (variability in properties) and where there is bedding or structure, vertical and horizontal variations in hydraulic properties will exist.

Travel times for contaminants through the unsaturated zone can be very rapid. In the UK, there is evidence of rapid transport of particles ranging from 0.1-6.0 μm (diameter) through 20m of unsaturated Chalk in less than 3 days after irrigation (BGS unpublished data). Investigation showed that the movement of particulates had been along fissures, both vertical and horizontal. Matrix pore waters revealed the presence of a solute tracer but no particulates indicating that the particles had preferentially migrated along the fissures. Examination of the surfaces of the fractures revealed sorbed particles (in this case fluorescent microspheres).

Other examples of microbial transmission through the unsaturated zone of the Chalk are given by Foster (1993). Municipal wastewater disposal into the Chalk has been practised for many years in Southern England. The methods used include discharge through soil drains, land spreading, over-irrigation of non-agricultural plots and lagoon infiltration. At one site (Whitchurch) investigation has revealed that the 10-15 m thick soil and unsaturated zone are effective at reducing concentrations of pathogenic microbes. However, contamination was identified in the saturated zone below the site indicating some transmission. Faecal coliform concentrations were reduced by between 2 and 3 orders of magnitude from 100,000 to one million CFU/100ml to 1000 CFU/100ml by the time the wastewater recharge had reached the water table. There was little evidence of any contamination, faecal or viral, at distances >200m down gradient of the site boundary.

Other studies of migration through the unsaturated zone have been carried out by Powelson et al (1990) who found that in soil columns, 39% of the bacteriophage injected on to a column was lost (due to inactivation) and the distribution on the column showed an exponentially declining profile with depth. A further study by Cochet et al, (1990), using columns of silty sand which were subjected to intermittent flooding with sewage sludge, showed virus removal in the first 20 mm of soil by between 63 and 90%.

Lee (1993) investigated the contamination of a water supply well by *Giardia* spp. and *Cryptosporidium* spp. in a karstic environment. The karstic nature of the study area provided the potential for rapid infiltration of surface waters to the water table through fractures and fissures and connection between the surface and the well was proven. Particle size analysis revealed that the full range of particle sizes found in the surface waters was not however present in the well. There were cut offs at both low and high ranges and it was concluded

there had been adsorption of smaller particles and straining of larger ones. The size range of the particles that were transported through the system included *Giardia* and *Cryptosporidium.*

In another case of *Cryptosporidium* contamination of a water supply well from Triassic sandstone in the UK (Bridgman et al, 1995) the well water was found to be contaminated by infiltration from agricultural land overlying the sandstone. Only a thin soil and drift cover were present, mining activity had resulted in additional fracturing of the sandstone and there was a failure of the well casing due to corrosion. This combination of pathway and receptor factors led to a rapid transport of the contaminants to the well with limited opportunity for attenuation.

4.6.2 Saturated zone

On reaching the saturated zone, the microbial contaminants will be subjected to the same processes described earlier but under conditions of natural or artificially induced flow. The physical shape of the microbe may also play an indetermined role in movement eg rod, spiral, coccus. Groundwater flow will transport the microbes by advection and they will also undergo diffusion and dispersion as a result of the aquifer heterogeneity and variation in groundwater velocity within the aquifer.

The groundwater flow through the aquifer is defined by Darcy's law. This enables a groundwater velocity, v, to be determined through an aquifer using the equation:

$$v = -(K/n_e).dh/dl$$

where K is the hydraulic conductivity of the aquifer material, n_e the kinematic porosity and dh/dl the hydraulic (head) gradient. (Kinematic porosity is that proportion of the saturated pore space which contributes to flow and is the value important when considering groundwater flow velocity. Effective porosity is also commonly used for the same quantity, although in the past it has been used for specific yield). It can be seen that the groundwater velocity increases with a decrease in kinematic porosity. This relationship is very important in fractured and fissured rocks and in particulate transport where kinematic porosity can be much smaller than the total porosity due to size exclusion and the dual porosity/permeability nature of rocks.

As aquifer material is naturally heterogenous, a spectrum of velocity regimes will exist because of the variability of pore sizes, pore geometry and variation in hydraulic conductivity. This spectrum of velocities acts to transport contaminants at different rates and leads to dispersion. The result is to lead to a spreading of contaminant concentration in space and time (Figure 4.2). This spreading during transport is referred to as hydrodynamic dispersion and it takes effect from the pore-scale upwards. Spreading may also provide nutrients and energy sources for microbial use leading to more growth.

In pollution studies, the spreading phenomenon tends to reduce concentrations. However, the process is three-dimensional and so spreading of pollutants is not only in the direction of flow but laterally and vertically. This may result in contamination of increasingly large aquifer volumes as the pollutant moves down gradient. The choice of parameter values for the purpose of modelling is made difficult by the heterogeneous nature of the medium and because values vary with scale. In addition to dispersion, molecular diffusion also occurs. This is the movement of particles, molecules and ions as a result of gradients in concentration, pressure and temperature and is defined as Brownian motion. Although

molecular diffusion is usually considered to be of negligible importance compared to hydrodynamic dispersion, its influence can be seen in dual-porosity media, e.g chalk, where within the saturated system water is mobile in part of it (fissures) and relatively immobile in the rest (matrix). An exchange of contaminants between these parts of the system can take place due to molecular diffusion and have implications for contaminant transport and aquifer remediation. Lawrence et al (1992) showed that slow diffusion of solvent from matrix water gave rise to intermittent long-term pollution of groundwater with the matrix acting as a secondary source of pollution. The effects of matrix diffusion can also be observed in tracer test breakthrough curves where tailing on the breakthrough curve cannot be attributed to hydrodynamic dispersion alone (Lloyd et al. 1996 and Ward, 1990).

With respect to microbial contaminants, the same transport processes will control their distribution and fate in addition to the physico-chemical controls described earlier. A number of investigators have studied migration of microbes within the saturated zone and some of these are described below.

Microbiological agents (bacteria, yeasts and bacteriophage) have been used to study transport processes in the subsurface for a long time through their use in tracer testing. However the importance of studying the transport of the microorganisms themselves has only recently become apparent. In recent years techniques have been improved to enable practical investigation of microbial transport in both the laboratory and the field.

Whilst laboratory studies are easier than field studies, it is only possible to simulate environmental conditions for very simple systems. However, an iterative approach of laboratory then field testing optimises field testing and focusses on particularly important factors.

Laboratory transport studies usually involve column experiments where glass, perspex or steel columns are packed with aquifer material (or soil). Groundwater is then circulated through the column and the microorganism injected into the column. In most cases, a conservative, non-reactive tracer is also used for comparison. Table 4.6 (adapted from Harvey, 1997) gives a list of reported column studies which have focused on characterising the factors controlling subsurface microbial transport behaviour.

Field investigations designed to examine such behaviour in the saturated zone have generally involved the introduction of microorganisms (tracer) into the aquifer, often with a conservative tracer. Different test methods can be used in field tests and these include radially diverging forced gradient tests, radially converging forced gradient tests, dipole forced gradient tests and natural gradient tests (see detailed examination of tracer test methodologies in Barker et al (1995)). Table 4.7 lists a number of field tracer tests in granular and fractured rocks using microbial tracers.

The very few published results of field experiments in the UK to study microbial contamination reflects the lack of research in this area. The work that has been published describes North American geological environments which do not reflect the characteristics of UK aquifers because of the preponderance of intergranular flow aquifers in the former and dual porosity in the latter. From the published work however, it has been found that the potential exists for migration of microbiological contaminants in the saturated zone over large distances under favourable conditions.

4.7 Modelling Microbial Transport in Groundwater

In order to predict the movement of microbial contaminants in the subsurface, mathematical models can be developed to simulate and describe the movement. These have the advantage that the impacts of microbial contaminants can be examined before contamination has taken place in order that an appropriate groundwater protection policy can be adopted. On the other hand, the complexities of the processes which affect the transport and survival of the microbes, which are often poorly understood, makes accurate modelling extremely difficult. In addition, without accurate parameter values for the models little confidence is placed in their outcome.

Recent advances made in modelling have been achieved through a better understanding of the processes involved in microbial and colloid transport directly as a result of the increasing number of laboratory and field studies. However, greater numbers of field studies are still required to improve confidence in the models being used as predictive tools.

In general, models describing microbial movement are based on the advection-dispersion equation (derived form Fick's first law (Freeze and Cherry, 1979)) which is modified to account for retention processes, growth and mortality etc. As described earlier, the velocity of the microbes can be retarded relative to the water velocity by a number of processes. These processes lead to a distribution of microbes between the solid phase of the medium and pore water (and also gas phase in the unsaturated zone).

Current modelling developments (Bales et al, 1997, Tan et al, 1994 and Lindqvist et al, 1994) are focusing on combined kinetic-equilibrium two-site models. These originate from filtration theory used to describe removal of colloids during packed-bed filtration in water-treatment applications (Harvey and Garabedian, 1991). The governing equations for one-dimensional colloid transport in a porous medium are:

$$\theta \frac{\partial C}{\partial t} + \rho_b \left[\frac{\partial S_1}{\partial t} + \frac{\partial S_2}{\partial t} \right] = \theta D \frac{\partial^2 C}{\partial x} - v\theta \frac{\partial C}{\partial x} - \lambda C$$

$$S_1 = K_{p1} C$$

$$\rho_b \frac{\partial S_2}{\partial t} = \theta k_1 C - \rho_b k_2 S_2$$

$$D = \alpha v$$

where C is the microbe concentration in the aqueous phase, S_1 and S_2 the concentrations bound to the surface for fast and kinetically limited sites respectively, θ is porosity, ρ_b is the dry bulk density of the solid material, D the longitudinal dispersion coefficient, v the average groundwater velocity, λ the decay constant resulting from inactivation or die off, k_1 the pseudo-first-order rate coefficient for sorption, k_2 the pseudo-first-order desorption rate

coefficient and longitudinal dispersivity. (Where there is microbial growth an additional term will be required).

A one-dimensional approach is applied because of the complexity of three-dimensional modelling. In general, this is adequate for relatively simple systems such as those that can be simulated in the laboratory, but in the field, where heterogeneity is greater, the one-dimensional approach becomes limiting (Bales et al, 1997). Attention is therefore moving towards three-dimensional modelling but until better field test data is available, model assessment and validation will be limited.

Concentration gradients of chemicals (nutrients, dissolved gases etc.) can induce chemotactic responses in motile microorganisms. Chemotaxis describes the process by which whole organisms respond to chemical stimuli. The responses can be either towards the chemical stimuli (positive chemotaxis) or away from the chemical stimuli (negative chemotaxis). Chemotaxis can have a major impact on microbial transport and survival in subsurface environments, but is rarely investigated in microbial models.

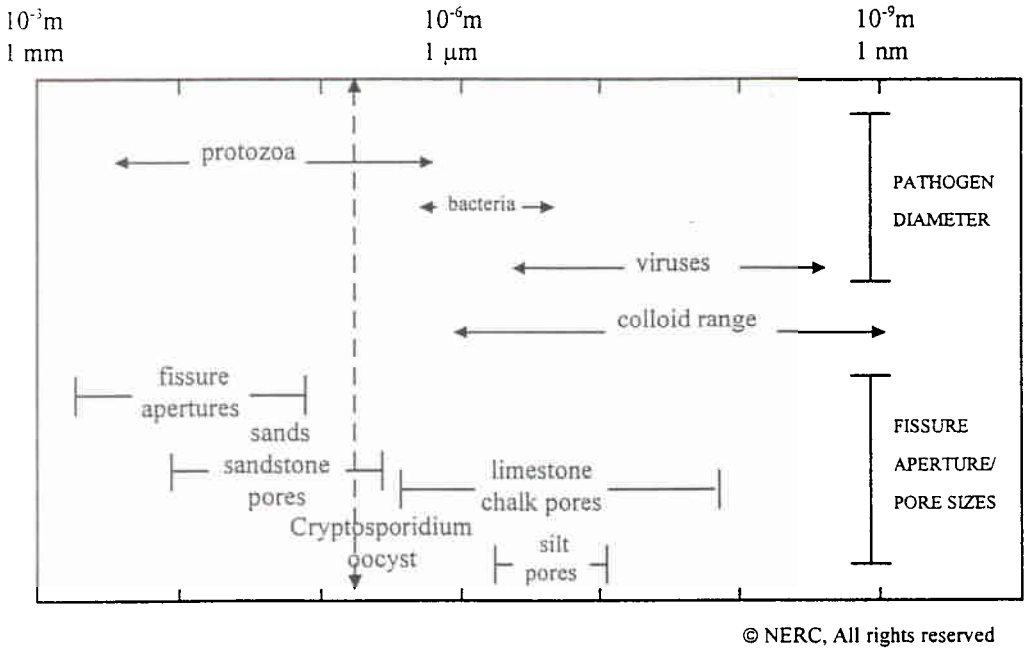


Figure 4.1. Pathogen diameters compared to aquifer matrix apertures

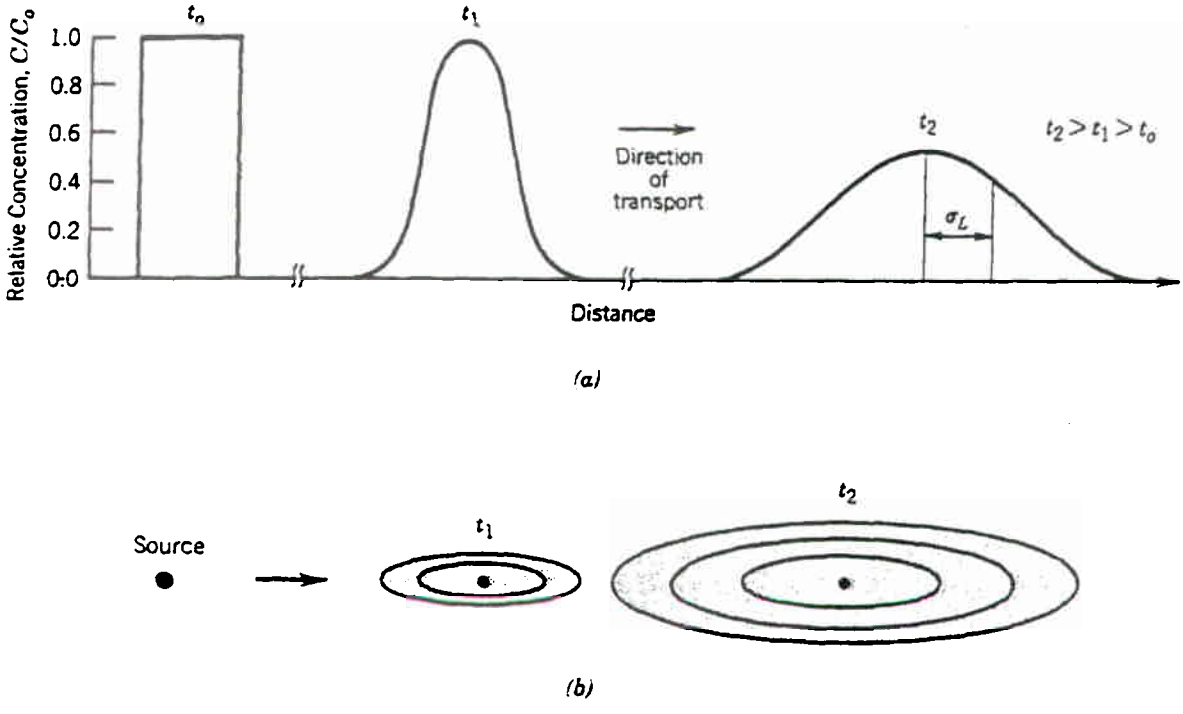


Figure 4.2. Dispersion effect of spreading

Table 4.1. Factors affecting fate and transport.

Characteristics of the microbes	Aquifer/soil (environment) properties
Size	Groundwater flow velocity
Shape	Dispersion
Density	Grain size
Inactivation rate	Kinematic/effective porosity
Adsorption	Organic carbon content (solid)
	Temperature
	Chemical properties of groundwater (pH etc)
	Mineral composition of aquifer/soil material (eg clay content)
	Predatory microflora (bacteria, fungi, algae etc)
	Moisture content
	Pressure

Table 4.2. Sizes of selected microbe.

Bacteria	
E coli	0.5 µm x 1.0 µm x 2.0 µm
Salmonella typhosa	0.6 µm x 0.7 µm x 2.5 µm
Shigella sp.	0.4 µm x 0.6 µm x 2.5 µm
Viruses	
Psittacosis virus	0.25 µm diameter
Bacteriophage virus	0.1 µm diameter
Poliomyelitis	0.01 µm diameter
Protozoa	
Cryptosporidium	4.0 - 6.0 µm diameter (oocysts)
Giardia	7.0 - 12.0 µm diameter
Enteroamoeba histolitica	20 - 25 µm diameter

Table 4.3. UK aquifer properties. (The value ranges presented are for indication only).

	Cretaceous Chalk	**Permo-Triassic Sandstone**
Lithology	microporous carbonate	quartz grains with silica and/or carbonate cement
Groundwater flow regime	fissure flow dominates	intergranular with some local, limited extent, fissure flow
Matrix porosity (%)	25 - 45	15 - 30
Characteristic pore size μ(m)	0.2 - 1.0	5 - 50
Fracture aperture (mm)	0.1 - 10	1 - 10
Matrix hyd. cond. (m/s)	$2x10^{-9}$ - $6x10^{-8}$	$6x10^{-6}$ - $1x10^{-4}$
Fracture hyd. cond. (m/s)	$1x10^{-6}$ - $1x10^{-1}$	$1x10^{-1}$ - $1x10^{1}$
Unsaturated zone flow rates (m/yr)	0.3-1.4	0.6-2.3
Natural saturated zone groundwater flow velocity (m/day)	1-400	difficult to ascertain

Table 4.4. Factors affecting microbe survival and half-life.

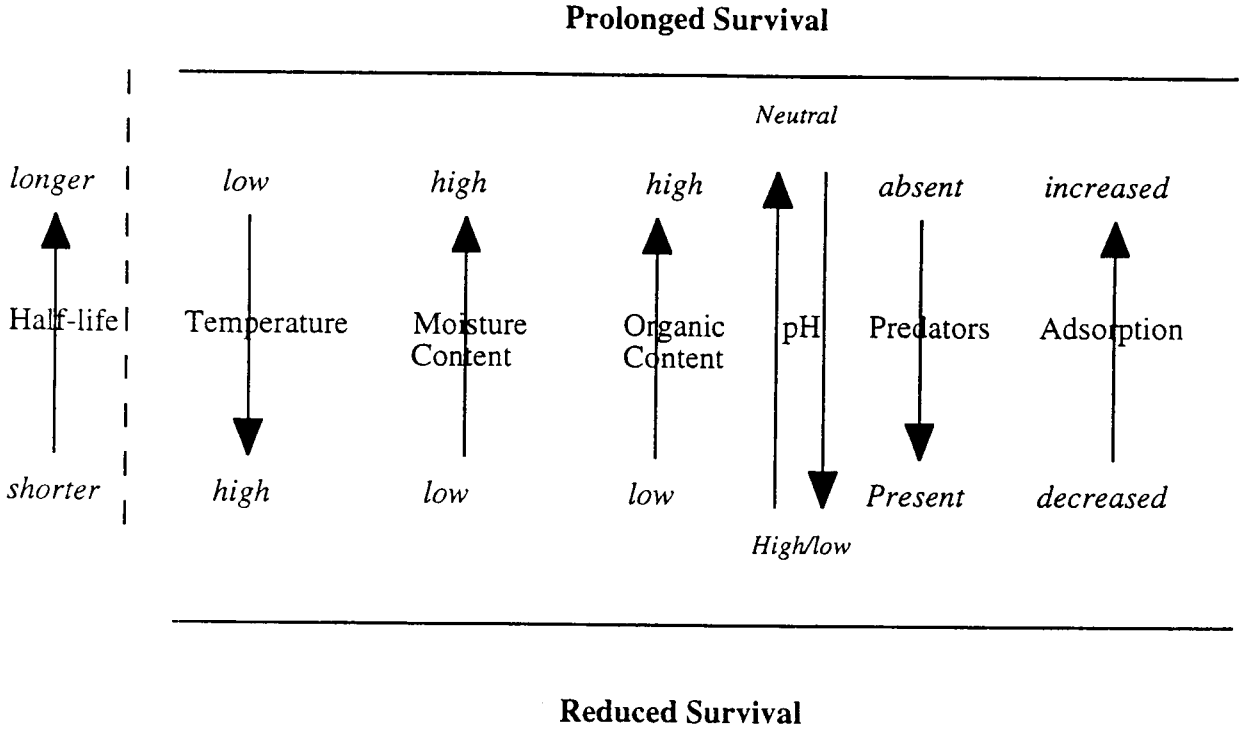


Table 4.5. Examples of half-lives of pathogenic microbes derived from experimentation. (Environmental conditions not specified).

Microbe	Decay constant (hr^{-1})	Half-life (hr)	Reference
Viruses			
Poliovirus (in groundwater)	0.0013	533.2	(Bitton et al, 1983)
	0.0088	78.8	(Keswick et al, 1982).
Viruses (in well water)	0.0004 - 0.0037	1732.9-187.34	(Bitton & Harvey, 1985)
Enteroviruses	0.004	173.3	(Gerba. 1985)
PSD-2 and MS-2 (*E. coli*) bacteriophage in groundwater	0.033	21.0	(Dowd & Pillai,1997)
Bacteria			
Salmonella spp.	0.0078	88.9	(Dowd & Pillai,1997)
(in groundwater)	0.055	12.6	(Gerba, 1985)
Klebsiella spp.	0.0013	533.2	(Dowd & Pillai,1997)
(in groundwater)			
Escherichia coli	0.038	18.2	(Gerba, 1985),
	0.013	53.3	(Keswick et al, 1983)
Streptococcus spp.	0.015	46.2	(Gerba, 1985)
	0.0096	72.2	(Keswick et al, 1983)
Shigella spp.	0.028	24.8	(Gerba, 1985)
Fecal coliforms	0.064	10.8	(Gerba, 1985)

Table 4.6. Laboratory column experiments used for investigating transport of microbiological contaminants.

Parameter(s) investigated	Geologic medium	Microorganism(s) used	Reference
Bacteria			
Density-dependant	Sandy soil	Aquifer isolate	Bengtsson attachment and Lindqvist,1995
Flow velocity	Glass beads *fluorescens*	*Pseudomonas*	Camper et al, 1993
Effect of ionic strength/survival	Aquifer sand	*Salmonella* sp.and *Klebsiella* sp	Dowd and Pillai, 1997
Heterogenity	Quartz sand	Aquifer isolates	Fontes et al, 1991
Ionic strength	Aquifer sand	*Pseudomonas* sp.	Gannon et al, 1991(b)
Relative mobility	Loamy soil	19 strains of bacteria	Gannon et al, 1991(a)
Effect of chemical	Glass beads	*Alcaligenes paradoxus*	Gross and treatments Logan 1995
Unsaturated flow	Loamy soils *Pseudomonas* sp.	*Burkholderia,*	Heckman et al, 1995
Model evaluation	Rounded quartz	Aquifer isolates	Hornberger et al, 1992
Hydrophobicity Sandy and clay soil properties		*Lactobacillus* spp.	Huysman soil and Verstraete, 1993
Transport properties	Sandstone	*Pseudomonas, Bacillus* and *Clostridium* spp	Jang et al, 1983
Facilitating PAH transport	Aquifer sand	*Bacillus subtilis*	Jenkins and Lion, 1993
Effect of. sterilisation tech	Sandstone	Landfill isolates	Jenneman et al, 1986
Permeability and motility	Sandstone	*Bacillus* and *Enterobacter* spp	Jenneman et al, 1985
Surface residence time	Aquifer sand	Subsurface isolate	Johnson et al, 1995
Survival	Aquifer sand strain B13	*Pseudomonas* sp	Krumme et al, 1994
Facilitating DDT transport	Sand	*Pseudomonas* and *Bacillus* spp,	Lindqvist and Enfield 1992
Sorption isolates /de-sorption	Sand	Gram-neg. aquifer	McCaulou et al, 1994
Effect of Aquifer temperature	Sediment	Subsurface isolate	McCaulou et al, 1995
Selectivity and of plugging	Sandstone	-	Raiders et al, 1986 depth

Table 4.6. Laboratory column experiments used for investigating transport of microbiological contaminants (continued).

Parameter(s) investigated	Geologic medium	Microorganism(s) used	Reference
Effect of nutrients motility	Sand	*Escherichia coli*	Reynolds et al, 1986. on
Cell hydrophobicity and surface charge	Glass and Teflon	Pseudomanads and coryneforms	Rijnaarts et al, 1993
Groundwater chemistry	Aquifer sand	Uncultured aquifer population	Scholl and Harvey, 1992
Mineralogy and chemistry	Coated sand	Aquifer isolates	Scholl et al, 1990
Effect of macropores	Soil	*Escherichia coli*	Smith et al, 1985
Clogging efficiency	Aquifer sand	Aquifer and soil isolates	Vandevivere and Baveye, 1992
Viruses			
Matrix diffusion	Fractured tuff	MS-2 (coliphage)	Bales et al, 1989
Sorption	Silica	PRD-1 and MS-2 phage)	Bales et al, 1991
Chemical perturbations	Silica beads	MS-2 and poliovirus	Bales et al, 1993
Organic matter	Loamy sand	MS-2 (coliphage)	Powelson et al, 1991
Protozoa			
Comparison of field and laboratory transport	Aquifer sand	Unidentified flagellates	Harvey et al, and 1995
Microspheres			
Heterogeneity	Aquifer sand carboxylated	0.2-4.8 m	Harvey et al, 1993
Optimal transport size	Aquifer sand	0.7-6.2 m carboxylated	Harvey et al, 1995
Influence of oil	Sandstone	Unspecified (negative charge)	Jang et al, 1983
Temperature effects	Aquifer sediment	1.5 μm carboxylated	McCaulou et al, 1995
Artificial fractures	Sand	1 μm latex microspheres	Toran and Polumbo, 1992

Table 4.7. Selected field tracer tests in which microbial tracers have been used.

Geologic medium	Tracer/microorganism	Type	Scale	Location and Reference
Fractured media				
clay-rich till	MS-2 and PRD-1 (bacteriophage)	1	4 m	NA (McKay et al, 1993)
crystalline fractured rock (granite)	*Escherichia coli*	2	13 m	NA(Champ and Schroeter, 1988)
North sea oil deposits	str. 4502 (sulphate reducing bacteria)	2	1-4 km	(E Beeder et al, 1996)
Chalk (UK)	MS-2, *Serratia marcesens*, *Enterobacter clocae* (bacteriophage)	4	4 kmUK	(Ward et al, 1997)
Chalk (UK)	MS-2 (bacteriophage) and microspheres	3	20 mUK	(BGS unpublished data)
Granular media				
alluvial sand	f2 (bacteriophage)	2	5 m	NA (Bales et al, 1989)
alluvial gravel	E. coli PB922 (rifampicin-resistant)	1	42 m	NA (Sinton, 1986)
glacial sand	PRD-1 and microspheres	2	3 m	NA(Bales et al, 1997)
sand and gravel	indigenous bacteria	1	6 m	NA (Harvey and Garabedian, 1991)
sand and gravel	PRD-1 (bacteriophage)	1	12 m	NA(Bales et al, 1995)
sand and gravel	32P-labelled PRD1	1	4 m	NA(Pieper et al, 1997)
sand and gravel	indigenous flagellates X (rifampicin-resistant)	1	1 m	NA(Harvey et al,1997)
sand and gravel	T7 and f1 (bacteriophage)	1	-	E (Rossi et al, 1994)
sand (High Si, low pH)	vaccine-type polio virus	3	2 m	NA(Jansons et al,1989)

Notes:

Test type: 1 - Natural gradient, 2 - Forced gradient, 3 - Artificial recharge, 4 - Natural/Forced (prevailing) gradient.

Location: NA - North America, E - Europe, UK - United Kingdom

4.8 References

Adams, B and Foster, S S D. (1992). Land-surface Zoning for Groundwater Protection. J. Inst. Water Environ. Management, 2(6), 312-319.

Bales, R C, Hinckle, S R, Kroeger, T W, Stocking, K and Gerba, C P. (1991). Bacteriophage Adsorption During Transport through Porous Media: Chemical Perturbations and Reversibility. Environ. Sci. Technol., 25, 2088-2095.

Bales, R C, Gerba, C P, Grondin, G H and Jensen, S L. (1989). Bacteriophage Transport in a Sandy Soil and Fractured Tuff. Appl. Environ. Microbiol., 55, 2061-2067.

Bales, R C, Li, S, Maguire, K M, Yahya, M T and Gerba, C P. (1993). MS-2 and Poliovirus Transport in Porous Media: Hydrophobic Effects and Chemical Perturbations. Water Resources Research, 29(4), 957-963.

Bales, R C, Shimin, L, Yeh, T-C J, Lenczewski, M E and Gerba, C P. (1997). Bacteriophage and Microsphere Transport in Saturated Porous Media: Forced-gradient Experiment at Borden, Canada. Water Resources Research, 33(4), 639-648.

Bales, R C, Shimin, L, Yeh, Maguire, K M, Yahya, M T, Gerba, C P and Harvey, R W. (1995). Virus and Bacteria Transport in a Sandy Aquifer, Cape Cod, MA. Groundwater, 33(4), 653-661.

Barker, J A, Williams, A T and Ward, R. S. (1995). Groundwater Tracer Studies. National Rivers Authority R&D Interim Report 587/2/S. Prepared by the British Geological Survey.

Beeder, J, Nilsen, R K, Thorstenson, T and Torsvik, T. (1996). Penetration of Sulfate Reducers Through a Porous North Sea Oil Reservoir. Appl. Environ. Microbiol., 62, 3551-3553.

Bengtsson, G and Lindqvist, R. (1995). Transport of Soil Bacteria Controlled by Density-Dependent Sorption Kinetics. Water Resources Research, 31, 1247-1256.

Bitton and Farrah, (1980) *Am. Soc. Microbiol. News, 46, 622.*

Bitton, G., Farah, S. R., Ruskin, R. H., Butner, J. & Chou, Y. J. (1983). Survival of Pathogenic and Indicator Organisms in Ground Water. Ground Water, 21(4), 405-410.

Bitton, G and Gerba, C P. (1984). Groundwater Pollution Microbiology, Wiley and Sons, ISBN 0-471-09656-3.

Bitton, G. & Harvey, R. W. (1992). Transport of pathogens through soils and aquifers. In *Environmental Microbiology*. Edited by R. Mitchell: John Wiley & Sons, 103-124.

Bridgman, S A, Robertson, R M P, Syed, Q, Speed, N, Andrews, N and Hunter P R. (1995). Outbreak of Cryptosporidiosis associated with a Disinfected Groundwater Supply. Epidemiol. Infect., 115, 555-566.

Camper, A K, Hayes, J T, Surman, P J, Jones, W L and Cunningham, A B. (1993). Effects of motility and Adsorption Rate Coefficients on transport of Bacteria Through Saturated Porous Media. Appl. Environ. Microbiol., 59, 3455-3462.

Champ, D R and Schroeter, J. (1988). Bacterial Transport in Fractured Rock - A Field Scale Tracer Test at the Chalk River Nuclear Laboratories. Water Sci. Technol., 20, 81-87.

Cochet, C, Derangere, D and Rousselle, T. (1990). Soil Absorption Systems and Nitrogen Removal. Water Science and Technology, 22(3-4), 109-116.

Dowd, S E and Pillai, S D. (1997). Survival and Transport of Selected Bacterial Pathogens and Indicator Viruses under sandy Aquifer Conditions. J. Environ. Sci. Health, A32(8), 2245-2258.

Fontes, D E, Mills, A L, Hornberger, G M and Herman, J S. (1991). Physical and Chemical Factors Influencing Transport of Microorganisms through Porous Media. Appl. Env. Microb., 57(9), 2473-2481.

Foster, S S D. (1993). The Chalk Aquifer - Its Vulnerability to Pollution. In *The Hydrogeology of the Chalk of North West Europe*, Ed: Downing, R A, Price , M and Jones, G P. Clarendon Press, Oxford, ISBN 0-19-854285-2, 93-112.

Foster, SSD and Hirata, R (1988) Groundwater pollution risk assessment- a methodology using available data. CEPIS-PAHO Lima, Perú

Fourie, A B and van Ryneveld, M B. (1995). The fate in the subsurface of contaminants associated with on-site sanitation: a review. *Water SA* 21, 101-111.

Freeze, R A and Cherry, J A. Groundwater. (1979) Prentice Hall, USA.

Gannon, J T, Mingelgrin, U, Alexander, M and Wagenet, R J. (1991). Bacterial Transport through Homogeneous Soil. Soil Biol. Biochem., 23(12), 1155-1160.

Gerba, C P. (1985). Microbial contamination of the subsurface. In *Groundwater Quality*, pp. 53-67. Edited by C. H. Ward & P. L. McCarty: John Wiley & Sons.

Gerba, C P, Goyal, S M, Cech, I and Bogdan, G F. (1981). Environ. Sci. Technol., 15, 940-953.

Gerba, C P, Rose, J B and Singh, S N. (1985). Waterborne Gastroenteritis and Viral-Hepatitis. CRC Critical Reviews in Environmental Control, 15(3), 213-236.

Gerba, C P, Yates, M V, Yates, S R. (1991). Quantification of factors Controlling Viral and Bacterial transport in the Subsurface. In *Modeling the Environmental Fate of Microorganisms*, Ed: Hurst, C J. Am. Soc. Microbiol., Washington D.C., 77-86.

Goldscmid, J, Zohar, Dm Argaman, Y and Kott, Y. (1973). In S H Jenkins, Ed., *Advances in Water Pollution Research*. Pergammon Press, New York, 147-160.

Grolimund, D, Borkovec, M, Barmettler, K and Sticher, H. (1996). Colloid-Facilitated Transport of Strongly Sorbing Contaminants in Natural Porous Media: A Laboratory Column Study. Environ. Sci. Technol., 30, 3118-3123.

Gross, M A and Logan, B E. (1995). Influence of Different Chemical Treatments on Transport of *Alcaligenes paradoxus* in Porous Media. Appl. Environ. Microbiol., 61, 1750-1756.

Harrison, I. (1996). A Study of Colloid Mobility in a Glacial Sand Formation. *PhD Thesis*, Nottingham Trent University, UK.

Harvey, R W. (1997). Microorganisms as Tracers in Groundwater Injection and Recovery Experiments: A Review. *FEMS Microbiology Reviews* 20, 461-472.

Harvey, R W and Garabedian, S P. (1991). Use of Colloid Filtration Theory in Modeling Movement of Bacteria through a Contaminated Sandy Aquifer. Environ. Sci. Technol., 25, 178-185.

Harvey, R W, Metge, D W, Kinner, N E and Mayberry, N. (1997). Physiological Considerations in Applying Laboratory-Determined Buoyant Densities to Predictions of Bacterial and Protozoan Transport in Groundwater: Results of In Situ and Laboratory Tests. Environ. Sci. Technol., 31, 289-295.

Harvey, R W, Kinner, N E, MacDonald, D, Metge, D W and Bunn, A. (1993). Role of Physical Heterogeneity in the Interpretation of Small-scale Laboratory and Field Observations of Bacteria, Microspheres and Bromide Transport through Aquifer Sediments. Water Resources Research, 29(8), 2713-2721.

Heckman, W E, Heijnen, C E, Burgeres, S L G E, van Veen, A and van Elsas, J D. (1995). Transport of Bacterial Inoculants Through Intact Cores of Two Different Soils as Affected by Water Percolation and the Presence of Wheat Plants. FEMS Microbiol. Ecol., 16, 143-158.

Hornberger, G M, Mills, A L and Herman, J S. (1992). Bacterial Transport in Porous Media: Evaluation of a Model suing Laboratory Observations. Water Resources Research, 28, 915-938.

Hurst, C J, Gerba, A P and Cech, I. (1980). Effects of Environmental Variables and Soil Characteristics on Virus Survival in Soils. Appl. Environ. Microbiol., 40, 1067-1079.

Huysman, F and Verstraete, W. (1993). Water-facilitated Transport of Bacteria in Unsaturated Soil Columns: Influence of Cell Surface Hydrophobicity and Soil Properties. Soil. Biol. Biochem., 25, 83-90.

Jang, L K, Chang, P W, Findley, J E and Yen, T F. (1983). Selection of Bacteria with Favourable transport Properties Through Porous Rock for the Application of Microbially-enhanced Oil Recovery. Appl. Environ. Microbiol., 46, 1066-1072.

Jansons, J, Edmonds, L W, Speight, B and Bucens, M R . (1989). Survival of Viruses in Groundwater. Wat. Res., 23(3), 301-306.

Jenkins, M B and Lion, L W. (1993). Mobile Bacteria and Transport of Polynuclear Aromatic Hydrocarbons in Porous Media. Appl. Environ. Microbiol., 59, 3306-3313.

Jenneman, G L, McInerney, Crocker, M E and Knapp, R M. (1986). Effect of Sterilisation by Dry Heat or Autoclaving on Bacterial Penetration Through Berea Sandstone. Appl. Environ. Microbiol., 51, 39-43.

Jenneman, G L, McInerney and Knapp, R M. (1985). Microbial Penetration through Nutrient-Saturated Berea Sandstone. Appl. Environ. Microbiol., 50, 383-391.

Johnson, W P, Blue, K A, Logan, B E and Arnold, R G. (1995). Modeling Bacterial Detachment during Transport Through Porous Media as a Residence-Time-Dependent Process. Water Resources Research, 31, 2649-2658.

Keswick, B H, Gerba, C P, Secor, S L and Cech, I. (1982). Survival of Enteric Viruses and Indicator Bacteria in Groundwater. J. Environ. Sci. Health, A17(6), 903-912.

Krumme, M L, Smith, R L, Egestorff, J, Thiem, S M, Tiedje, J M, Timmis, K N and Dwyer, D F. (1994). Behaviour of Pollutant-Degrading Microorganisms in Aquifers: Predictions for Genetically Engineered Organisms. Environ. Sci. Technol., 28, 1134-1138.

Lawrence, A R, Stuart, M E, Barker, J A, Chliton, P J, Goody, D C and Bird M J. (1992). Review of groundwater pollution of the Chalk aquifer by halogenated solvents. R&D Report 130/6/A, National Rivers Authority, Anglian Region.

Lee (1993). Contamination of a well by the direct influence of surface water, Proc of 1993 Annual Conf. Am. Waterworks. 215-231.

Liew, P and Gerba, C P. (1980). Themostabilization of Enteroviruses by Estuarine Sediment. Appl. Environ. Microbiol., 40, 205-308.

Lindqvist, R, Cho, J S and Enfield, C G. (1994). A Kinetic model for Cell Density Dependent Bacterial Transport in Porous Media. Water Resources Research., 30(12), 3291-3299.

Lindqvist, R and Enfield, C G. (1992). Biosorption of Dichlordiphenyltrichloroethane and Hexachlorobenzene in Groundwater and its Implications for Facilitated Transport. Appl. Environ. Microbiol., 58, 1362-1372.

Lloyd, J W, Greswell, R, Williams, G M, Ward R S, Mackay, R and Riley M S. (1996). An integrated study of controls on solute transport in the Lincolnshire limestone. Quarterly Journal of Engineering Geology, 29, 321-339.

McCaulou, D R, Bales, R C and McCarthy, J F. (1994). Use of Short-Pulse Experiments to study Bacteria Transport through Porous Media. J. Contam. Hydrol., 15, 1-14.

McCaulou, D R, Bales, R C and Arnold, R G. (1995). Effect of Temperature-Controlled Motility on Transport of Bacteria and Microspheres through Saturated Sediment. Water Resources Research, 31, 271-280.

McKay, L D, Cherry, J A, Bales R C, Yahya, M T and Gerba, C P. (1993). A Field Example of Bacteriophage as Tracers of Fracture Flow. Environ. Sci. Technol., 27, 1075-1079.

Moore, R S, Taylor, D H, Sturman, L S, Reddy, M M and Fuhs, G W. (1981). Poliovirus Adsorption by 34 Minerals and Soils. Appl. Enviro. Microbiol., 42(6), 963-975.

Pieper, A P, Ryan, J N, Harvey, R W, Amy, G L, Illangasekare, T H and Metge, D W. (1997). Transport and Recovery of Bacteriophage PRD1 in an Unconfined Sand Aquifer: Effect of Anthropogenic Organic Matter. Environ. Sci. Technol., 31(4), 1163-1170.

Powelson, D K, Simpson, J R and Gerba, C P. (1990). Virus Transport and Survival in Saturated and Unsaturated Flow Through Soil Columns. J. Env. Quality, 19(3), 396-401.

Powelson, D K, Simpson, J R and Gerba, C P. (1991). Effects of Organic Matter on Virus Transport in Unsaturated Flow. Appl. Environ. Microbiol., 57, 2192-2196.

Raiders, R A, McInerney, M J, Revus, D E, Tobati, H, Knapp, R M and Jenneman, G E. (1986). Selectivity and Depth of Microbial Plugging in Berea Sandstone Core. J. Ind. Microbiol., 1, 195-203.

Reddy, K R, Khaleel, R and Overcash, M R. (1981). Behaviour and Transport of Microbial Pathogens in Soils Treated with Organic Wastes. J. Environ. Qual., 10, 255-266.

Robertson, J B, Edberg, S C. (1997). Natural protection of Spring and Well Drinking Water Against Surface Microbial Contamination. 1. Hydrogeological Parameters. Critical Reviews in Microbiology, 23(2), 143-178.

Reynolds, P J, Sharma, P, Jenneman, G E and McInerney. (1989). Mechanisms of Microbial Movement in Subsurface Materials. Appl. Environ. Microbiol., 55, 2280-2286.

Rijnaarts, H H M, Norde, W, Bowerm E J, Lykema, J and Zehnder, A J B. (1993). Bacterial Adhesion Under Static and Dynamic Conditions. Appl. Environ. Microbiol., 59, 3255-3265.

Scholl, M A and Harvey, R W. (1992). Laboratory Investigations on the Role of Sediment Surface and Groundwater Chemistry in Transport of Bacteria Through a Contaminated Sandy Aquifer. Environ. Sci. Technol., 26, 1410-1417.

Scholl, M A, Mills, A L, Herman, J S and Hornberger, G M. (1990). The Influence of Mineralogy and Solution Chemistry on the Attachment of Bacteria to Representative Aquifer Materials. J. Contam. Hydrol., 6, 321-336.

Severson, K. J., Johnstone, D. L., Keller, C. K. & Wood, B. D. (1992). Hydrogeological Parameters Affecting Vadose-Zone Microbial Distributions. Geomicrobiology Journal, 9, 197-216.

Sinton, L W. (1986). Microbial Contamination of Alluvial gravel Aquifers by Septic Tank Effluent. Water Air Soil Pollut., 28, 407-425.

Smith, M S, Thomas, G W, White, R E, Ritonga, D. (1985). Transport of *Escherichia coli* Through Intact and Disturbed Soil Columns. J. Environ. Qual., 14, 87-91.

Sobsey, M. D., Shields, P. A., Hauchman, F. H., Hazard, R. L. & Caton III, L. W. (1986). Survival and transport of hepatitis A virus in soils, groundwater and wastewater. Water Science Technology 18, 97-106.

Tan, Y, Gannon, J T, Baveye, P and Alexander, M. (1994). Transport of Bacteria in an Aquifer Sand: Experiments and Model Simulations. Water Resources Research, 30(12), 3243-3252.

Toran, L and Palumbo, A V. (1992). Colloid Transport Through Fractured and Unfractured Laboratory Sand Columns. J. Contam. Hydrol. 9(3), 289-303.

Vandevivere, P and Baveye, P. (1992). Relationship Between Transport of Bacteria and their Clogging Efficiency in Sand Columns. Appl. Environ. Microbiol., 58, 2523-2530.

Wan, J, Wilson, J L and Kieft, T L. (1994). Influence of the Gas-Water Interface on Transport of Microorganisms through Unsaturated Porous Media. Appl. Env. Microb., 60(2), 509-516.

Ward, R S. (1990). Artificial tracer and natural 222Rn studies of the East Anglian Chalk aquifer. PhD Thesis, School of Environmental Sciences, University of East Anglia.

Ward, R S, Hassison, I, Leader, R U and Williams, A T. (1997). Fluorescent Polystyrene Microspheres as Tracers of Colloidal and Particulate Meterials: Examples of their Use and Developments in Analytical Technique. In: *Tracer Hydrology* 97, Ed: Kranjc, A. Proceedings of the 7th Int. Symp. on Water Tracing, Slovenia, 99-103.

Yates, M V, Gerba, C P and Kelley, L M. (1985). Virus Persistence in Groundwater. Appl. Environ. Microbiol., 49(4), 778-781.

Yavuz Corapcioglu, M and Haridas, A. (1984). Transport and Fate of Microorganisms in Porous Media: A Theoretical Investigation. J. Hydrol. 72, 149-169.

5. SEPTIC TANKS AND PACKAGE TREATMENT PLANTS (PTPS)

5.1 Introduction

A potential source of microbiological contamination of groundwater is from the disposal of treated sewage effluent. The majority of properties in the U.K. are connected to the main sewer and the sewage is treated at a large sewage works and the effluent from the works is disposed of to a surface water course. The sewage works achieves good removal of suspended solids, Bio-chemical Oxygen Demand (BOD) and micro-organisms and the effluent is further diluted by the receiving water. The quality and quantity of effluent entering the receiving water is strictly controlled and the risk to groundwater, through the passage of micro-organisms from the river bed into the ground, is small.

If a property is not connected to the main sewer then there are several alternatives for the disposal of sewage. These include Package Treatment Plants (PTPs), septic tanks and cesspools. PTPs are capable of treating sewage to a similar extent as a full-scale sewage treatment plant and the effluent from a PTP may be disposed of to a surface water course, if there is a suitable stream or river nearby, or to the ground. Although PTPs can treat the sewage to a great extent, the risk of a PTP contaminating the receiving water is greater than for a full-scale works because PTPs are rarely operated or maintained properly and are more vulnerable to changes in flow and toxic shocks. Discharge of treated effluent from a PTP to the ground will be via a soakaway and the correct sizing, design and installation of the soakaway is vital in preventing contamination of the groundwater by micro-organisms. If the effluent from a PTP is discharged to a surface water then the risk to groundwater is lessened by the dilution of the receiving water.

Septic tanks only achieve a small degree of sewage treatment and rely on the soakaway to complete the treatment and to prevent contamination of the groundwater. The risk of contamination of the groundwater strongly depends on the correct sizing, design and installation of the soakaway. Septic tanks have the advantage that they have no moving parts and require very little maintenance or attention from trained personnel but septic tanks can not treat the sewage to the same degree as a well operated PTP and the risk to groundwater depends almost entirely on the effectiveness of the soakaway. Cesspools act as a storage tank for sewage and do not discharge the sewage to the ground. Cesspools must be emptied on a regular basis. If properly constructed and watertight then cesspools should not pose any risk to groundwater.

5.1.1 Background

Septic tanks and Package Treatment Plants (PTPs) are used for the disposal of sewage in many small communities in England and Wales. About 4 % of properties in England and Wales are not connected the to public sewer and it has been estimated that over 800, 000 cesspools, septic tanks and small sewage treatment works are in use. It has also been estimated that at least 7,000 such systems are being installed per year (Payne and Butler, 1993). Septic tanks and PTP system are increasingly being used for the sewerage of new housing developments.

The performance of septic tanks and PTPs relies on many factors including their design, maintenance, installation and the way in which they are operated. The operation and maintenance of septic tanks and PTPs is often poor, frequently for reasons that are easily avoided. Inadequate performance of the septic tanks and PTPs leads to contamination of ground water, production of unpleasant odours and public health problems. There are problems with both new and old installations due to improper design, use and maintenance, a lack of understanding and a lack of knowledge about how the systems should be installed, maintained and operated.

Septic tanks must discharge to land or underground strata whereas PTPs, which treat the sewage to a greater extent, can discharge to surface water or to land. Particular consideration was given to the soakaways of septic tanks and PTPs, as proper design of the soakaway was perceived to be very important in the prevention of groundwater contamination. PTPs can potentially contaminate groundwater through discharge to the surface water too - but the greatest risk of contamination is where the PTP discharges directly to the ground.

5.1.2 Objectives and structure of this section

There are many different septic tank and PTP designs available, but there is little knowledge about their relative performance in terms of potential to contaminate groundwater. This section sets out to highlight the best design and operational practice for septic tanks and PTPs in order to prevent the contamination of groundwater.

The section starts with an assessment of the public health problems caused by septic tanks and PTPs. It then lists the types of septic tanks and PTPs available and describes the processes upon which they are based. This is followed by a discussion of the problems encountered with septic tanks and PTPs, based on the available information and discussions with septic tanks and PTP manufacturers. The section ends with a list of the Best Practice Guidelines for the design, operation and maintenance of septic tanks and PTPs to avoid groundwater contamination.

The whole of this section of the report relates to the treatment of domestic sewage and not to industrial factories or plants. A brief discussion of the additional constraints on designing for hotels, schools and other similar situations is given in Section 8.

5.1.3 Development of Best Practice Guidelines

Best practice guidelines were developed by:

- A literature search of databases for information related to septic tanks and PTPs
- A search of the Internet for relevant Websites
- An assessment of the literature recommended by the EA in its project specification
- An appraisal of the current British Standard on the design of small sewage treatment works and cesspools
- An assessment of manufacturers' literature
- Discussions with selected manufacturers

The academic literature contained a great deal of information on the microbiological contamination of the groundwater due to septic tanks. However the literature rarely identified

the direct causes of the contamination (in terms of poor design, under-sizing or misuse and neglect of the tanks). The most important sources of information in this regard were the CIRIA Technical Report 146 and the discussions with the manufacturers.

It was initially hoped that there would be enough reported experience to assign scores to each of the design and operational elements that were identified in being important in preventing groundwater contamination. However it soon became apparent that there was insufficient data to assign meaningful scores to the risk associated with the design elements. Much more research is necessary to develop risk factors.

The approach taken in this section was based on the assumption that, if the septic tanks or PTPs were under-performing in any way (including the poor removal of solids and chemicals) then the risk of microbiological contamination was increased, because of the increased potential for blockage of part of the septic tank or PTP system.

The Best Practice Guidelines were to a large extent based on the British Standard 6297, the information gathered from the academic literature, the CIRIA Technical Report 146 (Payne and Butler, 1993)) and the discussions with manufacturers. The list of manufacturers visited for this report is given in the Appendix.

5.2 Public Health Problems Arising From The Use Of Septic Tanks and PTPs

Published figures from the U.S. show that septic tanks are the most frequently reported sources of groundwater contamination and that they contribute approximately 2 to 3 x 10^9 m^3 of waste to the subsurface every year (OTA, 1994). The overflow or seepage of sewage from septic tanks or cesspools was responsible for an estimated 43% of the reported disease outbreaks and 63% of reported illnesses caused by the use of contaminated untreated groundwater from 1971 to 1979. The usage of septic tanks in the U.S. is higher than in Britain: approximately one third of domestic waste in the U.S is disposed of in on-site septic tank systems (Wilhelm et al, 1994). Although the usage in the U.K. is not so extensive the potential for groundwater contamination is clear and the misuse of septic tanks is evident from discussions with the manufacturers. Although the problems of chemical contamination from septic tanks and PTPs can have important environmental implications, and longer-term effects on human health, the presence of microbiological contamination has the most important direct effect on public health as contaminated groundwater is a significant vehicle for the transmission of disease (Pedley and Howard, 1997).

The principal categories of pathogenic organisms found in wastewater are bacteria, viruses, protozoa and helminths. The bacterial pathogenic organisms that can be excreted by man cause diseases of the gastrointestinal tract such as typhoid, paratyphoid, dysentery, diarrhea and cholera. Because there are large numbers of pathogenic organisms present in sewage and most of them are difficult to isolate and identify, the coliform organism is commonly used as an indicator organism (Section 3).

5.3 Septic Tank Systems

A septic tank system consists of two separate unit processes and the success of the whole system (in terms of minimizing the risk of microbial contamination of groundwater) depends on each of the processes. Septic tanks can receive the wastewater from single dwellings or from several hundred people, depending on the size of the tank

The first unit process is the septic tank, which can consist of a one, two or three compartment chamber. The second unit process is the soakaway (also called the percolation area, drainfield, disposal field, leachfield or tile drainage field).

Figure 5.1 shows a typical septic tank and Figure 5.2 shows schematic diagram of a septic tank and its associated soakaway. The soakaway can be an excavated pit or, preferably, a specially constructed trench system that contains perforated pipes that discharge the sewage into the ground.

A soakaway pit can be used when the ground is porous (such as gravel, sand or chalk). A soakaway pit consists of an excavation filled with large pieces of inert material or an unfilled excavation that is lined with dry-laid brickwork or pre-cast concrete rings.

A soakaway trench system typically consists of series of narrow, relatively shallow trenches filled with a porous medium, usually gravel, which contains perforated pipes. The sewage passes through the perforations, into the trenches and percolates into the ground. The porous medium is used to maintain the structure of the trenches, provide partial treatment of the effluent, distribute the effluent to the infiltrative soil surfaces and to provide temporary storage during peak flows. A schematic diagram of a soakaway trench is given in Figure 5.3. Figure 5.3 also shows a typical 'herringbone' arrangement of the drainage pipes.

The soakaway can also be replaced or followed by a number of alternatives, such as constructed wetlands, pond systems and biological treatment plants.

5.3.1 Purposes of the septic tank

Septic tanks can be rectangular in shape but are more commonly cylindrical or 'onion-shaped'. Both cylindrical and 'onion-type' septic tanks can contain up to three compartments which provide separate areas for treatment of the sewage. Schematic diagrams of cylindrical and 'onion-shaped' tanks are shown in Figure 5.4.

The septic tank has three purposes. First it separates suspended solid particles from the liquid phase of the sewage into three different phases: a sludge layer that forms at the base of the tank, a scum layer floating on the surface of the liquid and a separate liquid layer. This separation is important as any subsequent discharge of solids has the potential to block the soil pores and consequently the soakaway itself. Secondly the tank stores the sludge and the scum and the tank must be big enough to avoid affecting its performance as a settling tank. Solids separated from the sewage are not removed for disposal immediately and must be stored for extended periods. Finally the tank acts as an anaerobic digester to partially break down the organic matter in the sewage. However septic tanks are not very efficient at digestion and the process is incomplete. The organic load and faecal bacteria populations are reduced only to a limited extent.

5.3.2 Purpose of the soakaway

Partially treated sewage then leaves the septic tanks by gravity, siphon or intermittent pumping and enters the soakaway. The partially treated sewage then passes on to the unsaturated or vadose zone of the ground. Here aerobic bacteria break down the organic constituents to a large extent and the pathogenic organisms may be removed. Evaporation and transpiration will remove some contaminants. A biological 'mat' will develop beneath the distribution pipes and this mat affects the passage of sewage into the soil and has some effect

on the contaminants in the sewage. Removal mechanisms and the biological mat are discussed in more detail in the next section.

One of the main design parameters when sizing the soakaway is the percolation value Vp. Vp is obtained from the percolation test, which is described in Section 5.7.3.4.

5.4 Chemical and microbiological removal mechanisms in the septic system

There are three main zones in septic systems that affect the organic nature and the pathogens in the sewage: the anaerobic zone, the aerobic zone and the second anaerobic (denitrification) zone. This section describes the effect of these zones on the chemical and microbiological constituents of the sewage. Although this report is primarily concerned with the microbiological contamination by septic tanks, a discussion of the removal of both the chemical and microbiological constituents of sewage is necessary because their removal is interdependent.

5.4.1 Anaerobic Zone

The anaerobic zone of a septic system consists of the septic tank and a layer of accumulated organic matter directly beneath the distribution pipes, which is called the **biological mat**. The anaerobic zone provides both physical and biochemical treatment of the wastewater and are described in detail below.

Physical treatment

Physical treatment in the septic tank involves the removal of the larger solid particles from the wastewater: the denser-than-water particles settle to the bottom of the septic tank and form sludge, while the floating particles remain at the top of the tank and form scum. Sludge and scum accumulate at rates of about 40 l/person/yr and must be periodically pumped from the tanks for disposal to landfill or sewage treatment plants. The septic tank also acts as a balancing tank to even out any variations in inlet sewage quality.

Chemical treatment

The biochemical treatment of the sewage in this zone is limited by the supply of oxygen. Although the septic tank is vented, the diffusion coefficient of oxygen into water is low and diffusion is further prevented by the presence of the scum layer. In the absence of oxygen, microorganisms obtain energy from the fermentation and anaerobic oxidation of organic matter. The large organic molecules in the wastewater undergo a series of reactions that break them down into simpler molecules such as amino acids, sugar, fatty acids, acetates, hydrogen and carbon dioxide. In the final steps of anaerobic digestion, methanogenic bacteria use acetate or carbon dioxide and hydrogen to produce methane and carbon dioxide. Gas bubbles collected from septic tanks are found to consist mostly of methane, carbon dioxide and nitrogen. The BOD of the wastewater leaving the septic tank is reduced and the degree of treatment within the tank is affected by the residence time in the tank. Removal of BOD by both settling and digestion will increase with increased settling time.

Removal of pathogens

The pathogenic organisms in the sewage are removed to some extent by their passage through the septic tank. In a study (Laak et al, 1974) to count the faecal bacterial populations in effluents from five septic tanks, it was found that the mean population densities were 3.4 x

10^6 per 100 ml for total coliforms, 4.2 x 10^5 per 100 ml for faecal coliforms, 3.8 x 10^3 per 100 ml for faecal streptococci and 1 x 10^4 per 100 ml for *Pseudomonas aeruginosa*. The potential for contamination of groundwater is clear and the passage of the wastewater through the unsaturated zone is necessary to complete the treatment process.

Effect of the biological mat

If the soil beneath the gravel that surrounds the distribution pipes is finer than the gravel itself then a biological mat, 2 to 5 cm deep, forms over time. As the wastewater enters the finer soil, some sediment, suspended particles and organic matter are strained out. Anaerobic digestion of the wastewater continues to a lesser degree in the biological mat. The anaerobic conditions in the mat allow the formation of ferrous sulphide, which gives the mat its characteristically black appearance. The biological mat decreases the seepage rate of sewage into the drain field, especially if the drainage field is sited in coarser grained soils. As material accumulates, the hydraulic conductivity of the layer decreases which often causes the ponding of effluent above the biological mat. In many situations, the hydraulic conductivity of the biological mat, not of the soil, becomes the controlling variable in the infiltration of the wastewater.

Bacteria are retained in the septic tank systems primarily by straining and the biological mat appears to be the most important element of the septic tank system for this. In general the mobility of both bacteria and viruses are much greater in saturated flow than unsaturated flow, making unsaturated conditions below septic tanks desirable for both oxygen supply and pathogen retention.

5.4.2 Aerobic zone

The aerobic zone exists where the sewage flows down from the biological mat to the water table through the unsaturated or vadose zone. Where oxygen is available, microorganisms are capable of almost completely oxidizing the reduced wastewater constituents. The soil provides the bulk of the sewage treatment by the processes of physical filtration, chemical reaction and biological transformations.

Chemical treatment

The reactions that take place in this zone include the oxidation of organic matter to carbon dioxide and water, nitrification of ammonia to nitrate, the oxidation of sulphide to sulphate and carbonate buffering reactions. In an oxygenated environment the oxidation reactions occur directly beneath the biological mat. However the presence of a plentiful supply of oxygen does not preclude the occurrence of anaerobic reactions and in particular denitrification (the conversion of nitrate to nitrogen) can occur in anoxic microzones. If dissolved oxygen is present in the effluent when it reaches the water table, or if the groundwater itself contains dissolved oxygen, then aerobic oxidation can occur in the groundwater itself. When oxygen is limited or unavailable, the redox reactions described above occur only partially, or not at all, and the organic carbon and ammonium loads to the sediments and groundwater increase. An increased load of organic matter can cause system clogging and failure and can therefore affect the removal of pathogens.

In order to maintain aerobic conditions, a drainage field must meet the requirements for oxygen. The ability of a soil to supply oxygen is affected by many factors, including the geometry of the drain-field, the depth to ground water below the distribution pipeline and the soil texture. Researchers have observed that system clogging progresses faster in

continuously inundated soils i.e. soils that are applied with sewage all the time and this, and the above issues, are discussed in more detail in Section 9.

Removal of pathogens

Several mechanisms combine to remove bacteria from wastewater percolating through the soil. The physical process of straining and the chemical process of adsorption (bonding and chemical interaction) appear to be the most significant. Additional mechanisms include the competition for nutrients and the production of antibiotics by high populations of actinomycetes. *Pseudomonas* and *Bacillus*, in the aerated zone beneath the clogged layer formed at the soil trench or soil bed interface in a soil absorption system.

Physical straining occurs when the bacteria are larger than the pore spaces in the soil. Partial clogging of soil spaces by organic particles in the septic tank system effluent increases the efficiency of straining. Finer soil materials such as clay or silt generally function better for bacterial straining because of their small pore sizes.

Adsorption is the other mechanism in the removal of bacteria by soil. The process of adsorption appears to be significant in soils having pore-size openings several times larger than typical bacteria. Since most soils also carry a net negative charge, one might expect rejection rather than attraction of bacteria on soils. However adsorption will occur in water with high ionic strength and neutral or slightly acidic pH and these are typical characteristics of septic tank effluents. Cations such as calcium and magnesium sometimes saturate the surface of the bacteria making them susceptible to removal by negatively charged soil particles.

The mechanisms by which viruses are removed from the unsaturated zone are different from those responsible for bacterial removal. Viruses are variably charged particles and are more affected by adsorption onto clay particles and thus virus retention should increase as the permeability of the soils decrease.

5.4.3 Second anaerobic (denitrification) zone

The aerobic zone can convert ammonia in sewage to nitrate and can therefore create a potential health hazard and the possibility of eutrophication. The second anaerobic (denitrification) zone can convert nitrate to nitrogen and the bacteria that are capable of doing this are present in many subsurface environments. These bacteria require anoxic conditions to reduce nitrate and such conditions are best maintained in saturated or near-saturated sediments due to the limited oxygen diffusion in such environments. In ground water, the most common factor that limits denitrification is the lack of mobile carbon, which is necessary for the denitrification bacteria to replicate and grow.

5.4.4 Importance of the design of the soakaway

The correct sizing and design of the soakaway pit or trench system is vital for the prevention of contamination. Section 5.7 gives more details on the soakaway design factors that can reduce the potential for microbiological contamination of groundwater.

5.5 Package Treatment Plants (PTPs)

The term Package Treatment Plant or PTP refers to a range of commercially available, prefabricated units. They use a number of processes which are different in detail, but all include some form of settlement of solids and enhanced biological decomposition of the

sewage by aeration. PTPs can treat waste directly or they may be connected to a septic tank. They treat the sewage to a higher standard than septic tank systems and this normally allows direct discharge to a watercourse.

Modern PTPs are compact and those based on the activated sludge process produce less sludge than other methods. Primary settlement can be eliminated and secondary sludge can be stored for long periods under aerobic conditions with very little nuisance from odour. PTPs are, however, more expensive than septic tanks and maintenance costs and operating costs are higher. Power is needed to operate the plants.

Table 5.1, which is based on information from the CIRIA Technical Report 146, has been extended to give the range of PTPs available from the information received from the manufacturers. They are discussed below.

5.5.1 Activated sludge units

PTPs based on the activated sludge process provide aeration of crude unsettled sewage with activated sludge. Activated sludge is a flocculent microbial mass produced when sewage is continuously aerated. There are two types of activated sludge units: **contact stabilization** plants and **extended aeration**.

The contact stabilization process involves treatment in four distinct compartments. In the first compartment, sewage, which is usually screened or macerated, is aerated in contact with activated sludge for a period of between 0.5 to 2 hours. The mixed liquor then passes on to the settlement compartment. After settlement, the supernatant liquor (the treated effluent) is discharged and the sludge is transferred to a third re-aeration compartment where it is aerated for a period of several hours. During this time oxidation of the absorbed organic material occurs. A large portion of the activated sludge is then returned to the first compartment. There may be a fourth compartment where surplus sludge is further aerated to oxidize it as completely as possible before the sludge is removed for disposal.

The extended aeration process involves treatment in two compartments: an aeration or mixed liquor compartment and a settlement compartment. Sewage, which will normally be screened or macerated, flow to the aeration compartment where it is mixed with activated sludge and aerated. The sludge is then separated from the mixed liquor in the second compartment, which is usually integral with the first compartment but separated from it by a partition. The sludge is recycled to the aeration compartment either by gravity pump or by air-lift. The supernatant liquor, treated effluent, leaves the plant over a weir.

The right combination of sewage, activated sludge, air and contact time is required for satisfactory operation of these activated sludge units. Activated sludge units are far more complicated to operate and maintain than septic tanks and require regular inspection, maintenance and control.

5.5.2 Trickling or Percolating Filters

Trickling or percolating filters are commonly used as the basis for PTPs. These plants operate by circulating raw or comminuted sewage through a ventilated bed containing mineral or plastic media. The media supports microorganisms for the aerobic digestion of organic matter. This type of PTP is similar to the filters of full-scale sewage treatment works. The filter requires ample ventilation and an efficient system of under-drains leading to an outlet. Advantages of trickling filters include the absence of moving parts and the quantity of

sludge produced is lower than for septic tanks. Disadvantages include their vulnerability to changes in flow and toxic shocks (e.g. a shock load of domestic chemicals).

5.5.3 Rotating biological contactors (RBCs)

RBCs are increasingly specified instead of septic tanks for treatment of sewage from single households. Sewage in a RBC moves horizontally through a tank in which large diameter media filled disks rotate on a horizontal shaft. Approximately 40 % of the surface area of each disk is in contact with the sewage at any one time. As the disks rotate, sewage is absorbed into the biofilm on the surface and then passed through the air and aerobic digestion takes place.

The advantages of RBCs are that full aerobic treatment can be provided with a headloss equivalent to a septic tank. The power requirements are low and recirculation of the sewage is not necessary. Maintenance is quite straightforward compared with an activated sludge unit and desludging is required typically three to four times a year. RBCs are resistant to shock loads but will not work if they are allowed to stand idle for extended periods. Disadvantages of RCBs include the need for a rotaing crank shaft which can snap or seize and their need for protection against the environment and to prevent odour migration. A diagram of an RBC is given in Figure 5.5.

5.5.4 Submerged Aerated Filters (SAFs)

An SAF system works by treating the sewage is treated in three stages: primary settlement, biological filtration and humus settlement. Raw sewage enters the first settlement chamber where much of the solid matter settles to the bottom to form sludge. Partially settled sewage passes into the second chamber where further settlement takes place and the settled swage then passes onto the biological filter. The biological filter contains high voidage plastic media where the oxidizing bacteria are attached. A flow of air is introduced below the plastic media via a series of bubble diffuser to provide the oxygen supply to enable the biomass to grow. The treated effluent then passes into the humus settlement chamber where final settlement takes place. The humus sludge is transferred to the primary chamber for co-settlement with incoming sewage.

5.5.5 The need for primary settlement tanks

PTPs are often designed to treat settled, and not raw, sewage. The proper operation, maintenance and sizing of primary settlement tanks has been identified by several manufacturers as being crucial to the successful running and performance of the PTPs. Some of the PTPs, especially those for small populations, have integral primary settlement tanks. The larger PTPs need primary settlement tanks upstream of the unit. BS 6297 states that a primary settlement tank is needed for populations greater than 100. The efficiency of a primary settlement tank is dependent on the velocity of the flow through the tank, which is dependent on the tank dimensions.

5.5.6 The need for secondary settlement tanks

Secondary settlement tanks, usually known as humus tanks when used in conjunction with biological filters, are essential components of secondary sewage treatment when a 30:20 quality effluent is required (see section 5.4.8). They are installed either as separate units or as integral parts of PTPs. It may be advantageous to arrange for re-circulation of some of the final effluent through the biological filters. Secondary sludge may also be transferred to the

primary compartments of septic tanks for storage and final disposal with the primary sludge. In the case of activated sludge units, sludge requires to be continuously withdrawn from the settlement tank for return to the aeration tank. Recirculation can buffer shock loads which inevitably occur early in the morning and can allow the PTP to operate successfully if the users of the systems go away for a few days.

5.5.7 Discharge from PTPs

Unlike septic tanks, which must discharge to ground, PTPs can discharge to surface water or to ground. If PTPs discharge to ground then there is a formula that is used to calculate the total area of the soakaway from the value found in the percolation test. This formula is taken from BS 6297 and allows the soakaway to be 20 % smaller than a soakaway receiving septic tank effluent from an equivalent population. The formula is presented in Section 7.

5.5.8 Assessment of the performance of PTPs

The performance of PTPs is, even now, assessed using the parameters used in the Royal Commission standard set earlier this century as a target for sewage treatment works. The standard is typically presented as 30:20:20, which means the effluent from the plant contains a maximum of 30 mg/l of suspended solids, 20 mg/l of BOD and 20 mg/l of ammonia. The microbiological assessment of these units is very limited and it is rare for the EA to request a microbiological standard as a consent. The manufacturers that were contacted were not aware of the microbiological removal achieved by their plant.

Literature suggests that a reduction of 1 log or 90% removal of *E. coli* across the plants is a typical figure but this is a broad approximation.

5.6 Regulations and Guides Covering the Design, Installation and Operation of Septic Tanks and PTPs

Several sets of regulation and codes of practice already control the building of septic tanks and PTPs. These are presented in the CIRIA leaflet 'Septic Tank Systems'. A Regulator's Guide'

5.7 Identification of Design and Operational Factors Leading to Groundwater Contamination By Septic Tanks and PTPs

The literature, and in particular the CIRIA Technical Report 146, was reviewed with respect to the problems associated with septic tanks and PTPs that may cause groundwater contamination. This section discusses the factors that lead to problems with septic tanks and PTPs and forms a basis for the Best Practice Guidelines that are presented in Section 7.

The main problems with septic tanks are discussed in the following sections.

5.7.1 Blocked soakaway

This is a very common problem which occurs either because the ground conditions are unsuitable, or because of solids carryover from the septic tank. Blockages can occur quickly (where the ground conditions are unsuitable) or after a long time (when the septic tank fills up). Blockages most often occur in winter when the ground is saturated. Blockages can also be caused by the poor design of the outlet of the septic tanks, which allows the easy carry-over of solids. Solids can be carried over from PTPs because of the poor performance of the

secondary settlement tanks, the poor performance of the PTP or the overloading of the PTP because of the under-designing or poor operation of the primary tank.

One study from the literature (Anis Al-layla and Al-Rawi, 1989) showed that there were faulty inlet/outlet arrangements occurred in many septic tanks i.e. inlet and outlet pipes were 'directed' at each other and short-circuiting could easily take place. This can lead to raw sewage passing straight through the tank and consequent contamination of groundwater.

5.7.2 Inadequate drainage field

The most common reasons for the drain field being inadequate is that it has not been designed or constructed properly. Sometimes the soakaway is constructed without carrying out a percolation test (see Section 5.7.3.4) or using the results of a percolation test which was not carried out properly and which has given misleading results. Even with good supervision it is a crude test because of the variation in local and seasonal conditions. It is commonly found that the length of drain identified in the test is too long for the area of ground - but this rarely leads to an alternative means of sewage disposal being sought. Another circumstance where the drainage field can be inadequate is where a dwelling has been extended and the water use has increased without any modification to the existing drains.

5.7.3 Sizing, operation and maintenance of septic tanks and PTPs

Several manufacturers highlighted the correct sizing of the septic tank or PTP as being vital for the correct operation of the sewage treatment process.

CIRIA Technical Report 146 (Payne and Butler, 1993) states that many reported problems are caused by failure to desludge the tanks properly and the main reason for this is thought to be ignorance or negligence on part of the owner. Owners may seek to avoid the cost of maintenance until a noticeable problem develops. Several local authorities have overcome this problem by providing a free desludging service.

Tanks that are jointly owned frequently receive inadequate maintenance because the reponsibilities are not clear. When houses change hands the new owners may not be told about the septic tanks. This may lead to a change in pattern of water use and the tank becomes too small for the population it serves.

The manufacturers frequently reported that PTPs were not operated, maintained or desludged properly. This caused problems with the operation of the units and led to concerns over public health and nuisance. It was reported that septic tanks were desludged much less frequently than once a year and desludging was only initiated when there was an apparent problem with the units. Septic tanks were frequently neglected altogether.

Septic tank and PTP manufacturers state that the use of detergents and disinfectants need not be limited but care should be taken to use them in accordance with the manufacturers' instructions.

The effect of discharge of water softening recharge effluent to a septic tank is complex: the presence of large concentrations of sodium ions can have a deleterious effect on the soil structure in a soakaway. However there are reports that the presence of calcium and magnesium in the recharge effluents can have a beneficial effect on the soil.

If greases and oils are allowed to enter the septic tank, there is also the possibility that they can be discharged along with the septic tank effluent to the soil absorption system. Greases and oils, along with suspended solids, tend to accumulate on the surface of the soil absorption

system ultimately leading to a reduction in the infiltration capacity. Greases and oils are particularly troublesome because of their persistence.

Surface water should be separated from sewage as it would dilute the sewage, overload the system and lead to scouring of biomass.

5.7.4 Proliferation of tanks

In some areas there is a proliferation of tanks discharging to land, which quickly leads to pollution of ground and surface water. In another publication (CIRIA, 1998), CIRIA states that 'many systems in one area may fail where an individual system would succeed.

5.7.5 High water table

Septic tanks should not be installed in areas with high water tables - but they often are. It is recommended that the distance between the drainage pipes and the water table should be no more than 1 m. There is no requirement that the distance to the water table should be measured, unlike the requirement for a percolation test. However, in the recent CIRIA leaflet (CIRIA 1998) it is suggested that a trial hole test (to measure the distance to the water table) and a thorough visual inspection should be carried out. Research is needed to identify the distance between the soakaway system and the highest level reached by the water table that avoids the pollution of the groundwater.

5.7.6 Package treatment plants

Fewer problems with PTPs were reported in CIRIA Technical Report (146 Payne and Butler, 1993), presumably because solids removal is better and there is less risk of blocking the soakaway. The main problems identified by the manufacturer were related to undersizing of the plant.

5.7.7 Ease of operation

Septic tanks have the advantage that they have no moving parts and are easy to use and maintain once properly installed. PTPs require careful operation and control; they are more complicated, frequently have moving parts, need specialist repairs and are more prone to breakdown. Nethertheless PTPs are capable of treating sewage to a higher degree and will be appropriate for situations where septic tanks would not provide sufficient treatment or where discharge to ground is not possible. However it is true to say that the treatment system of choice should be the simplest system available: the system that has the fewest moving parts and controls to go wrong.

5.7.8 Summary of findings

It is clear that the main aspects of a septic tank system which can lead to contamination of groundwater is the sizing and design of the soakaway and the lack of maintenance and operational care when using the tanks. Best Practice Guidelines for the prevention of groundwater contamination are presented in Section 7.

5.8 Best Practice Guidelines for Septic Tanks and PTPs

5.8.1 British Standards

The BS 6297:1983 on 'Design and installation of small sewage treatment works and cesspools' has been used as a basis for the development of these should be followed wherever possible.

The BS 6297 deals with the design and installation of sewage treatment works suitable for the domestic discharge from domestic and industrial communities ranging from single households up to about 1000 population equivalent.

5.8.2 Collection of information

The following information should be collected before designing and installing septic tanks and small sewage works in order to be able to design the works properly:

a) the requirements of the local building control and planning authority

b) the requirements of the Environment Agency

c) the minimum and maximum number of persons (resident and non-resident) to be served

d) average 24 hour water consumption, and any special conditions affecting the composition of sewage and peak rates of flow

e) existence of infiltration water

f) particulars of the site including: distance from nearest habitable building, prevailing winds, ground and other levels, information as to the nature of the ground including the level and variations in the water table and access for vehicles and plant

g) particulars of outfall, e.g. tidal or inland waters, rivers, streams, ditches or soakage, also the proximity, highest known flood level and minimum flow of any stream or other watercourse to which discharge of the effluent is possible

h) conditions under which the works will normally operate and be maintained

i) possibility of the need for future extensions of the works or of their elimination by a comprehensive scheme

j) availability of electric power and mains water

k) facilities for eventual disposal of sludge and screenings

A multiplicity of small sewage treatment works in a limited area, especially for single houses, is undesirable. Greater efficiency of operation as well as economy of construction can be achieved by collective drainage and treatment arrangements.

The designer should make adequate provision, where appropriate, for unusual pollution loads such as the use of waste disposal units.

5.8.3 Materials used in the construction of septic tanks and PTPs

All materials used in the construction of septic tanks and small sewage treatment works should comply with the relevant British Standards. Where no British Standard exists, materials should be suitable and adequate for the purpose for which they are used.

Septic tanks and other tanks that form part of a PTP should be watertight and have robust secure covers. Where possible, products should be in receipt of a third-party accreditation certificate, such as a British Board of Agreement Certificate. Pre-cast concrete tanks should be constructed to the appropriate British Standard.

5.8.4 Siting of Septic Tanks and PTPs

PTPs should be as far from habitable dwellings as possible and that the direction of the prevailing wind should be taken into account when siting the works. A small treatment works serving more than one premise and incorporating conventional biological treatment, should be a minimum of 25 m away from any dwelling and this should be progressively increased for larger works. Septic tanks and PTPs should be sited 15 m away from habitable any dwelling.

Good road access should be provided to enable the tank-emptying vehicle to operate within its suction-lift capability. The sludge contractors vehicle will probably have a reach of about 40 m but a maximum distance of 30 m between the tank and suitable access for the desludging vehicle is recommended. A distance of 10 m is preferable.

PTPs should not be located in an area subject to flooding or where the water table can rise to such levels as to cause flow into the treatment units. The same applies to septic tanks but with septic tanks a more detailed consideration of the siting of the soakaway should be undertaken.

Wherever possible, pumping should be avoided by locating the plant lower than the premises to be served. If pumping is inevitable then it is preferable to pump settled sewage rather than crude sewage.

It is recommended that septic tanks should be at least 10 m away from the nearest watercourse and 1 m above the local water table. The drainage field should be at least 50 m from a well, borehole or spring.

5.8.5 Control over the use of domestic chemicals

The intrusion of surface water into the drainage system should be prevented as this causes dilution of the sewage and large loads on the septic tank or PTP.

Manufacturers' guidelines should be followed on what is possible to discharge into tanks. Table 5.2 gives one manufacturer's list of recommendations for what is acceptable to discharge into their PTPs.

Domestic use of detergents and disinfectants is not detrimental but excessive use may have a harmful effect on the performance of the works. The use of detergents and disinfectants need not be limited but care should be taken to use them in accordance with the manufacturers' instructions.

5.8.6 Grease and oil interceptor tanks

Excessive quantities of grease and oil may cause malfunction of a small sewage treatment works. In such cases arrangements should be made, where practicable, for grease and oil to be removed at source or for them to be excluded from the sewerage system. The use of conventional septic tanks as interceptor tanks (as an additional stage) has proven to be very effective.

5.8.8 Inlet flow

Measurement of flows to small works is difficult but should be carried out wherever possible. To minimize blockages it is more satisfactory to measure flow of the final effluent. Where continuous measurement is not installed, a facility such as a V-notch weir should be provided to permit the use of portable measuring equipment when required.

5.8.9 Installation

Workmanship should be of good standard and methods of working should be in accordance with BS codes of practice and other appropriate codes.

Manufacturers' instructions should be followed precisely and special care should be taken when installing the septic tanks or PTPs in wet conditions. However if the water table is too high (see Section 5.7.2) then the systems should not be installed on that site.

Wherever possible inlet and outlet pipes should be built into walls as work proceeds. Where the wall through which the pipe passes form part of a liquid retaining structure, special care should be taken to ensure that there is no leakage through the wall along the line of the pipe.

5.8.10 Capacity of septic tanks

The calculation for the total capacity of septic tanks for the population covered by this code should be made on the basis of the number of persons to be served. The following formula is recommended for general use, where desludging is carried out at not more than 12-monthly intervals:

$$C = 180P + 2000$$

where: C is the capacity of the tank in litres with a minimum capacity of 2720 litres and P is the design population with a minimum value of 4.

This formula allows for proportionately larger retention at the lower populations in order to cover the surges in flow, which are experienced in small systems.

The capacity of the primary chamber should be at least two-thirds of the total capacity of the tank.

Where waste disposal units are used, additional sludge solids are discharged with the sewage and the capacity of septic tanks should be increased by 70 litres for each person served.

The capacity obtained from the above calculation is a minimum value which can be increased to take account of high consumption fittings, projected water usage and infiltration rates.

5.8.11 Inlet and outlets of septic tanks

The design of septic tank inlets and outlets should be such as to introduce the crude sewage and to remove the clarified liquid with the least possible disturbance of the settled sludge or the surface scum.

Inlet and outlet pipes should not be directly in line with each other.

In general, the inlet and outlet pipes from an onion-type septic tank should be designed to minimize turbulence on inlet and minimize solids carry-over from the outlet.

5.8.12 Gradient from dwelling

The incoming drain or sewer should be precisely in line with the centre of the septic tanks for a distance of at least 6 m to minimize the impact of the flow of sewage into the tank. Where the incoming gradient has a steep gradient, at least the last 12 m should be laid at a gradient not steeper than 1:50 in order to minimize turbulence.

5.8.13 Design of the soakaway for septic tanks and PTPs - percolation tests

The soakaway trench system should be very carefully designed. The porous or perforated pipes laid in the trenches should have a uniform gradient, which should not be steeper than 1:200.

The pipes should be laid out in a loop or 'herringbone' or other suitable pattern (See Figure 5.3). The pipes should be laid on a 150 mm layer of clinker, clean gravel or broken stone 20 to 50 mm grade and the trenches filled to a level 50mm above the pipe and covered with strips of plastic materials laid to prevent the entry of silt. Polythene sheet or membrane can be used as a cover for the pipes. The remainder of the trench can be filled with normal soil.

Pipes should be laid at a minimum depth of 500 mm below the surface. If the level of the water table rises in the winter to within 1 m of the proposed irrigation system it is not normally advisable to use subsurface irrigation.

The flow area of the sub-surface drainage trench required to disperse effluents from septic tanks may be calculated from:

$$A = P \times V_p \times 0.25$$

Where A is the trench area, P is the number of persons served by the tank and V_p is the percolation value obtained from the percolation test.

For effluents that have received secondary treatment followed by secondary settlement, this area can be reduced by 20 % i.e.

$$A = P \times V_p \times 0.20$$

The area determined should be used as a basis for the total floor area of the pit or the floor area of the soakaway and therefore the total length of drain.

The percolation test is described in detail in BS 6297. In essence the test measures the time taken for an excavated hole to empty of water. The percolation test finds a value V in seconds that can be used to determine the area of soakaway as shown above. The BS states that if the percolation value V_p is greater than 140 s then the soil is not suitable to be used as a soakaway. If V_p is between 140 to 100 seconds then underdrains are necessary and details of these are given in BS 6297.

The percolation test should not be carried out when there are abnormal weather conditions such as heavy rain, severe frost or drought. It also suggests carrying out the test three times in three different locations on the route of a land drain, or at least three tests on separate days on the site proposed for a soakaway pit. CIRIA suggest carrying out at least one percolation test during adverse conditions e.g. during or after a period of heavy rainfall.

The suitability of land for use as a drainage field should not be based entirely on a percolation test. CIRIA suggest trial hole tests should be carried out along with a thorough visual

inspection of the site. Details of these tests can be found in their leaflet: 'Septic tank systems. A regulator's guide' (CIRIA, 1998).

5.8.14 Operation and maintenance of septic tanks and PTPs

Owners of septic tanks and PTPs need authoritative guidance on how their tanks work and the maintenance requirements.

Septic tanks and PTPs should be desludged at the frequency recommended by the manufacturer and by an authorized desludging contractor. The plant should be serviced at the frequency recommended by the manufacturer by an authorized engineer.

The design of the septic tank or PTP tank should allow access to the tank in order that cleaning can be carried out efficiently.

5.8.15 Preliminary treatment for PTPs

Rags and floating debris will inevitably form part of the flow reaching the works and in place to prevent blocking and fouling.

5.8.16 Primary and secondary settlement tanks for PTPs

Settlement tanks may be of the horizontal or upward flow type. Upward flow tanks have several advantages in terms of desludging and ease of maintenance.

Design capacities for upward flow and horizontal flow tanks are given in BS 6297 and should be adhered to.

The design principles for secondary settlement are similar to those for primary tanks but where re-circulation of final effluent is adopted as part of the biological filtration process it will be necessary to enlist specialist advice to increase the surface area and capacity of secondary settlement tanks. Similarly as with primary tanks, two designs are possible: upward flow and horizontal flow. Guidance for the design of secondary tanks is given in BS 6297.

Primary settlement tanks should be operated and maintained properly

5.8.17 Design aspects of PTPs

Activated sludge units

BS 6297 gives design equations for capacities of tanks, residence times in the tanks and air supplies for activated sludge plants. The BS states that specialist advice should be obtained when using activated sludge units. The BS gives general advice on the sizing of the settlement tanks for activated sludge.

Trickling or percolating filters

The most suitable method of distribution for use in small installations is a series of fixed channels or a rotating-arm distributor.

The design equation for the volume of mineral media necessary for treatment of the sewage is given in BS 6297 and is:

$$V = 1.5\ P^{0.83}$$

where: V is the volume of media in m^3 and P is the design population

When waste disposal units are installed the volume of medium obtained from the formula should be increased by 30 %. Introducing recirculation may reduce the volume of media but specialist advice is necessary for this. This also reduces the risk of the media drying out during periods of low flow.

The mineral filter media should comply with BS 1438 (described in BS 6297).

The depth of mineral media should be 1800 mm with an absolute minimum of 1200 mm.

Plastic media is often used in PTPs. Plastic media is available in a range of shapes and sizes and there is no simple formula for the calculation of media necessary. When plastic media is used, it is essential to follow the manufacturers' recommendations with care.

Rotating Biological Contactors or RBCs

RBCs are frequently used in PTPs and incorporate primary and secondary settlement in the units. The recommendations of the manufacturer concerning selection of plant, maintenance and operation should be followed with care.

Wherever possible installations using RBCs should be supplied by gravity and means provided to minimize surges in flow, especially with PTPs. Where crude sewage is being pumped it is important that the average frequency of pumping should not be less than four times per hour throughout most of the day.

The design should facilitate the transfer of excess film, shed from the rotating surfaces, from the treatment zone to a secondary settlement unit, either by positive mechanical means or by ensuring that sufficient turbulence is induced to carry it forward in the effluent stream. Some systems incorporate novel or patented features in order to do this and it is important to justify claims made by the manufacturer.

The rotational speed should not exceed 0.35 m/s to avoid stripping of the biomass. Biological film builds up more thickly on the surfaces nearest the inlet to the treatment zone and the spacing between adjacent surfaces of discs in this region should be designed to prevent the bridging of the gaps between surfaces.

Proper provision for overload-protection of the motor should be made to allow for the uneven distribution of biomass. Uneven biomass distribution may arise from the disc drying out due to power failure. Automatic restart for the motor should be provided for after a power failure.

Structures supporting the rotor bearings and drive should have adequate long-term rigidity to maintain alignment. Bearing, drive chains and sprocket should be protected from moisture and provided with easy access for lubrication and adjustment.

Secondary tanks for use with RBCs should conform to the guidelines described in Section 7.4.2.

The loading of the RBCs should not exceed 5 g BOD per m^2 of settled sewage or 7.5 g BOD per m^2 of crude sewage in order to achieve the 30:20 standard.

5.8.17 Operation and maintenance of PTPs

The operation and maintenance of PTPs is described above. In general PTPs should be operated and maintained according to the manufacturers'instructions and these instructions should be strictly adhered to.

5.8.18 Sludge treatment for PTPs

Sludge is continuously produced by settlement tanks and needs to be removed at frequent intervals. Drying beds are an option but can give rise to odour and insect nuisance and pose problems regarding clearance of the dried sludge. It is recommended that sludge is removed of by tanker and disposed of properly.

5.9 Recent and Future Research Into Septic Tank Design

Most of the literature found on septic tanks was from the U.S A. Comparatively few studies have been published in the U.K compared with the U.S.A where research has been carried out to investigate ways to minimize the pollution from septic tanks. In the U.S.A. approximately one quarter of households use septic tanks, which is equivalent to approximately one trillion gallons of sewage per year disposed to the ground. The potential for groundwater contamination has been clearly demonstrated where the situation is on a much larger scale than in the U.K. However in the U.S.A. the sewage is more dilute because water consumption is greater in the U.S.A. and the total volume of sewage produced per person is greater. This factor complicates a direct translation of the results of these investigations to the situation in the U.K but a study of the literature provides a useful background and basis for suggestions for future research.

5.9.1 Design of the soakaway

The most important aspect of septic tank and PTP systems in the prevention of groundwater contamination is the design and operation of the soakaway. The ability of the sub-soil to receive and treat the wastewater is crucial to the success of the system i.e. to its ability to prevent groundwater contamination.

The NRA Severn-Trent and Southern regions developed a scoring system, which assesses the soil conditions in the site proposed for a soakaway. It scores the ground according the classification system presented in Table 5.3. If the final score is greater than 30 then the proposed scheme is judged not to be a potential source of groundwater contamination and can be allowed. This system was discussed with a member of staff in the Environment Agency who used the scoring system. It was clear that this numberic scoring is not strictly adhered to but is used as a useful initial screening tool. If the total score is just under 30 then there is a possibility that the installation can still be allowed and each individual situation is judged on it merits.

CIRIA (1998) recommends that the suitability of land for use as a soakaway should not be based entirely on a percolation test but that a wider assessment is necessary, including trial hole tests, which include the estimation of the depth to the water table, and a thorough visual inspection of the ground and the site.

Geary (1993) stated that the performance of on-site systems is related not only to the level of treatment afforded by the septic tank, but also to the acceptance capability of the disposal area that is receiving the effluent. Land capability criteria for on-site systems should include consideration of the permeability of the soil, the size of the allotment, the available land for effluent disposal, the slope of the site, its flooding frequency, the depth to the water table and the depth to bedrock. The suitability of a site for on-site disposal should be rated according to the poorest site rating for an individual criterion. Geary also concluded that the choice of suitable land for disposal and treatment of septic tank effluent is more complicated than a simple percolation test, supporting the use of additional tests such as the trial hole test and

visual inspection recommended by CIRIA. The trench design of soakaways allows more diffusion than a bed design because of increased sidewall diffusion of oxygen into the unsaturated zone. Wilhelm et al (1994) also suggest that wastewater strength should be taken into account when designing soakaways.

5.9.2 The influence of the biological mat

The influence of the biological mat is very important. Wilhelm et al (1994), in an extensive paper which describes in detail the passage of sewage through a septic tank system, stated that the current U.S. regulations on septic tank design (this equally applies to the U.K.) imply that the main design parameter is the percolation rate of the soil, a parameter whose actual physical meaning is difficult to define. Based upon this rate, wastewater loadings are prescribed which should provide unsaturated flow. However this approach fails to appreciate that the infiltrative capacity of the biological mat, not the soils properties, often defines the permeability and the systems ability to transmit wastewater. If this is confirmed then the percolation test value may be irrelevant and a better indicator of the draining ability of the system should be found.

5.9.3 Effect of the soakaway

Viraghavan and Warnock (1976) showed that even with a soil absorption system operating well, groundwater contamination is possible because the soil is not equally effective at removing all constituents of the percolate. In this study groundwater adjacent to the test site was monitored for fifteen months. The unsaturated soil had the ability to remove a high percentage of coliforms and faecal streptococci present in the effluent but groundwater quality was significantly affected by the duration of the loadings of the soakaway and by seasonal variations. The most important environmental factor was depth to groundwater table as this limits the unsaturated zone depth available or pollutant absorption for adsorption before reaching the water table. An indication of the minimum depth to water table is an important parameter in determining whether a site is suitable for the disposal of septic tank effluent or PTP effluent and this has been recommended by CIRIA (1998). It is worth noting that the Groundwater Protection Policy (GPP) explicitly recognises the role of the unsaturated zone in attenuating microbial polutants but does not include depth to water as a criterion in classifying and mapping aquifer vulnerability.

Some research has been carried out into the depth of ground needed to remove pathogens from an effluent. In a very detailed study Hagedorn et al (1981) found that approximately 30-90 cm of sub-soil material beneath the base of the soakaway trench was adequate for complete bacterial removal of septic effluents, provided that the sub-soil material has both a layer permeable to effluent flow and another region adequately restrictive to form a clogged zone. However it was also stated that the soil depths required for removal of enteric bacteria from septic tank effluents had been derived from a somewhat limited body of information which is not consistent. The conclusions must be that the removal of bacteria is site specific. The minimum water table depth of 1 m (recommended for the U.K.) may need adjusting according to the type of soil in which the soakaway is sited and may not simply depend on the hydraulic conductivity of the soil.

Evidence to support the above data was found from investigations of the purification efficiency of 19 subsurface systems (Bouma et al, 1976). This paper concluded that septic systems that exhibited proper hydraulic functioning also served to purify the wastewater. Bacterial filtration was determined by dissecting a soakaway and counting the indicator organisms present in the sub-soil at various distances below the soakaway trench. The large

populations of faecal coliforms, total coliforms and enterococci present in the wastewater were reduced to control levels at a distance of 61 cm below the soakaway. The most abrupt population decline occurred in the biological mat located at the interface of the soakaway trench and the soil. The biological mat was highly efficient in trapping and holding bacterial species present in the wastewater and served as the primary barrier to the subsurface escape of the bacteria. However, if the mat was too thick or dense and restricted the hydraulic functioning of the system, then the effluent could not enter the soil and became ponded in the trench and subsequently spilled out onto the soil surface. It has been reported (Laak et al, 1974) that in a properly functioning absorption field, the faecal indicator and potentially pathogenic bacteria were almost completely removed after a relatively short distance through unsaturated soil. Thus there is a soil transition zone: from the high bacterial populations in the biological mat, which equal or exceed the concentrations in the septic tank effluent, to the reduced populations in the deeper zones, to the final zone where the populations become similar to those found in the unpolluted sub-soil.

5.9.4 The hydraulic conductivity of the soakaway

The relative conductivity of the soil was found to be an important factor. Laak et al (1974) studied the relative permeabilities of septic tank systems. They concluded that if a soil has a coefficient of permeability of less than 5×10^{-5} cm/sec then the volumetric capacity of the system governs the size of the subsurface soakaway. Seasonally high water tables or impervious strata may retard the flow and reduce the quantity of wastewater that can be carried away from the subsurface disposal area. In this case an elevated bed can be designed to increase the potential hydraulic gradient. Laak et al also concluded that soakaways can be designed with higher loadings in soils having a greater coefficient of permeability than above if increased pre-treatment is used. Long-term loading rates for different soil permeabilities were plotted on a graph that could be used for sizing fields.

Hagedorn et al. proposed that septic systems submerged in stormflow saturated soils represent one potentially large source of faecal contamination but the magnitude of these incidents has only been evaluated to a limited extent. It is important to assess what happens when stormflow conditions exist and what the contamination from septic tanks is during these times.

Artificial drainage has been shown to improve the hydraulic functioning of septic tank systems located in soils underlain by slowly permeable soil horizons and subjected to seasonally high water tables. However U.S. health guidelines which specify the minimum distance between the disposal area and the intercepting tile lines or drainage ditches were established intuitively with little data support (Hagedorn et al, 1981). In one study that set out to define this distance more reliably, it was shown that although artificial drainage was effective, horizontal movement of bacteria was substantial.

5.9.5 Alternative soakaway systems

Hagedorn suggested that research should be carried out into alternative systems that can function in soils that are not suitable for septic tanks. These systems include:

a) elevated mound systems where the septic tank effluent is injected into a mound to increase its passage through the ground

b) use of intercepter tile lines around soakaway to draw off the effluent

c) pressure distribution to evenly spread the effluent over the soakaway

d) deep ploughing to break up the restrictive zone beneath the soakaway

e) using another material to fill in the bed after removing the original soil

In a study to examine the field performance of conventional and alternative septic systems in wet soils, Cogger and Carlile (1984) examined low-pressure pipe systems (LPP) and soil replacement systems. LPP systems pump effluent under low pressure through small-diameter pipes into a drainfield consisting of shallow narrow trenches. The drainfield is dosed one to three times daily. It allows distribution of effluent through the entire absorption area and resting periods between doses to maintain aerobic conditions. Shallow placement of the trenches keeps the system as far as possible above the water table. For the mound system, effluent is pressure dosed in the same manner into a carefully constructed mound of fill, so that it is pre-treated before it reaches the natural sub-soil. The LPP is designed to provide aerobic treatment in sub-soils with high water tables or shallow restrictive horizons. Shallow trenches and dosing and resting cycles are used to maintain aerobic conditions. Improved treatment can be provided by LPP systems under high water table conditions. Pressure dosed mound systems have provided excellent treatment of effluent in high water table conditions when properly designed and operated.

Mote et al (1990) undertook laboratory testing of parallel and serial methods for distributing effluent. Serial methods of distributing effluent mean that the initial stretch of the soakaway trench gets overloaded and areas downstream only receive the effluent which cannot be received by the initial stretch. Parallel methods of distribution of effluent can distribute the flow of effluent more effectively. The tests showed that there was no difference between parallel and serial distribution in terms of the size of the filter field or number of seepage trenches required to satisfactorily convey the wastewater through the soil. However, from a water quality viewpoint the parallel method of distribution was much better than serial distribution.

Soil replacement systems are built in fine textured soils by removing the natural soil to a depth of 1 to 1.5 m and replacing it with sandy fill. The absorption area is then installed in this area. However the two soil replacement systems studied did not perform any better, probably due to the system being highly loaded.

5.9.6 Distance between septic tank soakaways and drinking water wells

In a couple of papers, Yates and Yates (1988, 1989) calculate the distance necessary between septic tanks and drinking water wells to prevent groundwater contamination. The paper reports that the single most important method of limiting groundwater pollution by septic tanks is to restrict the density of systems in this area. Setback distances of as low as 15 m were calculated. The distance between the soakaway and a well of 50 m (recommended in the U.K.) is considerably greater than this figure but it is important to note that much U.S. research relates to intergranular flow aquifers, whereas the UK is dominated by consolidated aquifers in which rapid flow through fractures may be important.

5.9.7 Search of the Internet

A search of the Internet found one Website that advertised an internal filter for use with septic tanks. The filter is installed over the outlet of the septic tank inside the tank and provides further filtering of the effluent and prevents carry-over of some solids. In addition, in theory the filter develops its own biomass that reduces the organic load leaving the tank.

5.10 Summary and Suggestions for Research

The appropriateness and adequacy of the percolation test as an indicator of the suitability of the ground to receive septic tank or PTP effluent is a subject of some concern. Other influential factors include depth to water table and the relative conductivity of the biological mat compared with the receiving soil. The appropriate minimum depth of unsaturated zone should be investigated further. There are very limited data on the microbiological quality of effluents produced by septic tanks and PTPs, despite its obvious significance with respect to groundwater contamination. Of particular concern is the observation that in the UK septic tanks are primarily used in rural areas, where small private water supplies are most common, and in upland areas, where flow in many aquifers is predominantly along shallow fracture systems.

The contamination of groundwater by septic tanks in the U.S.A. is well documented but there is very little data on the microbiological contamination of groundwater by septic tanks in the U.K. It is important to establish the extent of this contamination by studying existing septic tank and PTP systems throughout the country, in a range of geological conditions, and especially the microbiology of the groundwaters in the vicinity of the systems. This survey would provide essential backgound information and a basis for prioritising further work.

The findings of this report show that the main need is for a more detailed investigation of the soakaway system, as used currently, and for other novel methods of transferring the effluent into the ground.

5.10.1 Development of existing septic tank system design and methods of use

The following sections give a list of suggested areas for future research.

Investigation of the characteristics of the ground that affect the treatment of the effluent:

a. Nature of the sub-soil
b. Depth to the water table
c. Depth to bedrock below the drift
d. Slope of the site and the associated drainage
e. Flooding frequency
f. Material of drainage pipe
g. Shape and fill of the soakaway trench
h. Seasonal variations in the performance of soakaway
i. Areal density of septic tanks to control total loading per unit area

Quantification of contamination inherent in/consequent upon various design and operational aspects of septic tanks and PTPs. A controlled comparison could provide the basis for the development of a scoring system similar to but better than the NRAs ground scoring system

Assessment of microbiological removal of pathogens from the different PTPs in order to estimate the potential microbiological loading on the unsaturated zone

Investigation of the effect of intermittent flows to soakaways with septic tank/PTP effluent

Investigation of the effect of biological mat on hydraulic conductivity of the system and its effect on performance system

5.10.2 Investigation of novel septic tank design system and methods of use

Several other novel methods of treatment were revealed in the literature search and are suggested as possible areas for research.

The use of evapotranspiration beds where the soil conditions are not suitable for use as a soakaway

The use of elevated mound systems where the septic tank effluent is injected into a mound to increase its passage through the ground

The use of interceptor tile lines around soakaways to draw off the effluent

Deep ploughing to break up the restrictive zone beneath the soakaway

The use of another material to fill in the bed after removing the original soil

Investigation of low pressure pipeline (LPP) systems

Investigation of filters or novel products which could be used in conjunction with septic tanks

5.11 Conclusions

1. The published literature on the removal of microbes or pathogens by septic tanks and PTPs is very limited. This is an area that needs much more research in order to estimate the microbiological loading on the ground (and potentially on underlying groundwater resources). For instance, there are no microbiological standards when setting consent conditions for the discharge of septic tanks and PTPs, and this seems an obvious regulatory omission for an issue so closely related to public health.

2. Septic tanks are only intended to partially treating sewage and rely on the soakaway to complete the treatment process. PTPs are capable of treating sewage to a much higher standard than septic tanks. If PTPs then discharge to the ground the main risk of groundwater contamination is from the design and operation of the soakaway.

3. The importance of the soakaway as an integral part of a septic tank or PTP system is not always recognized. If the risk of groundwater contamination is to be minimized then the system must be viewed as a whole and not as two independent units.

4. The area of soakaway design and optimization is an important area for research. A more detailed list of future research areas is given in Section 9 above. There is a need for a better sub-soil suitability index than the simple percolation test and this has been achieved to some extent by CIRIA's trial hole test and recommendation for a thorough visual inspection. However more research needs to be carried out into the ability of different sub-soils to effectively receive and treat septic tank and PTP effluent, especially with respect to pathogens and nutrients.

5. The Best Practice Guidelines for Septic Tank and PTPs are presented in Section 5.7 above. The guidelines are based on BS 6297, the findings from literature and the discussions with manufacturers. It is apparent that the design criteria for septic tank and PTP systems are well established but their implementation and control is not. These aspects can present a significant risk to groundwater.

6. The manufacturers that were contacted all offered service contracts but these are not mandatory and were frequently not taken up. It is recommended that a service contract should be compulsory. CIRIA suggests that there should be a local register of owners of septic tanks and PTPs. Registered owners would then be required to take out a maintenance contract with an approved operator. The increasing use of household chemicals should be examined in the light of their effect on the microbiological performance of septic tanks and PTPs.

7. The main risk factors when designing and using septic tanks and PTPs relating to the potential for groundwater contamination were identified as:

a) Correct sizing of the septic tank system or PTP

b) Separation of surface water runoff from the sewage

c) Proper operation of the septic tank or PTP including control over what is discharged into the septic tank or PTP

d) Proper maintenance including appropriate desludging and maintenance of the mechanical and electrical plant

e) Appropriate sizing and siting of the soakaway for both septic tanks and PTPs

f) Education of the users of septic tanks and PTPs about efficient use and importance of maintaining the biological health of the system.

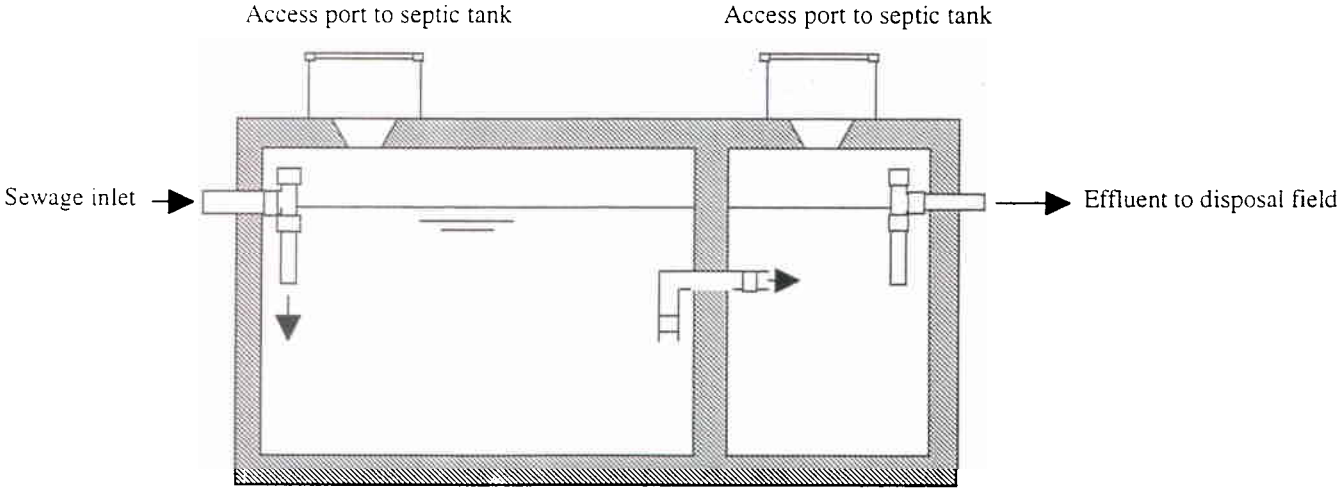


Figure 5.1. Typical two chamber septic tank system

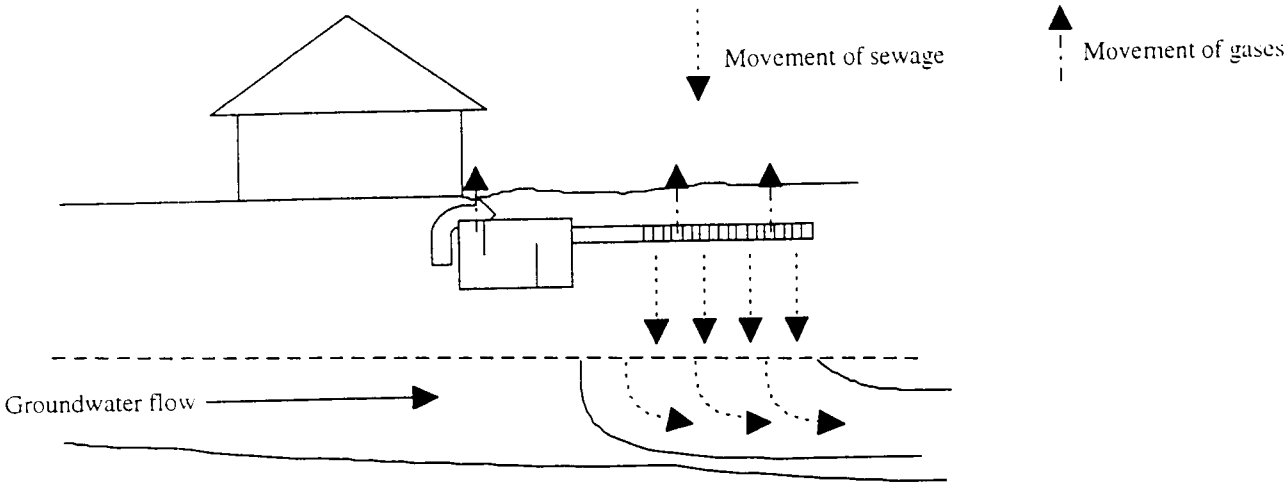


Figure 5.2. **Schematic cross section of a conventional septic system including septic tank, distribution pipe and groundwater plume**

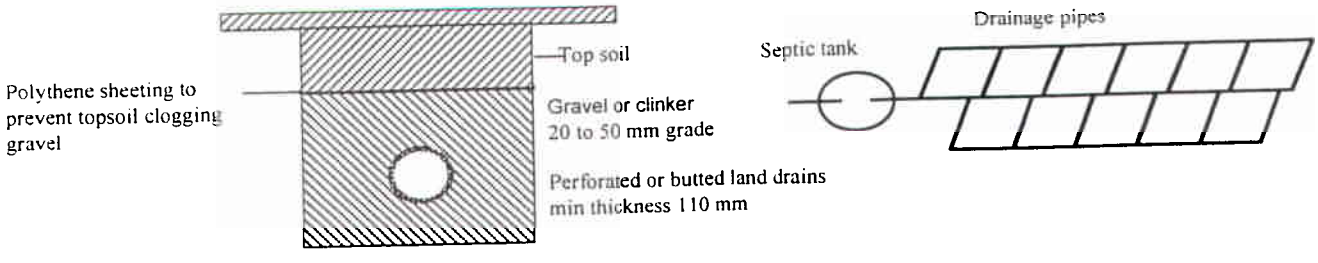
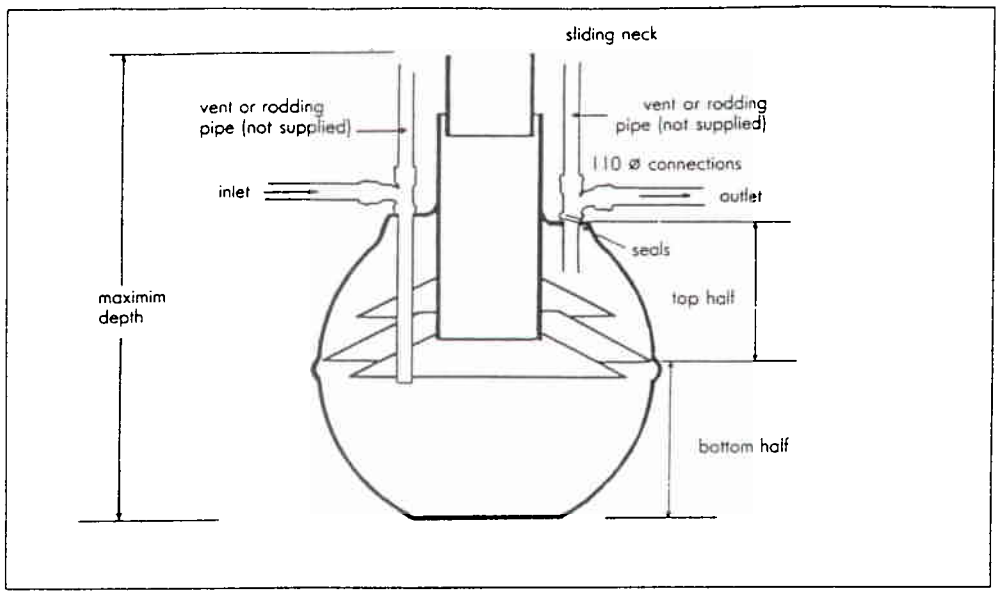


Figure 5.3. **Typical layout of the drainage pipes in gravel-filled trenches and 'herringbone' drainage system**



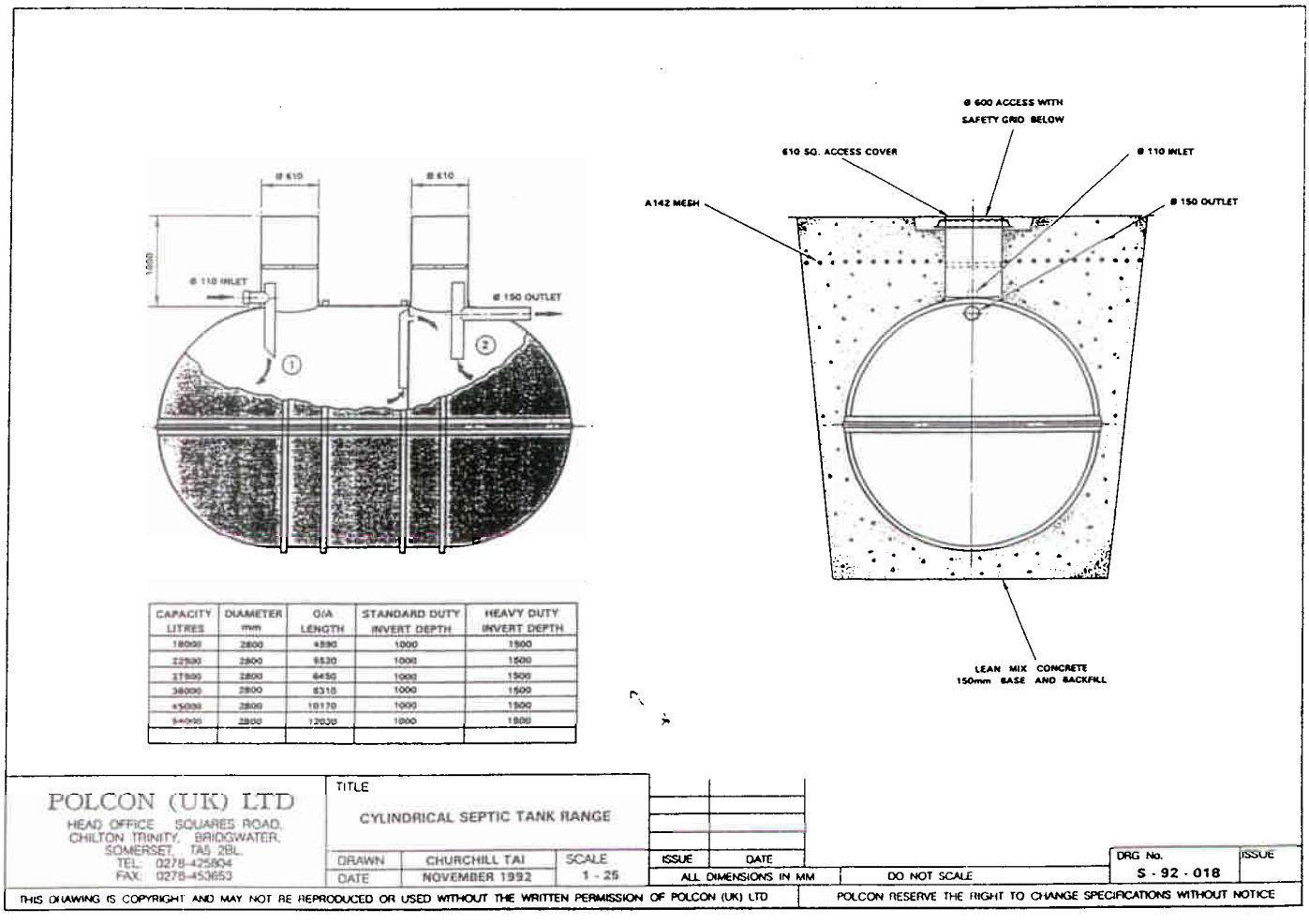


Figure 5.4. **Schematic diagrams of cylindrical and 'onion shaped' septic tanks**
***see acknowledgements section**

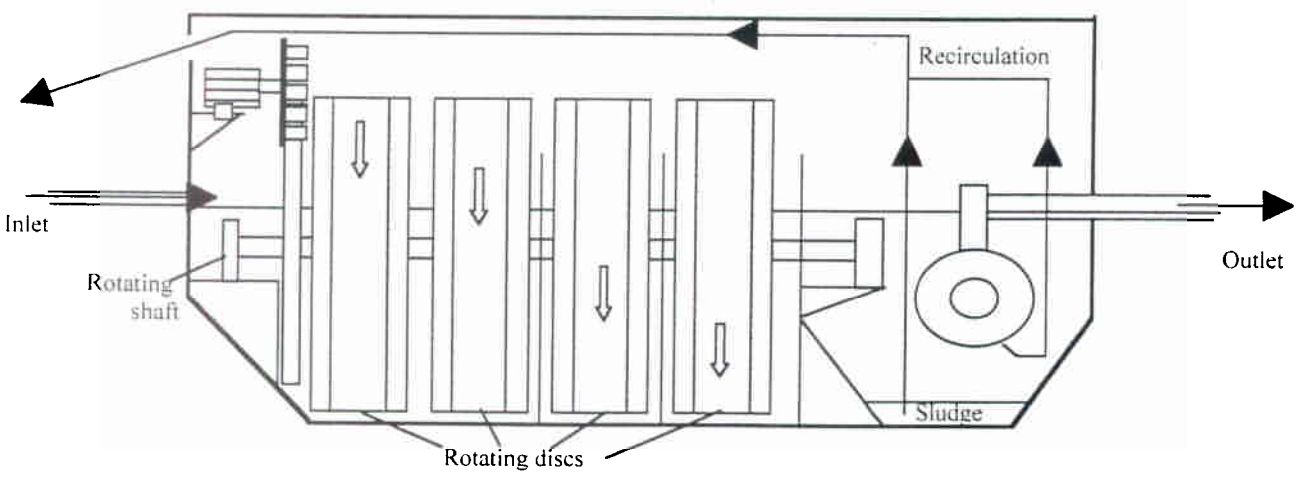


Figure 5.5: Rotating biological contactors (RBCs)

Table 5.1: Types and features of Packaged Treatment Plants

Type of plant	Size range	Advantages	Disadvantages
Activated sludge: Contact stabilisation	Pre-fabricated plant. Sizes from 30 to 20000 persons	No primary sludge formed.Small quantity of secondary sludge which is partly stabilised. No odour nuisance. Compact.Reserve activated sludge always available. 30:20 effluent can be achieved.	Regular power and maintenance required for aeration and pumping. Power failure can be serious. Surge flows can cause loss of activated sludge. Noise can be a problem.
Activated sludge: Extended aeration.	Pre-fabricated plant Sizes from 17 to 30000 persons	No primary sludge formed. Small quantity of secondary sludge which is partly stabilised. No odour nuisance.30:20 effluent can be achieved	Highest power requirement of all types. Regular maintenance required for aeration and desludging. Regular inspection advised.
Trickling/percolating filters	Pre-fabricated plant available to serve 15 450 persons	No primary sludge formed. Can treat intermittent flow. Compact plant possible. 30:20 effluent standards can be achieved.	Some odour problems reported. Final to sludge difficult to dewater. High power requirements. Efficient operation depends on reliable power supply, regular inspection and maintenance. Can recycle effluent to maintain treatment during periods of low flow.
Rotating biological contactor	Pre-fabricated plant available. Sizes from 5 to 40000 persons	Power consumption and headloss required are both low. 30:20 effluent standards can be achieved. Compact and inconspicuous. Fly nuisance can be eliminated.	Sludge removal and motor maintenance needed every three months. Sensitive to overloading. Power failure causes total loss of efficiency.
Submerged aerated filter	Prefabricated plant available, typically for 5 to 3500 persons	Compact. 30:20 standards can be achieved. No moving parts within the treatment unit.Low maintenance costs.	Power consumption high. Regular inspection needed

Table 5.2. Instruction sheet for use with septic tanks and PTPs.

Do's and Don'ts

Do:

✓ Think before you put anything down the sink, toilet or drains.

✓ Tell your guests/visitors/staff that you have a specialist sewage treatment plant and tell them how they can avoid harming it.

✓ Read the lable and use the manufacturers' recommended doses for all household cleaning products.

✓ Use cleaning products little and often so the plant isn't overloaded.

✓ Spread your clothes washing throughout the week.

✓ Stick to the same washing, dishwasher powders and other cleaning products - the bacteria in the plant will work more efficiently with products they are used to.

✓ Use liquid cleaners for clothes washing and for dishwashers.

Don't:

X Spring clean and use large amounts of cleaners and chemicals in one day.

X Have a 'washing day' - spread your washing throughout the week.

X Use household bleach and other strong chemicals indiscriminately.

X Keep changing your brands of household cleaners and washing powders.

X Tip bottles of medicine, mouth wash etc down the toilet.

X Put sanitary towels, tampons, disposable nappies, baby wipes, cotton wool, incontenence pads etc down the toilet.

X Over flush the toilet unnecessarily - use a water-saving flush if it's fitted.

X Pour fat or grease from cooking down the sink or drains.

X Change the oil in your chip pan and pour it down the sink.

X Use your waste disposal unit like a rubbish bin - use it sparingly.

X Pour garden chemicals or car engine oil down the drains.

Table 5.3. Point scoring system for septic tank soakaway assessment

Distance of soakaway from abstraction

Distance	*Points*
<50	-40
50-100	-20
101-150	-10
151-200	0
201-300	10
301-400	20
401-600	30
601-800	40
>800	50

Strata

Type	*Points*
Carboniferous/Jurassic/ Magnesian Limestone	-10
Namurian Sandstones (or other fissured sandstones)	0
Triassic Sandstone Gravel/sands	20
Boulder clay/Drift	25
Marl/clay/shale	40

Unsaturated zone (m)

Depth	*Points*
<5 or unknown	0
5-10	2
11-15	4
16-20	6
21-25	8
26-30	10
>30	12

For significantly fissured strata score 0

Abstraction rate

Rate (m3/day)	*Points*
>4500	0
1000-4500	5
100-999	8
10-99	10
<10	40

Position relative to Abstraction position

Position	*Points*
Up groundwater gradient	-5
Adjacent or unknown	0
Down groundwater gradient	5

Depth of soil beneath septic tank outlet

Depth (m)	*Points*
0 or unknown	0
some	5
2	10

Quantity of effluent discharge

Assess score according to above system and multiply total by factor

Dwelling equivalent	*Factor*
1-2	1
3-5	0.8
6-10	0.6
10	0.5

5.12 References

Anis Al-layla, M. and Al-Rawi, S.M. (1989) Evaluation of septic tanks performance of some parts of Mosul city in Iraq. *J. Environmental Science and Health*, A24(5), pp. 543-556

Bouma, J., Zieball, W.G., Walther, P.G, Olcott, E. McCoy and Hole, F.D. (1972). Soil absorption of septic tank effluents. Univ. of Winconsin Ext. Cir. No. 20

CIRIA Septic tank systems. A regulator's guide. CIRIA leaflet 1998.

Cogger, C.G. and Carlile, B.L. (1984) Field performance of conventional and alternative septic systems in wet soils. *J. Env. Qual.*, 13(1), pp. 137-142

Geary, P. M. Evaluation of on-site disposal options. (1993) *Wat. Sci. Tech.*, Vol. 27, No. 1, pp. 59-62

Hagedorn, C., McCoy, E.L. and Rahe, T.M. (1981). The Potential for Groundwater Contamination from Septic Effluents. *J. Environmental Quality*, 10 (1), Jan-Mar, pp. 1-8

Laak, R., Healy, K.A. and Hardisty, D.M. (1974). Rational basis for septic tank design. *Ground Water*, 12(6), pp. 348-352. Nov-Dec 1974

Mote, C.R., Mucke, F.A. and Allison, J.S. Septic tank efficiency. (1990) *Journal of Environmental Health*, Mar/Apr, pp. 283-287

Office of Technology Assessment 1984. Protecting the nation's groundwater from contamination. U.S. Congress, Washington, D.C. OTA-0-233

Payne, J.A. and Butler, D. (1993) Septic tanks and small sewage treatment works: A guide to current practice and common problems. CIRIA Technical Report 146.

Pedley, S. and Howard, G. (1997) Microbiological contamination of groundwater. Quarterly *Journal of Engineering Geology*, 30, pp. 179-188

Viraghavan, T. and Warnock, R.G. (1976) Groundwater quality adjacent to a septic tank system. *J. American Water Works Association*, pp. 611-614. November 1976

Wilhelm, S.R., Schiff, S.L. and Cherry, J.A. (1994) Biogeochemical Evolution of domestic waste water in septic systems:1. Conceptual model. *Ground Water*, 32(6), pp. 905-916

Yates, M.V. and Yates, S.R. (1988) Virus survival and transport in ground water. *Wat. Sci. Tech.* 20(11/23), pp. 301-307

Yates, M.V. and Yates, S.R. (1989) Septic tank setback distances: A way to minimise virus contamination of drinking water. *Ground Water*. 27(2), pp. 202-208

Appendix 5.1

List of manufacturer visits

Clearwater and Polcon,
Clearwater Industrial Park,
Bristol Road, Bridgwater ,
Somerset, TA6 4AW.

Contact: Vaughan Crofts, Andre Moreau
Date visited: 16th February 1998

Entec (Pollution Control) Ltd.,
West Portway,
Andover,
Hampshire,
SP10 3LF

Contact: Steve Bungay
Date visited: 17th February 1998

WPL Ltd.,
14/15, Bridge Industries,
Broadcut,
Fareham,
Hampshire,
PO16 8SX

Contact: Richard Munden
Date visited: 24th February 1998

6. BIOREMEDIATION

6.1 Introduction

The growth of industrial activity in the UK during the last 200 years has generated a wide variety of chemical waste products that have been discharged to the environment with a minimum level of control. Although current legislation sets quality standards for waste discharges, illegal and accidental discharges contribute significantly to the chemical burden in the environment. The problem is exacerbated by the rapid increase in the potential number of pollutants. The US EPA's Toxic Substances Control Act Chemical Inventory currently includes over 72,000 chemicals, with approximately 2300 new chemicals submitted to the US EPA every year. Few data exist for the release of total toxic chemicals into the environment. However, US figures for 1990 show that manufacturers (Ember 1992) released approximately 2.2 billion kg of toxic chemicals into the environment. Many of these chemicals will become associated with the hydrological cycle from where they may be eventually transported into groundwater systems.

Bioremediation, sometimes referred to as biorestoration, is a managed or natural process in which microbiological processes are used to degrade or transform contaminants to less toxic or non-toxic forms, thereby remedying or eliminating environmental contamination. Bioremediation is a complex process and its application is based on an understanding of the microbiology, biochemistry, metabolic processes, structure and function of natural microbiological communities, chemistry of the pollutants and engineering its application. The use of microorganisms to remediate contaminated water is not a new idea in the field of applied microbiology. Microorganisms have been used for many decades to remove, or reduce the level of organic and toxic chemicals in domestic and manufacturing waste effluents.

During the last few decades the potential for combining microorganisms and process engineering to remediate contamination of other environmental systems has been recognised, and there has been a rapid growth in bioremediation as an industry. Many excellent reviews, and several textbooks, describing the science and engineering of bioremediation have been published (Bonaventura and Johnson, 1997; Crawford and Crawford, 1996; Hinchee and Olfenbuttel, 1991a; Norris et al, 1994; Young and Cerniglia, 1995). In addition, there have been a considerable number of reports, published in scientific and technical journals, which deal, in detail, with different aspects of the bioremediation process. Several of these authors note that bioremediation is, perhaps, the only viable method for the treatment of some types of contaminated groundwater. Furthermore, they predict that there will continue to be increases in the use of this technology to amend groundwater pollution.

This section will review the current state of technology for the bioremediation of groundwater. It will not attempt to duplicate the excellent reviews that have already been published. Instead it will introduce the technology by drawing from examples of the main groups of chemical pollutants that have been treated in groundwater, the microorganisms that have been used, and the methods that have been applied to treat contaminated groundwater. Biochemical pathways have been elucidated for the metabolism of many of the major organic contaminants. These will be discussed only where they have direct consequences for the quality of groundwater. It should be noted that most of the published literature describes the results of US studies. The relevance of this data to UK applications has not been fully assessed.

6.2 Drivers For The Introduction Of Bioremediation

Growth in the application of remediation technologies, including bioremediation, has been accelerated by environmental, legislative and social factors, which have emerged during the last decade. In the UK these include:

- Water Resources Act
- Environmental Protection Act
- EC directive on groundwater
- Civil liability
- Contaminated land register
- Obligation of the developer to remediate
- Public awareness and the role of environmental organisations
- Development pressure

Although development pressure and public awareness of environmental pollution have created the appropriate climate for remediation technologies to flourish, the introduction of legislation to maintain environmental quality has been a major incentive behind the growth of research into the development and application of these technologies. The Chemical Industries Association (CIA, 1993) summarises the relevant legislation as follows:

Water Resources Act 1991

- Section 81
- It is an offence to cause or knowingly permit 'any poisonous noxious or polluting matter to enter any controlled waters'.
- Section 104
- All groundwaters in the UK are considered to be 'controlled waters'.
- Section 161
- Gives powers to the Environment Agency to undertake preventative remedial work the cost of which can be reclaimed from the polluter retrospectively if necessary.

- Environmental Protection Act 1990
- Section Part IIA

  Gives local authorities the duty to investigate nuisances allegedly caused by the owner/occupier and serve an abatement notice accordingly.
- Section 82 Private individuals aggrieved by nuisances may take action through the local magistrate's court. Does not apply where a waste management license is in force.

  Sections 79/80

Directive on Groundwater 80/68/EEC

Requires the protection of all groundwater irrespective of its use

Prohibits the discharge of certain substances identified in List I and limits the discharge of others specified in List II.

Civil liability

Civil liability is of most concern to industry because of its potential unlimited financial consequences. Claims for the clean up of underground water could be very high. Experience in the USA has been that claims can amount to tens of millions of dollars at some sites.

6.3 Microorganisms and Biochemical Degradation of Pollutants

A substantial number of reports have been published which demonstrate the ability of microorganisms to amend groundwater pollution due to a variety of organic and inorganic chemicals, including metals. Most of these reports, however, concentrate upon the degradation of a relatively few compounds: mainly chlorinated solvents, and aliphatic and aromatic hydrocarbons, indeed petroleum and creosote are most often the pollutants of concern, composing about 60% of the sites where bioremediation is being used in field demonstrations or for full-scale operations (Hinchee and Olfenbuttel, 1991).

In the following pages, each of the major groups of contaminants will be reviewed. Particular emphasis will be given to those compounds for which microbiological degradation in groundwater has been demonstrated conclusively, and the metabolic process elucidated. Many compounds, which are considered to be of environmental significance, will be omitted if they do not contribute to the understanding of bioremediation and its impact upon the microbiology of groundwater.

6.3.1 Halogenated Organic Compounds (HOC)

Halogenated organic compounds comprise a large group of chemicals, including some of the most useful and economically important chemicals available to industry and agriculture. They are used in a broad range of industrial processes, for example dry-cleaning, solvents for the cleaning and polishing of metals, refrigerants, insulators and lubricants: in agriculture they have widespread application as fungicides, herbicides and pesticides. Although many HOCs are produced intentionally, others are formed as a waste by-product of chemical syntheses and industrial processes (Adriaens and Vogel, 1995). An inevitable consequence of the extensive use of these compounds is that they are now amongst the most widely distributed chemical pollutants found in the environment (Vogel et al, 1987; Kuhn and Suflita, 1989; Neilson, 1990).

Some marine bacteria have been shown to synthesise HOCs as a by-product of normal metabolism. However, there are very few HOCs synthesised naturally in terrestrial environments (Wood, 1982). The presence of these compounds in groundwater, therefore, is the direct consequence of industrial or agricultural pollution.

A survey of organic species in the Birmingham aquifer (Rivett et al, 1990) demonstrated that the aquifer was contaminated to high levels by trichloroethene (TCE), and that other solvents, such as 1,1,1-trichloroethane (TCA) and tetrachloroethene (TeCE), were widespread in lower concentrations. Longstaff and co-workers (1992) reported similar levels of pollution in a chalk aquifer in the Luton/Dunstable area. Industrial activity in this area is dominated by the production of automotive components, which has resulted in the release of solvents into the aquifer underlying the urban area. An incident at the Harwell Laboratory in Oxfordshire arose due to inadequate disposal methods for chlorinated solvents. This was compounded by a lack of understanding of the vulnerability of groundwater to organic contamination. The result was a major pollution incident in the aquifer underlying the laboratory (Fellingham et al, 1993). The problem was identified when routine measurements in a public supply borehole 6km away detected concentrations of solvents above background levels. Other cities

in the UK where extensive groundwater pollution with chlorinated solvents has been identified include Coventry (Burston et al, 1993) and Nottingham (Barrett et al, 1997). The implication of such surveys is that wherever groundwater exists beneath industrial areas, organic contamination to some degree may occur.

The widespread distribution of HOCs in groundwater and the known or assumed toxicity of many of these compounds (Salkinoja-Salonen et al, 1995) has stimulated much research into the development of remediation techniques.

Bioremediation of groundwater contaminated with HOCs

The diversity of structures and the chemical inertness of HOCs pose particular problems for microorganisms. For an organohalide to enter one of the principal metabolic pathways, all halogen moieties must be removed and the carbon skeleton must be transformed into a common metabolic intermediate (Wackett, 1995). Bacteria succeed in transforming HOCs in three general ways:

- Metabolism into trunk pathways
- Use of halocarbons as final electron acceptors for ATP generating electron transport
- Via cometabolic pathways

A combination of laboratory studies and pilot experiments at field sites have demonstrated that only a limited number of chlorinated aliphatic hydrocarbons can support bacterial growth by serving as a source of carbon and energy (Janssen and Witholt, 1992). However, many more chlorinated compounds are biodegradable by cometabolic conversion (Mars et al, 1998). For several classes of HOCs, the species of bacteria that are involved in their transformation have been identified and the biochemical pathways involved in their degradation have been elucidated in the laboratory. In contrast, there are relatively few comprehensive studies of the degradation of HOCs, in the laboratory which combine the chemistry, biochemistry, microbiology and hydrogeology of the remediation site. The notable exception is Trichlorethene (TCE).

Trichloroethene is amongst the most frequently identified HOCs in groundwater in the USA (Semprini, 1995) and UK (Rivett et al, 1990, Burston et al, 1993, Barrett et al, 1997). As a consequence, considerable research effort has been channelled into the development and assessment of remediation techniques for this chemical. This work has produced two significant outputs. First, bioremediation has been acknowledged to be a practical and economically viable method for the remediation of TCE pollution in some groundwater. Second, these studies have provided an insight into the complex interaction between the microbiology of groundwater systems and the degradation of the TCE by aerobic and anaerobic pathways.

Anaerobic degradation of TCE

Chlorinated aliphatic compounds, such as TCE, TeCE, TCA, carbon tetrachloride (CTC) and chloroform (TCM) are resistant to biodegradation in subsurface aerobic environments (Wilson and Wilson, 1985). However, the results from early monitoring studies following the accidental release of alkyl halides into the environment showed that TeCE and TCE were progressively dechlorinated under anaerobic conditions (Chapelle, 1993). The microbial processes involved in these reactions were elucidated by McCarty and co-workers in a series of laboratory experiments carried out at Stanford University (Bouwer et al, 1981; Bouwer and

McCarty, 1983; Vogel and McCarty, 1985). Using batch experiments and continuous flow column experiments McCarty was able to demonstrate the rapid removal of TeCE, TCE and dichloroethene (DCE) as the contaminants passed down the experimental columns. Indeed, all of these compounds were removed in the top few centimetres of the column (Vogel and McCarty, 1985). In contrast, the subsequent transformation of vinyl chloride to ethene was found to be the rate-limiting step in the anaerobic degradation pathway leading to the accumulation of vinyl chloride in the effluent from the soil column.

Anaerobic degradation has also been identified as a potential method for the removal of TCE in groundwater (Komatsu et al, 1994). Studies in the USA and Japan have shown that biotransformation of TeCE and TCE to cis-DCE proceeds rapidly in natural anaerobic environments. Further transformation of cis-DCE was rate limiting and the compound was shown to accumulate in groundwater (Parsons et al, 1984; Hirata, et al, 1992). Clearly, the potential to accumulate vinyl chloride and cis-DCE as by-products of the degradation of more highly chlorinated aliphatic compounds creates as much of a problem in the groundwater as the original contaminant (Parsons et al, 1983; 1984). By increasing the supply of electron donors, such as methanol or gaseous hydrogen, to methanogenic cultures Freedman and Gossett, (1989), and DeStefano and co-workers (1991) demonstrated the complete dehalogenation of TeCE and TCE. Similar studies by Kamatsan et al (1994) showed that anaerobic enrichment cultures were able to dechlorinate cis-DCE to ethylene in the presence of yeast extract, glucose or proprionate. These results suggest that the anaerobic biodegradation of chlorinated aliphatic compounds may be rate limited by the level of electron donors in the substrate.

The complete biochemical pathway for the anaerobic degradation of chlorinated aliphatic compounds has been elucidated (Vogel and McCarty, 1985). The pathway is relatively simple and may be summarised as follows:

Tetrachloroethene ->Trichloroethene -> Dichloroethene -> Vinyl chloride -> Carbon dioxide

Aerobic degradation of TCE

Methanotrophic organisms possess the enzyme Methane Monooxygenase (MMO) which initiates the first step in the oxidation of methane when it is used as the sole source of carbon and energy for growth (Dalton et al, 1984). The MMO enzyme complex has a low substrate specificity and is able to initiate the oxidation of a wide variety of carbon compounds including methylated and brominated alkene, and chloro-, fluoro-, bromo- and nitro-methanes (Higgins, et al, 1980; Patel et al, 1979; 1982; Patel, 1984). In the presence of specific organic substrates some other bacterial species are induced to synthesise dioxygenase enzymes. Dioxygenase enzymes incorporate both atoms of the oxygen molecule into the products of their enzymatic reactions (Table 6.1). Theoretical pathways for the degradation of TCE by monooxygenases and dioxygenases are shown in Figure 6.1, whilst Table 6.1 lists the organisms currently implicated in TCE degradation.

Wilson and Wilson (1985) were the first to demonstrate that the MMO enzyme complex could be exploited for the biodegradation of TCE. Using unsaturated soil columns that had been enriched for methanotrophs by exposure to natural gas, they showed that TCE could be degraded in an aerobic environment to carbon dioxide without the accumulation of high levels of toxic intermediate compounds. Subsequent studies established that TCE does not serve as the primary carbon source for methanotrophs and, by itself, will not induce the synthesis of MMO. In order for TCE to be degraded, the indigenous methane-oxidising bacteria must first be stimulated (sometimes referred to as biostimulation) by the addition of

the primary substrate of the MMO complex. TCE, and other chlorinated ethenes, are degraded by cometabolism (Semprini et al, 1990). Brock et al., 1984 defined cometabolism as "a process in which microorganisms growing on one compound (primary substrate) produce an enzyme which fortuitously transforms another compound, from which they cannot obtain energy for growth".

The potential significance of these observations for groundwater remediation was recognised by McCarty, Semprini and co-workers. They undertook a comprehensive investigation of TCE degradation in situ with the aim of characterising the biochemical transformation of these compounds in groundwater. The test site used for these studies was a shallow aquifer at the Moffett Naval Air Station in California (Roberts et al, 1990).

The first series of field studies were designed to mimic the conditions that had been used to degrade TCE successfully in laboratory columns (Roberts et al, 1990; Semprini et al, 1990). First, the growth of indigenous methanotrophs was stimulated by injecting methane and oxygen enriched groundwater into the test zone in alternating pulses. After a few weeks of stimulation, methane concentrations gradually decreased and eventually became undetectable in groundwater at a distance of two metres from the point of injection. Once the active biostimulation conditions had been achieved, chlorinated ethenes were added to the injection water. The data from these studies showed that:

- The growth, or activity, of indigenous methanotrophs can be stimulated in the subsurface environment by the addition of methane and oxygen, and that stimulation can be used to promote the degradation of certain chlorinated aliphatic compounds.
- Partial transformation of vinyl chloride (90 to 95%); trans DCE (80 to 90%); cis DCE (45 to 50%); and TCE (20 to 30%), occurred within one to two meters of the injection point.
- The rate of biotransformation was dependent on the structure of the chlorinated compounds, with less chlorinated compounds more rapidly transformed.
- Active utilisation of methane was necessary for the transformation of chlorinated ethenes. At control sites, which were not stimulated by the addition of methane and oxygen, co-metabolism of the chlorinated ethenes was not observed.

The results of the field studies showed that enhanced, in-situ bioremediation of chlorinated aliphatic compounds is a potential method for aquifer restoration. Furthermore, in contrast to the anaerobic pathways of TCE metabolism, aerobic co-metabolism does not leave vinyl chloride as a toxic residue in the groundwater. However, the further application of the early methods used by Semprini and co-workers (1990) was restricted by the low rate of transformation of TCE.

Earlier studies had demonstrated that pure cultures of a methanotroph, *Pseudomonas cepacia* strain G4, were able to oxidise TCE to CO_2 (Nelson et al, 1986). However, the activity was dependent upon an unidentified component of the groundwater from which the organism was obtained. Analysis of the water used as the medium for TCE metabolism by strain G4, showed the loss of an organic compound which was identified as phenol (Nelson et al, 1987). Using chloramphenicol to inhibit the synthesis of proteins, Nelson and co-workers were able to show that strain G4 would only degrade TCE when induced with phenol before addition of chloramphenicol, thus proving that TCE degradation was mediated by the same enzyme

complex. Furthermore, toluene, o-cresol, and m-cresol, could replace the requirement for phenol (Nelson et al, 1987).

Once again, the transition from laboratory observations to successful field trials was relatively simple. Using the groundwater test site at Moffett Naval Air Station, Hopkins and co-workers (1993) were able to demonstrate the efficient removal of TCE and cis-DCE after biostimulation of the indigenous methanotrophs with phenol and oxygen. Under these conditions over 90% removal of cis-DCE and TCE was observed within the two meter zone of biostimulation. This result compares with the much lower degradation rate of these two compounds following biostimulation with methane and oxygen (Semprini et al, 1990). Later studies confirmed that toluene could also be used as the primary carbon source.

At the Moffett Naval Air Station effective degradation of TCE and related compounds was achieved without the addition of other nutrients. However, similar studies at a second site, in Aiken, South Carolina, showed that TCE degradation, during stimulation with methane, was enhanced significantly by the addition of nitrogen and phosphorous as supplementary nutrients (Pfiffner et al, 1997). Microbiological analysis of the water showed that the density of methanotrophs increased by three to five orders of magnitude during the methane, toluene and nutrient injection phases. These results emphasise the importance of gathering data about the groundwater chemistry at the remediation site. At the Moffett Naval Air Station site the concentrations of nitrogen and phosphorus were clearly sufficient to support the increased level of growth and activity of the indigenous methanotrophs during stimulation. This may not apply at all sites, and additional nutrients may be required to effect the bioremediation process.

Oxygen is required for the aerobic cometabolism of TCE by bacteria, both as a cosubstrate for the oxygenase and as a terminal electron acceptor for cellular respiration. The rate of aerobic remediation of contaminated groundwater may, therefore, become limited by insufficient oxygen, as has been demonstrated in soil (Fan and Scow, 1993). The use of nitrate as an alternative electron acceptor has been investigated (Leahy et al, 1996), but its activity was found to vary according to the strain of bacteria being tested and the type of electron donor. Hydrogen peroxide has been shown also to be an effective source of oxygen, but its widespread use has been limited by cost, its toxicity to microorganisms and the potential for blockage of soil pores as a result of gas bubble formation (Hopkins and McCarty, 1995; Morgan and Watkinson, 1992). Further work will be required before a reliable alternative to oxygen can be recommended.

The principal difficulty of aerobic in-situ bioremediation of TCE is the requirement for a primary growth substrate to induce TCE-degrading enzymes. Stimulation of indigenous populations with a specific substrate may enrich for a population that is either unable to cometabolise the target compound or may do so slowly. In addition, many of the compounds shown to support TCE-transforming cultures pose mass transfer and/or regulatory problems with their addition to groundwater systems (Munakata-Marr et al, 1996). One strategy that has been used to overcome some of these difficulties is to augment the indigenous microbial populations with bacterial cultures known to transform the target compound. Although the addition of cultured bacteria will enhance the rate of degradation of the target compound, and may also reduce the duration of the lag-time before degradation is initiated, the maintenance of cultures *in situ* will still require the addition of a primary growth substrate. Munakata-Marr and co-workers (1996) have recently described a genetically modified strain of *Burkholderia (Pseudomonas) cepacia* G4 that is capable of uninduced constitutive degradation of TCE in the absence of phenol or toluene. Experiments in microcosms, under

conditions resembling an *in-situ* treatment scheme, have shown that the mutant organism will degrade TCE without primary substrate addition. To date, the mutant bacteria has not been tested in the field.

Microbiology of aerobic TCE degradation in groundwater

There have been very few detailed analyses of the microbiology of groundwaters at sites being treated for TCE contamination. Even simple measurements of bacterial densities in the groundwater before, during and after treatment have been omitted from many of the studies. The development of reliable methods to quantify bacterial numbers, and for the qualitative analysis of microbial populations, and population dynamics are in their infancy. Moreover, relating the analytical data to relevant bacterial populations at the remediation site is particularly difficult with the limitations of current knowledge. Some recent studies have tried to elucidate the dynamics of microbial communities at bioremediation sites.

Fries and co-workers (1997a; 1997b) have published the results of field studies at the Moffett Naval Air Station, which have attempted to understand the impact of cometabolic treatments on the community composition. Several methods were used to identify the microbial species present in the groundwater, including growth-dependant procedures, fatty acid methyl ester analysis, and DNA restriction fragment analysis. Two hundred and seventy three phenol and toluene degrading isolates were characterised during the course of the study, and these were grouped into 63 genetically distinct strains. Sixty percent of these strains were able to cometabolise TCE. Gram positive strains comprised 30% of the collection (Fries et al, 1997b). Six microbial taxa were dominant: three members of the subclass of the class *Proteobacteria* (*Comamonas-Variovorax, Azoarcus,* and *Burkholderia*), and three gram positive groups (*Bacillus, Nocardia,* and an unidentified group).

Population densities are subject to considerable temporal and spatial variation in any aqueous environment. Estimating bacterial numbers and demonstrating significant variation in numbers is, therefore, very subjective. Population densities at the Moffett Naval Air Station site were sensitive to the presence of toluene and its concentration. Reducing the level of toluene from 50ppm to 5ppm led to an increase of population density of two to three orders of magnitude; from approximately 10^3 cells per ml to 10^5 – 10^6 cells per ml (Fries et al, 1997b). Phenol concentrations had less of an impact on population density, but there was an increase of nearly 1 log unit when the phenol concentration was decreased from 50 to 5ppm. Whilst it may be anticipated that a change in bacterial density of two or three orders of magnitude will influence the rate of degradation of the cometabolised compounds, there is no conclusive evidence from these papers that there is a direct correlation between the two events.

The resilience of microbial populations to environmental stress and toxic disturbance at groundwater remediation sites has been investigated only once (Fries et al, 1997a). The addition of 1,1-DCE to groundwater was found to dramatically reduce the microbial growth and species richness, which corresponded to reduction in bioremediation effectiveness (Hopkins and McCarty, 1995). However, the originally dominant microbial groups were still dominant after 1,1-DCE was removed, indicating that the microbial community at the site was quite resilient to toxic disturbance (Fries et al, 1997a).

Despite an extensive search of the published literature no examples could be found of UK bioremediation studies that are comparable to those which have been carried out at the Moffett Naval Air Base. Indeed, very few UK studies have been published. Indirect evidence of TCE transformation in a UK aquifer has been reported by Burston et al (1993).

Both TCE and by-products of TCE degradation were measured in groundwater samples taken from an aquifer below Coventry. The mechanism of transformation of TCE in the aquifer was not investigated further by these workers, although, they suggest that the relatively high dissolved oxygen concentration in the groundwater precluded a biological process. This conclusion is premature and there is sufficient published data to suggest that biological processes in the aquifer are not involved in the transformation of TCE. First, it is unlikely that TCE will be the only contaminant in the groundwater (published UK urban groundwater quality surveys reveal that a mixture of chlorinated solvents and BTEX compounds is the norm; e.g. Burston et al, 1993, Rivett et al, 1990, Barrett et al, 1997) and further analysis may detect the presence of other compounds that may act as primary carbon sources for the MMO and dioxygenase enzyme complexes. Second, even within an aerobic aqueous environment, microbiological activity can produce localised anaerobic conditions (demonstrated by sewage contamination of the shallow groundwater in the Meadows area of Nottingham [Barrett et al, 1997]). TCE transformation in the aquifer by anaerobic pathways cannot be excluded.

6.3.2 Aromatic Hydrocarbons

This section will review the bioremediation in groundwater of a broad group of organic pollutants which are derived from petroleum and related products. In particular, it will concentrate upon the bioremediation of Benzene, Toluene, Ethylbenzene and Xylene (the BTEX compounds, the most soluble portion of petroleum products) and polycyclic aromatic hydrocarbons (such as naphthalene and anthracene, often found associated with creosote and coal tars). A substantial amount of information has been published about the fate of these compounds in the aqueous environment, including groundwater.

Over the past one hundred years there has been a continuous and rapid growth of the worldwide market for oil as a source of energy and raw materials. In the early 1990s the worldwide production of petroleum was reported to be 3500 million metric tons per year (Energy Information Administration, 1992). Today, many of the world's major economies are underpinned by the use of oil and its by-products.

The major sources of oil pollution are municipal and industrial wastes and run-offs, leaks in pipe-lines and storage tanks and the discharge of dirty ballast and bilge waters (Rosenberg and Ron, 1996). A 1990 survey of underground storage tanks in the U.S.A. suggested that as many as 78% may be leaking, constituting a major source of BTEX contamination of groundwater (Fetter, 1993). It is highly likely that these sources are also a major contributor to UK groundwater contamination.

Oil and refined oil products are comprised of a complex mixture of hydrocarbons, which will exhibit different properties when they come into contact with water. Table 6.2 shows the typical concentrations of hydrocarbons in water after contact with three products of distillation of oil: kerosene, diesel and fuel oil.

The ability of some microorganisms to utilise petroleum hydrocarbons as a source of carbon and energy for growth has been known for many years. Early studies of petroleum microbiology were motivated by the requirement to control the economic losses that may result from microbial activity in the production equipment and during storage of the crude and refined products (Herbert, 1985). The potential for microorganisms to compromise safety by blocking fuel lines was an additional stimulus to research in this field. More recently, the ability of microorganisms to degrade petroleum hydrocarbons has been harnessed as an effective mechanism for remediating environmental pollution of these compounds (Rosenberg and Ron, 1996).

Petroleum microbiology has been reviewed extensively. For a full discourse of this subject the reader is referred to Davis, (1967) and Atlas (1984).

Bioremediation of groundwater contaminated by aromatic hydrocarbons

Benzene, toluene, ethylbenzene and xylene are volatile monoaromatic hydrocarbons which are found together in crude petroleum and petroleum products. They are also produced and used in industry as solvents and as precursors for the synthesis of pesticides, plastics and synthetic fibres . They are considered to be one of the major causes of environmental pollution because of the widespread occurrence of leakage from underground storage tanks and spills at petroleum production wells, refineries, pipelines and distribution terminals (Fries et al, 1994). Toluene is one of the most frequently detected organic contaminants in the Nottingham aquifer, UK (Barrett et al, 1997). Many governments have established clean-up standards for these chemicals in groundwater because of their carcinogenic potential (Dean, 1985). However groundwater contaminated with BTEX is difficult to remedy because these compounds are relatively soluble when compared to the aliphatic, alicyclic and polycyclic hydrocarbons, and disperse rapidly once introduced into an aquifer.

Various types of data can be used to show that natural attenuation of these compounds does occur. Studies usually focus on three avenues of research: documenting the loss of contaminants from the site, showing that microorganisms in samples from the site have the potential to transform the contaminants, and demonstrating that this potential actually occurs in the field (Rao et al, 1996; Williams et al, 1997).

Anaerobic degradation

The degradation of BTEX compounds in aerobic environments has been studied extensively. Yet groundwater is seldom contaminated with just BTEX compounds: frequently they are accompanied by other organic contaminants, which are more readily degraded in aerobic environments (e.g. Barrett et al, 1994). Frequently oxygen becomes depleted, and at this point anaerobic degradation of BTEX compounds will be dominant and the microbiology of the groundwater will change. Despite anaerobic conditions predominating in many contaminated groundwaters, it is only comparatively recently that the anaerobic degradation of BTEX compounds has been studied in detail. These studies have been discussed in several excellent reviews (Evans and Fuchs, 1988; Grbic-Galic, 1990; Krumholz et al, 1996; Salanitro, 1993). A significant conclusion that emerges from this work is that anaerobic degradation of BTEX compounds can proceed at rates that are equivalent to those observed under aerobic conditions. Anaerobic degradation is thus a significant process in the remediation of BTEX contaminated groundwater.

In the absence of molecular oxygen, degradation of BTEX can only take place by utilising alternative electron acceptors such as nitrate, sulphate, carbon dioxide and possibly ferric iron or other metal oxides (Ball et al, 1991; Braddock and McCarthy, 1996). Both field results and laboratory data have been published which demonstrate the oxidation of BTEX compounds coupled to the reduction of each of the electron acceptors. Indeed, in situ degradation may proceed by more than one route, reflecting changes to the environment that can occur as the pollution plume moves away from the source (Braddock and McCarthy, 1996; Thierrin et al, 1995). Typical changes in the oxidation of organic compounds is illustrated in Figure 6.2.

Benzene is the simplest aromatic hydrocarbon and, perhaps, the most recalcitrant of the BTEX compounds. Until recently benzene was believed to be completely resistant to

degradation in anaerobic environments (Krumholz et al, 1996), a view supported by the majority of field and laboratory investigations. For example, Thierrin and co-workers (1993; 1994) have studied the degradation and retardation of aromatic hydrocarbons in anoxic groundwater contaminated by leakage from an underground storage tank. Their results suggest that whilst toluene, naphthalene and p-xylene were significantly degraded, possibly coupled with sulphate reduction, benzene did not show any measurable degradation during the time course of the investigation. However, two field studies have been published which suggest that benzene may be degraded in anoxic and anaerobic groundwater. Piet and Smeenk (1985) report the reduction of benzene concentrations in groundwater, relative to the infiltration water, at a field site in Amsterdam, and Cozzarelli et al (1990) have used the presence of phenol as indicative of benzene oxidation in Fe(III)-reducing regions of an aquifer in Minnesota. The data presented in both of these studies have limitations, which cannot eliminate the possibility of benzene degradation taking place by other mechanisms, but they provide sufficient evidence to suggest that anaerobic degradation of benzene is a possibility.

Anaerobic transformation of benzene has been reported in several laboratory microcosm experiments. The microbiological detail in these studies is sparse, but the degradation of benzene has been shown to occur in combination with the reduction of sulphate, nitrate, and transition metals and by methanogenic bacteria. (Krumholtz et al, 1996)

The degradation of the other BTEX compounds, toluene, xylene and ethylbenzene, in anaerobic environments has been investigated by several groups of workers, and summarised in reviews by Krumholz et al (1996) and Bowlen and Kosson, (1995). Because these compounds are already chemically reduced and stable hydrocarbons, further reduction, while thermodynamically possible, is not a primary mechanism of biodegradation, even under strict anaerobic conditions. In the absence of molecular oxygen, the initial transformation step may still be oxidative with the oxygen functionality derived from water (Bossert and Compeau, 1995). Of the three compounds, the anaerobic degradation of toluene has been studied in the greatest detail.

The degradation of toluene has been coupled with the reduction of oxidised nitrogen, sulphur and transition metal species (Krumholz et al, 1996). In addition, toluene disappearance has also been reported in methanogenic aquifer slurries at several test sites in the USA (for example Wilson et al, 1990; Beller et al, 1991). Frequently, the lag period before the onset of toluene decay was 100 days or more and the rate of decay of toluene was found to be relatively slow (Krumholz et al, 1996). The microorganisms that have been reported to degrade toluene under anaerobic conditions and the electron acceptors that have been coupled with the degradation are summarised in Table 6.3. Figure 6.3 illustrates the proposed pathways for the initial anaerobic transformation of toluene.

The anaerobic degradation of ethylbenzene has not been demonstrated conclusively, but has been inferred from observations made of the reduction in ethylbenzene concentrations in anaerobic environments. The depletion of ethylbenzene has been coupled to the reduction of sulphate in groundwater (Thierrin et al 1993) and there is indirect evidence of methanogenic degradation (Cozzarelli et al, 1990). In contrast, published evidence for the metabolism of ethylbenzene under nitrate reducing conditions is inconclusive. Several authors have reported negative results for the degradation of this compound in the presence of nitrate as a terminal electron acceptor (for example, Alvarez and Vogel, 1994). The microbiology of ethylbenzene degradation has not been published. In common with many other compounds, the microbiology and biochemistry of ethylbenzene degradation have been inferred from the metabolic processes that occur in parallel with its transformation.

The biodegradation of xylene has been reported from experiments carried out using acclimated laboratory cultures (Evans et al, 1991; Hutchins, 1993). However, the results from field studies, monitoring the remediation of contaminated groundwater, have not been as positive, but there are notable exceptions. In a field injection experiment at Seal Beach, California, Ball et al (1994) demonstrated the complete removal of m-xylene from an aquifer amended with nitrate to stimulate biodegradation. Similarly, Wilson et al (1990) have reported the transformation of m and p-xylene in aquifer material from Traverse City, Michigan. Methanogic conditions were dominant in the aquifer material used in this study.

Aerobic degradation

The aerobic biodegradation of aromatic hydrocarbons has been studied extensively, but most of the reported field studies have been concerned with the remediation of contaminated soils or surface waters. Major marine oils spills, for example the loss of the Exon Valdez with the subsequent release of crude oil into pristine coastal waters, have provided experimental field sites for trials of bioremediation methods. Although the results of these, and similar studies have been published widely, their application to the aerobic remediation of groundwater is uncertain.

The aerobic degradation of toluene by microorganisms serves as a model for the degradation of aromatic hydrocarbons. Five aerobic pathways for toluene degradation have been elucidated (figure 6.4). Aerobically, the catabolism of toluene proceeds through oxygenated intermediates to produce a 1,2-dihydroxylated aromatic compound. This is then subject to ring cleavage, and the products further degraded through one of the major metabolic pathways (Zylstra, 1994). The initial steps in some of the aerobic pathways degradation of toluene are catalysed by monoxygenase or dioxygenase enzymes. The biochemistry of these enzymes, and the microorganisms that have been shown to produce them, have been described previously in relation to the cometabolic degradation of TCE. (Section 6.3.1.3).

Degradation of Polycyclic Aromatic Hydrocarbons

Many bacterial, fungal and algal strains have been shown to degrade polycyclic aromatic hydrocarbons (PAH) containing from two to five aromatic rings (Tables 6.4 to 6.7). Bacterial cultures that grow on high molecular weight PAHs, of four or more fused rings, have been reported, but the results of these studies suggest that this breadth is achieved by cometabolism (Mueller et al, 1996). In general, high molecular weight PAHs are degraded slowly by indigenous microorganisms, which is due, in part, to the strong adsorption of these compounds to organic matter, and to their low solubility in water: both factors decrease the availability of PAHs for microbial degradation (Mueller et al, 1996). To overcome these limitations significant investments have been made towards the development of methods to remove adsorbed PAHs and increase their solubility. One of the most effective ways to achieve this has been to use surface active agents (Kile and Chiou, 1989, 1990; Edwards et al, 1991). The use of surfactants may be particularly significant for the remediation of contaminated groundwater.

6.3.3 Munitions and nitrotoluenes

In general, explosives are relatively simple C-N-O compounds, often cyclic with the nitrogen atoms in azo functionality or as part of nitro groups. Probably the most widely known representatives of this class of compounds are the nitrotoluenes: 2,4,6-trinitrotoluene (TNT), 2,4-dinitrotoluene (2,4-DNT) and 2,6-dinitrotoluene (2,6-DNT) (Swoboda-Colberg, 1995). These compounds have been produced in large quantities since World War I.

During the manufacture of explosives and the disposal of old munitions, large quantities of water become contaminated. The wash water was typically disposed of in unlined lagoons that facilitated the slow release of explosives from the soil in the lagoons into groundwater, lakes and rivers (Funk, et al, 1996). Munitions compounds persist in soil and groundwater and can still be found as contaminants at sites where munitions plants were operational in World War II (Toze, 1996). The mutagenicity of TNT, as well as its toxic effects on algae and fish, humans and other vertebrates, make it a serious environmental hazard (Funk et al, 1996). TNT causes a number of ailments in humans, including headaches, weakness, aplastic anaemia and toxic hepatitis.

The mechanisms of biodegradation of nitroaromatic compounds have been studied in both aerobic and anaerobic environments. One of the problems that has emerged from this work is that frequently these compounds are not completely metabolised, instead intermediate compounds may result via 'dead-end' pathways. This can lead to the accumulation and persistence of metabolic products in the environment, some of which may be toxic (Crawford, 1995).

Biochemical and microbiological data have been accumulated from laboratory experiments, and the use of microcosms to simulate the conditions at contaminated field sites. Degradation of TNT by the fungus *Phanerochaete chrysosporium* has been reported by several groups of workers (Bumpus and Tatarko, 1994; Fernando et al, 1990; Michels and Gottschalk, 1994; Stahl and Aust, 1993), but there is little evidence for the complete and productive degradation of TNT by bacteria (Vorbeck, et al, 1998).

Anaerobic microorganisms initiate the degradation of nitroaromatic compounds by reduction of the nitro groups. Preuss and co-workers (1993) observed the sequential reduction of the nitro groups on TNT by *Desulfovibrio* sp. in the presence of pyruvate and sulphate. TNT was shown to be the sole nitrogen source for this organism. Other workers have reported the reduction of nitro groups by other bacterial species. Gorontzy et al (1993) have shown that sulphate reducing bacteria, methanogenic bacteria and *Clostridia* will reduce the aromatic nitro groups to amino groups. However, no further conversion of the reduced compounds was observed.

There are relatively few reported case studies of the bioremediation of groundwater contaminated with munitions compounds. Toze, (1996) describes a study being carried out by the CSIRO Division of Water Resources at a decommissioned munitions processing plant in Australia. The underlying groundwater is anaerobic and situated in a semi-confined fractured basalt aquifer. Low concentrations of some of the breakdown products of TNT were detected in the groundwater, indicating that natural biodegradation of the munitions compounds probably occurred. Further analysis of the degradative processes have been carried out using microcosm studies with microbial consortia isolated from the soil and groundwater at the factory site. These studies have shown that microorganisms in the groundwater are capable of using TNT, 2,4-DNT and 2-NT, 3-NT and 4-NT as sole carbon and/or energy sources. The addition of carbon, phosphate or oxygen to the microcosms significantly increased the rate of transformation of these compounds.

6.4 Summary of Methods Used For The Remediation of Contaminated Groundwater

The remediation of many contaminants will occur naturally in some, if not all environments. However, natural remediation is frequently slow, or progresses only as far as metabolic intermediates that may be either toxic to the microorganisms, or exert a negative feedback upon preceding steps of the degradative pathway. In many cases, therefore, the successful bioremediation of a polluted groundwater will require some intervention to enhance the natural process. The intervention may take place in situ: treating the groundwater in the aquifer, or ex situ: abstracting the groundwater for treatment and then returning the treated water to the aquifer (frequently refered to as pump and treat systems). In situ bioremediation methods appear most often inpublished studies of the treatment of groundwater, and are the most likely to produce an impact on the microbiology of the groundwater.

6.4.1 Insitu bioremediation

In its simplest form, in situ bioremediation may proceed without any external intervention. Some polluted groundwater will contain adequate quantities of nutrients, electron acceptors and energy and carbon sources to support a metabolically active indigenous microbial population. The introduction of a pollutant into the system provides selective pressure for those organisms that will degrade the compound. This type of bioremediation has been referred to as *passive bioremediation* or natural attenuation. Passive bioremediation has been widely reported (for example Thierrin et al, 1995). Table 6.10 provides a summary of the compounds that have shown to degrade by passive bioremediation.

In situ groundwater bioremediation is most widely recognised as a technology that encourages the growth and reproduction of indigenous microorganisms to enhance the biodegradation of organic constituents in the saturated zone (Anon, 1995). Frequently this involves a delivery system for providing one or more of the following: an electron acceptor (oxygen, nitrate), nutrients (nitrogen, phosphorus) and an energy source (carbon). Braddock and co-workers (1997) have effectively demonstrated the importance of nutrient addition to groundwater at a petroleum-contaminated site in Northern Alaska. The study site was at a former naval research laboratory where accidental release of approximately 1300m^3 of various types of fuel had occurred in the vicinity of the airstrip. Both the groundwater and the soil showed evidence of petroleum hydrocarbon contamination over 20 years after the pollution incidents (Braddock and McCarthy, 1996). Nitrogen and phosphorus were added to the soil and groundwater in an attempt to stimulate microbiological activity (Braddock et al, 1997). Their analyses showed that nitrogen was found to be the limiting nutrient in the system, but the microbial population was maximally enhanced by the additions of both nitrogen and phosphorus. However, simply increasing the concentration of nutrients did not yield a comparable increase in the activity of the microorganisms. Braddock et al (1997) measured the effect of adding three concentrations of nutrient supplement and observed the greatest stimulation of microbial activity at the lowest, rather than the highest, level of nutrient addition. A similar, inverse relationship between nutrient concentration and the activity of indigenous populations of microorganisms has been observed during the cometabolism of TCE and other HOCs (Fries et al, 1997b). These reports, and others, emphasise the importance of understanding the effect of nutrient addition at specific sites to enhance successful in situ bioremediation.

A particular advantage of in situ bioremediation of groundwater is that it can be combined with other saturated zone remedial technologies (for example air sparging) and unsaturated zone remedial operations (for example soil vapour extraction and bioventing) (Anon, 1995).

6.4.2 Bioaugmentation

The remediation of contaminated groundwater using *in situ* biostimulation may not always be the most appropriate technique: suitable microorganisms may not be present at the site, or may be present in such low numbers that they take too long to become established. Bioaugmentation is a more extreme form of microbiological intervention to encourage in situ bioremediation. For this technique, microorganisms are selected for their ability to degrade the pollutant that is of concern and are grown in large numbers in fermenters within a laboratory. The microbiological cultures are added to the contaminated groundwater at injection wells or infiltration galleries. Nutrient formulations may be added simultaneously to help the organism establish and maintain itself in the groundwater. Both pure and mixed cultures have been used to augment the indigenous population of microorganisms.

There are few published examples of the use of bioaugmentation in the field. Pritchard (1992) has reviewed the use of microbial inoculation in bioremediation. All of the examples described in the review involve the inoculation of microbiological cultures into soil and surface water. Pritchard concludes that, in general, the trials have shown little effect upon the target chemical and/or there has been insufficient cause and effect information to demonstrate clearly that enhanced biodegradation was responsible for decreases in the target chemicals. Where measurements have been made of the fate of the inoculum, they show that the introduced organisms die off quickly in the field.

Other advantages and disadvantages of this system are summarised in Table 6.8. Bioaugmentation has the potential to become a valuable remediation technique, provided that it is well researched and applied in controlled and appropriate circumstances. The remediation of pesticide contaminated soils and groundwater is probably one of the best potential applications of this technology since, depending on the pesticide, natural enrichment of degrader populations does not often occur in the field.

6.4.3 Pump and treat technology

The use of bioreactors to remediate polluted groundwater has been discussed in several publications (for example Naidu et al, 1996). In practice the method involves pumping the polluted groundwater from one or more wells, treatment to remove residual dissolved constituents, and then passing the treated groundwater through one or more containment systems which retain the active microorganisms or their constituents. Many different designs for these systems have been proposed, and a few have been tried in the field. However, most of the designs are the subject of commercial confidentiality and are not available for reproduction in this report.

Both whole microorganisms and purified enzyme complexes have been used in laboratory scale models of bioreactors. The microorganisms, and the enzymes, can be maintained in the liquid phase in the reactor, or immobilised on surfaces. Rotating drum treatment systems have been used successfully in package treatment plants (see section 5), and similar processes have been used for the treatment of polluted water systems.

Since the bioreactors are contained systems the conditions in the reactor can be controlled very precisely to optimise the degradation of the groundwater contaminant. Temperature fluctuations can be avoided, and nutrient concentrations can be constantly monitored and

maintained at the optimum level for the microorganism being used in the reactor. Consequently, treatment times may be reduced when compared with the in situ methods of bioremediation. Furthermore, the bioreactor is designed to contain the microorganism, and if adequately maintained, will prevent the release of microorganisms into the environment. The microbiological impact of properly designed, and operated bioreactor systems on groundwater microbiology is expected to be minimal.

6.5 Conclusions

Microorganisms have been used to treat polluted water for at least 100 years. Indeed, sewage treatment is still the most important example of a managed biological treatment process. During the last twenty years the application of bioremediation methods for the treatment of other polluted environmental systems has been recognised and adopted by industry particularly in the U.S.A. as a cost-effective alternative to physical or chemical treatment methods. In an era of environmental awareness, bioremediation gives the perception of being a 'green' technology. Biotechnology companies emphasise that bioremediation overcomes the need for relocating the pollution to a landfill site, or transferring the pollutant to another environmental medium by incineration. Furthermore, the technology exploits the potential of indigenus microorganisms to transform pollutants into harmless products. In practice, bioremediation will succeed as a viable method in circumstances where it is the only practical solution, or it represents an economic benefit to industry. These benefits are most evident in the remediation of groundwater pollution.

The search for information to carry out this review was wide ranging, and undertaken without any intention to favour one type of study over another and included approaches to several UK companies (Table 6.9). However, the product of the search was heavily biased towards the methodology and biochemistry of bioremediation and the reports of laboratory microcosm studies. Very few examples were found of studies that attempted to measure the impact of the bioremediation process on groundwater microbiology. Such omissions may be anticipated: microbial ecology is a difficult subject and it is understandable if research groups avoid the topic when the principal aim of their work is to demonstrate remediation of a compound in groundwater. Indeed, it may be argued that if bioremediation leads to a significant improvement in the quality of a polluted groundwater and is safe, it is not necessary to fully understand the microbiological processes that are involved.

Although supporting evidence is sparse, a pollution event, and the intervention to bioremediate the pollutant, will have an impact on the groundwater microbiology. The physical and chemical conditions in a groundwater are not static, but inclined to fluctuate to a greater or lesser extent. As a result, the indigenous population of microorganisms may withstand some temporary change to the normal environmental conditions. In contrast a pollution event will have a major impact upon the quality of a groundwater, which may be sufficient to induce changes within the indigenous population of microorganisms. If the pollutant is toxic, it may be anticipated that the density of microorganisms in the groundwater will decline, leaving only those organisms that are resistant to the effects of the pollutant and others that have the capacity to transform the pollutant. A pollutant that is non-toxic may exert different selective pressures upon the indigenous population of microorganisms (Section 2), but produce a similar outcome by favouring a few species at the expense of others.

Bioremediation will have both direct and indirect effects upon the microbiology of groundwater. Techniques used for in situ bioremediation of groundwater deliberately set out to stimulate the growth and activity of a sub population of the indigenous microorganisms.

Bioaugmentation involves the direct introduction of selected and cultured species, or consortia, into the polluted groundwater to enhance remediation. When remediation is complete, and the additives that have been used to enhance remediation have been withdrawn, the conditions in the groundwater will change, and, possibly, revert to the conditions that prevailed before the groundwater was polluted. Once again, these changes will exert selective pressure on the microbial populations.

There can be no doubt that the pollution of groundwater and the subsequent bioremediation of the pollution will have an impact upon groundwater microbiology. However, there is a paucity of published case studies that can be used to assess the magnitude or nature of the microbiological impact, even at the most basic level. Until this information is available it will not be possible to carry out a meaningful risk assessment of groundwater bioremediation methods.

Several UK companies were approached to request information about bioremediation projects (Table 6.9). We did not receive a sufficient response from the selection of companies approached during this project to make a judgement of the commercial viability of groundwater bioremediation technology. Indeed only two companies replied. Kuaerner-Water have licenced a process for the treatment of borehole water for potable use by oxidising iron and manganese *in situ* using well injection to promote bacteriological activity. They report significant market resistance to the introduction of the process. However the recent proposal to concentrate housing development at brown field sites in the UK may provide the necessary stimulus to increase the application of groundwater bioremediation.

The groups of compounds that have attracted the greatest amount of research interest in the area of bioremediation are those, in general, which are restricted to groundwater in urban areas e.g. Chlorinated Aliphatics, BTEX. It would be reasonable to expect, therefore, that groundwater bioremediation projects will be concentrated in these areas. Consequently, any potential microbiological impact from bioremediation projects will be most acute in urban groundwater. While the principal source of groundwater for potable supplies is derived from rural areas, the impact of bioremediation projects will be minimal.

As rural sources of groundwater decline, and water authorities seek alternative sources closer to urban areas, the potential will emerge for bioremediation projects to impact upon public supplies. When this situation arises the Environment Agency must have the appropriate information available to be able to balance the risks from the microbiological impacts of bioremediation against those that occur due to the presence of the pollutant.

6.6 Recommendations For Future Work

Fundamental research is required to produce data that can be used to predict the impact of bioremediation methods on the microbiology of groundwater. As a priority, the Environment Agency should obtain detailed microbiological and chemical characterisatics before, during and after remedial action has been taken. To achieve this, development of appropriate continuous groundwater monitoring technologies is required.

The EA should attempt to maintain a database of groundwater bioremediation projects in the UK. Many projects are subject to commercial confidentiality and were not available to this project. If it is within their remit, the EA should insist that summary data from these projects is passed to the National Groundwater and Contaminated Land Centre for analysis and storage. This information will allow the EA to co-ordinate similar studies that are being

carried out at different sites to determine how representative the data from a single study can be.

Studies should also include monitoring of the medium and long-term impacts of bioremediation methods on the microbial ecology of groundwater systems.

Assessment of health and safety standards applied in bioremediation projects, need to be done, particularly where microbiological cultures are being handled on site.

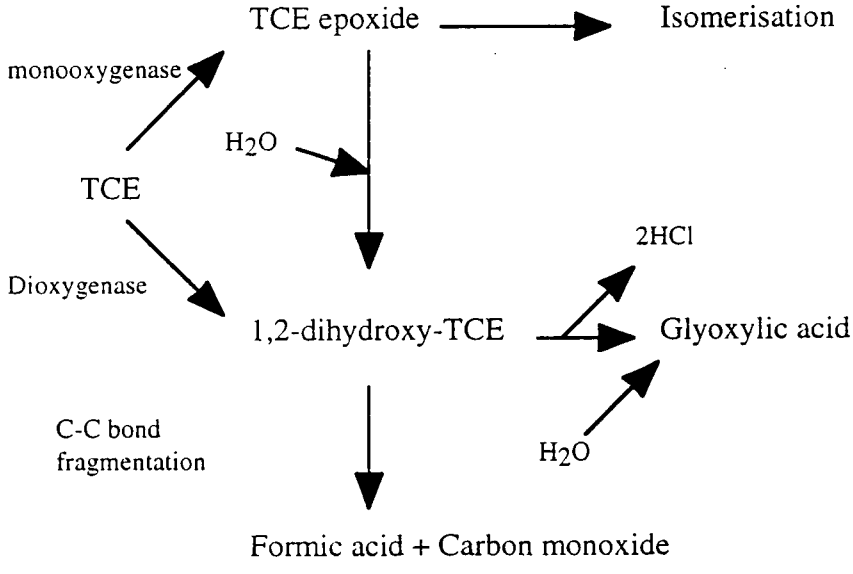


Fig 6.1. Theoretical pathways of TCE oxidation by monooxygenases and dioxygenases (from: Wackett, 1995)

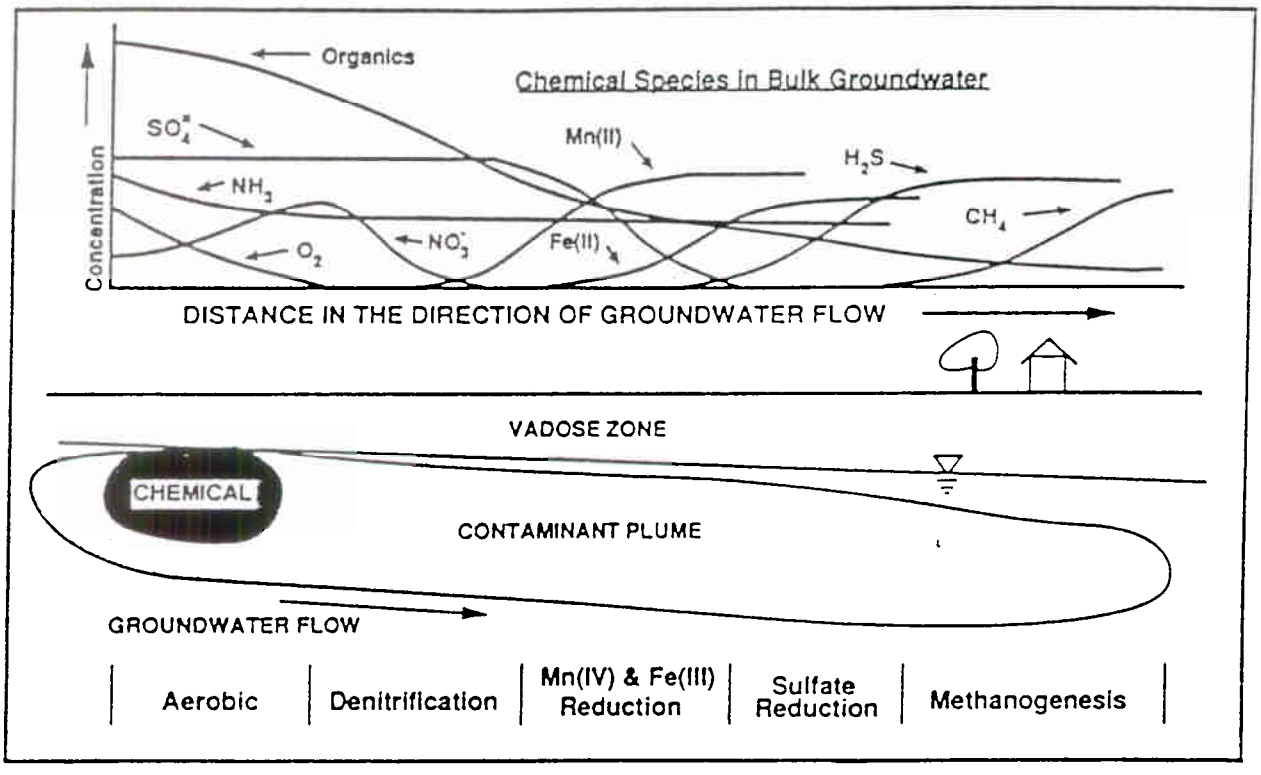


Figure 6.2. Possible microbially mediated changes in the chemical species and redox conditions in the direction of groundwater flow in the presence of organic contaminants (Bouwer, 1992)

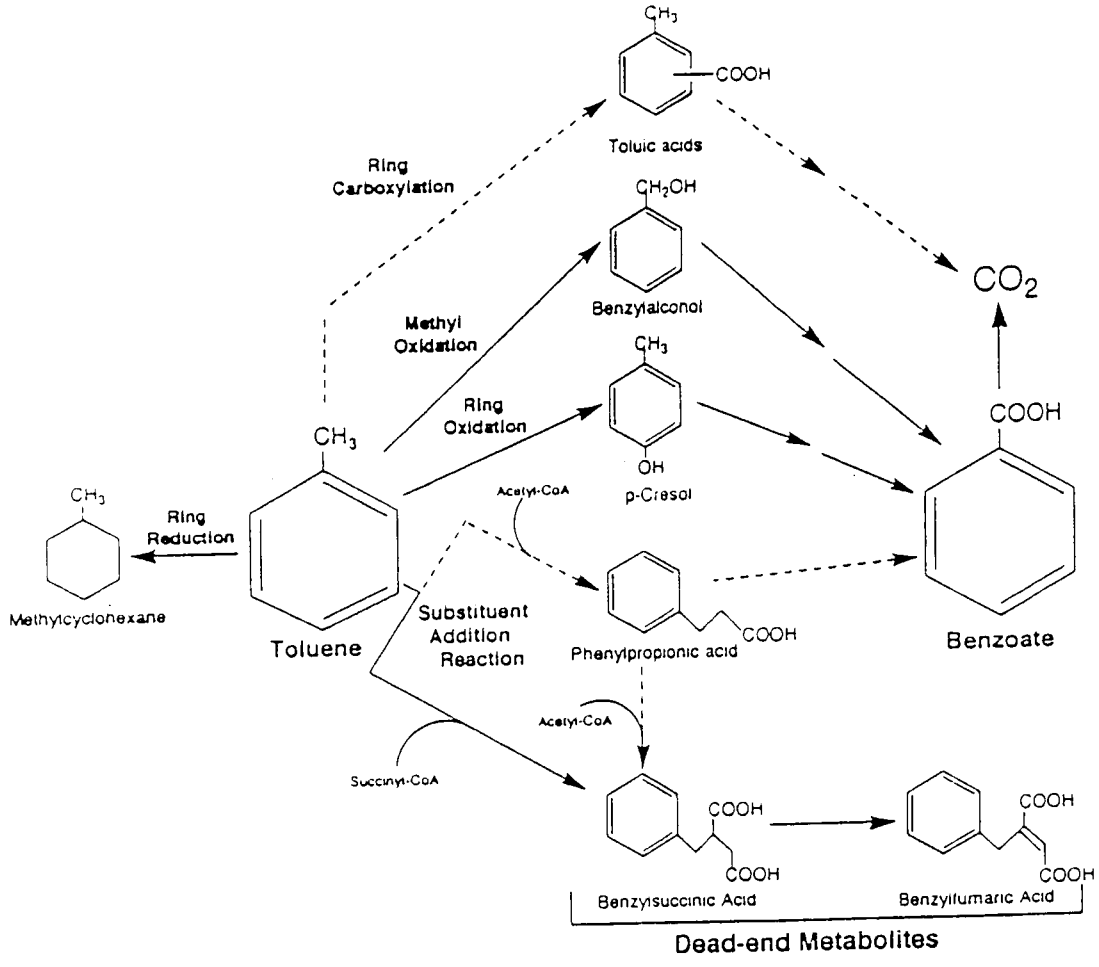


Figure 6.3. Proposed pathways for the initial anaerobic degradation of toluene. The dashed lines indicate pathways for which the identification of the intermediate is based on indirect evidence (Krumholz et al, 1996)

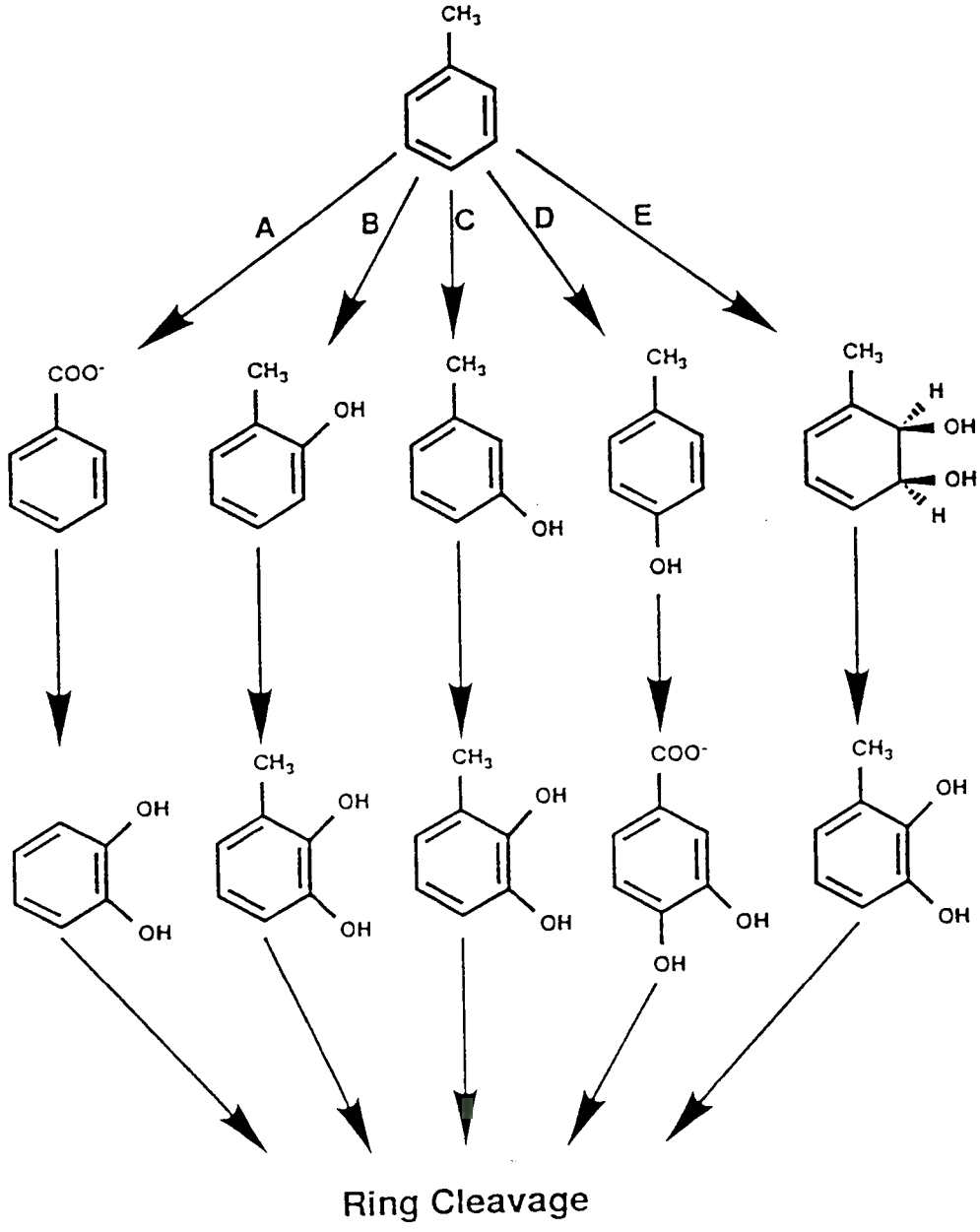


Figure 6.4. Catabolic pathways for the aerobic degradation of toluene (Zylstra, 1994)

Table 6.1. Oxygenases and organisms implicated in TCE oxidation and their relative *in vivo* rates (from Wackett, 1995)

Organism	Enzyme	Rate (nmol/min/mg)
Methylosinu trichosprium OB3b	Soluble methan monooxygenase	20-150
Burkholderia (Pseudomonas) cepacia G4	Toluene 2-monoxygenase	8
Nitrosomonas europeaea	Ammonia monooxygenase	1
Methylocytis parvus OBBP	Particulate methane monooxygenase	0.7
Mycobacterium sp	Propane monooxygenase	0.5
Alcaligenes eutrophus JMP 134	Phenol hydroxylase	0.2
Rhodococcus erythropolis	Isoprene oxygenase	0.2

Table 6.2. Concentration of hydrocarbons in water after contact with distillate products (Chapelle, 1993).

		Detection limit	Kerosene	Diesel	Fuel Oil
Benzene	µg/l	0.5	294	344	203
Ethylbenzene	µg/l	0.5	19	139	100
Toluene	µg/l	0.5	870	777	509
Xylenes	µg/l	0.5	1260	875	592
M-tert-but.Ether	µg/l	0.5	BDL	BDL	BDL
Ethylene-Dibromide	µg/l	0.005	BDL	BDL	BDL
Tot.pet.Hydrocarbons	µg/l	0.2	40	16.8	21.1
Napthalene	µg/l	0.5	356	6.6	40
1-methyl naphthalene	µg/l	0.5	193	66.2	107
2-methyl naphthalene	µg/l	0.5	225	108	152
Total BTEX	µg/l		2240	2140	1400
Total naphthalene	µg/l		774	181	299

Table 6.3. Isolates that have been documented to degrade toluene under anaerobic conditions (Krumholz *et al*, 1996)

Organism (strain)	Electron acceptor	Concentration (max)	Degradation rate
Geobacter metallireducens	Fe(III)	10mM	dt=18d(approx doubling time)
Pseudomonas sap. Strain T	NO_3, N_2O	0.3mM	5-12 µmol/min/l
Strain T1	NO_3, N_2O	3.0mM	1.8 µmol/min/l gr=0.14h^{-1}
Strain T1-pUK45-10C	NO_3	0.4mM	95 µmol/l/h
Pseudomonas sp. Strain SP	NO_3	0.5mM	nd
Pseudomonas sp. Strain K172	NO_3, N_2O	2.0mM	dt=24h 20-50 µmol/min/g
Pseudomonas sp. Strain S100	NO_3, N_2O	2.0mM	nd
Pseudomonas sp. Strain S2	NO_3, N_2O	2.0mM	nd
NO_3	0.5mM	nd	
Desulfobacula toluolica	SO_4	0.5mM	dt=27h
Azoarcus sp. Strain Tol-4	NO_3	0.54mM	dt=8-13h
Azoarcus sp. Strain Td-1	NO_3	0.54mM	dt=6-7h
Azoarcus sp. Strain Td-2	NO_3	0.54mM	dt=5-7h
Azoarcus sp. Strain Td-3	NO_3	0.54mM	dt=7-8h
Azoarcus sp. Strain Td-15	NO_3	0.54mM	dt=6-7h
Azoarcus sp. Strain Td-17	NO_3	0.54mM	dt=5-7h
Azoarcus sp. Strain Td-19	NO_3	0.54mM	dt=5-7h
Azoarcus sp. Strain Td-21	NO_3	0.54mM	dt=5-7h
strain PRTOL1	SO_4	nd	nd

dt = doubling time

a = approximate doubling time

nd = not determined

gr = growth rate

Table 6.4 Representative polycyclic aromatic hydrocarbons metaloised by different species of bacteria (Mueller et al, 1996)

Compound	Organisms
Napthalene	*Acinetobacter calcoacetius, Alcaligenes denitrificans, Mycobacteiumsp., Pseudomonas fluorescens, Pseudomonas pancimobilis, Pseudomonas vesicularis, Pseudomonas cepacia, Pseudomonas testosteroni, Rhodococcus sp., Corynebacterium renale, Moraxella sp., Streptomyces sp., Bacillus cereus*
Acenapthene	*Beijerinckia sp., Pseudomonas putida, Pseudomonas fluorescens, Pseudomonas cepacia, Pseudomonas sp.*
Anthracene	*Beijerinckia ap., Mycobacterium sp., Pseudomonas putida, Pseudomonas paucimobilis, Pseudomonas fluorescens, Pseudomonas cepacia, Rhodococcus sp., Flavobacterium sp., Arthrobacter sp.*
Phenanthrene	*Aeromonas sp., Alcaligenes faecalis, Alcaligenes denitrificans, Arthrobacter polychromogenes, Beijerinckia sp., Micrococcus sp., Mycobacterium sp., Pseudomonas putida, Pseudomonas paucimobilis, Rhodococcus sp., Vibrio sp., Nocardia sp., Flavobacterium sp., Streptomyces sp., Streptomyces griseus, Acinetobacter sp.*
Fluoranthene	*Alcaligenes denitrificans, Mycobacterium sp., Pseudomonas putida, Pseudomonas paucimobilis, Pseudomonas cepacia, Rhodococcus sp., Pseudomonas sp.*
Pyrene	*Alcaligenes denitrificans, Mycobacterium sp., Rhodococcus* sp.
Chrysene	*Rhodococcus* sp.
Benz [a] anthracene	*Alcaligenes denitrificans, Beijerinckia sp., Pseudomonas putida*
Benz [a] pyrene	*Beijerinckia sp., Mycobacterium* sp.

Table 6.5. Representative polycyclic aromatic hydrocarbons metabolised by different species of fungi (Mueller et al, 1996)

Compound	Organisms
Napthalene	*Absida glauca, Aspergillus niger, Basidiobolus ranarum, Candida utilis, Choanephora campincta, Circinella sp., Claviceps paspali, Cokeromyces poitrassi, Condiobolus gonimodes, Cunninghamella bainieri, Cunninghamella elegans, Cunnunghamella japonica, Emercellopsis sp., Epiciccum nigrum, Gilbertella persicaria, Gliocladium sp., Helicostylum piriforme, Hyphochytrium carenoides, Linderina pennispora, Mucor hiemalis, Neurospora crassa, Panaeolus cambodigensism, Panaeolus subalteatus, Penicillium chrysogenum, Pestalotia sp., Phlyctochtrium reinboldate, Phycomyces blakesleeanus, Phytphthora cinnamomi, Psilocybe cubensis, Psilocybe strictipes, Psilocybe stunzii, Psilocybe subaerunginascens, Rhizophlyctis harderi, Rhizophlyctis rosea, Rhixpus pryzae, Rhizopus stolonifer, Saccharomyces cerevisiae, Saprolegina parasitica, Smittium culicis, Smittium culisetae, Smittium simulii, Sordaria finicola, Syncephalastrum racemosum, Thamnidium anomalum, Zygorchynchus moelleri*
Acenaphthene	*Cuninghamella elegans*
Anthracene	*Bkerjandera ap., Cunnighamella elegans, Phanerochaete chrysosporium, Ramaria sp., Rhizoctonia solani, Trametes versicolor.*
Phenanthrene	*Cunninghamella elegans, Phenerochaete chrysosporium, Trametes visicolor*
Fluoranthene	*Cunninghamella elegans*
Pyrene	*Cunninghamella elegans, Phanerochaete chrysoporium, Crinipellis stipitaria*
Benz[a]anthracene	*Cunninghamella elegans*
Benz[a]pyrene	*Aspergillus ochraceus, Bjerkandera adusta, Bjerkandera sp., Candida maltosa, Candida tropicalis, Chrysosporium pannorum, Cunninghamella elegans, Mortierella verrucosa, Neurospora crasse, Penicillium sp., Phanerochaete chrysoporium, Ramaria sp., Saccharomyces cerevisiae, Trametes versicolor, Trichoderam viride*

Table 6.6. Representative polycyclic aromatic hydrocarbons metabolised by different species of cyanobacteria and algae (Mueller et al, 1996)

Compound	Organisms
Napthalene	*Oscillatoria sp. (strain JCM), Oscillatoria sp.(strain MEV), Microcoleus chthonoplastes, Nostoc, sp., Anabens sp. (strain CA), Anabena sp. (Strain IF), Agmenellum quadruplicatum, Coccochloris elabens, Aphanocapsa sp., Chlorella sorokiniana, Chlorella autotrophica, Dunaliella tertiolecta, Chlamysomonas angulosa, Ulva fasciata, Cylidrotheca sp., Synedra sp., Navicula sp., Porphyridium cruentum.*
Phenanthrene	*Oscillatoria sp. (Strain JCM), Agmenellum quadruplicatum*
Benzo[a]pyrene	*Selenastrum capricornutum*

Table 6.7. Microorganisms that metabolise aromatic hydrocarbons (Rosenberg and Ron, 1996)

Bacteria:	
Pseudonomas	*Aeromonas*
Moraxella	*Beijerinckia*
Flavobacteria	*Achrobacteria*
Nocardia	*Corynebacteria*
Acinetobacter	*Alcaligenes*
Mycobacteria	*Rhodococci*
Streptomyces	*Bacilli*
Arthrobacter	*Aeromonas*
Cyanobacteria	
Fungi:	
Chiridomycetes	*Oomycetes*
Zygomycota	Ascomycota
Basidiomycota	Deuteromycota
Microalgae:	
Porphyridium	*Petalonia*
Diatoms	*Chlorell*
Dunaliella	*Chlamydonomas*
Ulva	

Table 6.8: Advantages and disadvantages of in situ bioremediation of groundwater (from Anon, 1995)

Advantages	Disadvantages
Remediates contaminants that are adsorbed onto or trapped within the geologic materials of which the aquifer is composed along with contaminants dissolved in groundwater	Injection wells and/or infiltration galleries may become plugged by microbial growth or mineral precipitation
Application involves equipment that is widely available and easy to install	High concentrations of low solubility constituents may be toxic and/or not bioavailable
Creates minimal disruption and/or disturbance to on-going site activities	Difficult to implement in low permeability aquifers
Time required for subsurface remediation may be shorter than than other approaches (eg pump and treat)	Re-injection wells or infiltration galleries may require permits or may be prohibited. In the US some states require a permit for air injection into groundwater
Generally recognised as being less other remedial options	May require continuous monitoring and costly than maintenance
Can be combined with other technologies to enhance site remediation	Remediation may only occur in more permeable layers or channels within the aquifer
In many cases this techniques does not produce waste products that must be disposed of	

Table 6.9 UK companies that have been approached for information

Organisation	Title	Address
Abzorboil	Technical Director	11 Simon Evans Close, Cleobury, Mortimer, Kidderminster DY14 8AX
Alpha Environmental Systems Ltd	Technical Director	Unit 32 Loughanhill Ind Est, Coleraine, Co Londonderry BT52 2NR
Edge Group	Technical Director	Liburn House, 52 Dundonald Road,RamsgateKent CT11 9PU
Global Environmental	Technical Director	Thorncliffe Hall,Thorncliffe Park, Chapletown, Sheffield S35 2PQ
Hydroklear UK Ltd	Technical Director	Unit M, Floors St Floors St Ind Est, Johnstone, Services Renfrewshire, PA5 8PE
Mourik UK Ltd	Technical Director	Unit 2, Buckwins Sq, Basildon, Essex, SS13 1BJ
Alba Int Ltd	Technical Director	Leading Light Building, Midgeley, Goldsborough. N Yorks HG5 8NY
Bechtel	Technical Director	245 Hammersmith Rd, London, W6 8ZD
Biffa Waste Services	Technical Director	Potters Lane, Wednesbury, W Midlands, WS10 7NR
Bio-logic Remedation	Technical Director	Festival Business Centre, 150 Brand Street, Glasgow, Strathclyde, G51 1DH
Bio-Logix Environmental Ltd	Technical Director	International Centre, Sprindle Way, Crawley, W Sussex
Celtic Technologies Ltd	Technical Director	CBT Centre Road, Senghenydd, Cardiff, CF2 4AY
Davy International	Technical Director	Ashmore House, Richardson Road, Stockton on Tees Cleveland, TS18 3RE

Table 6.10: Compounds with potential for natural attenuation (from Spain 1997)

Hydrocarbons	Vinyl chloride
Acetone	1,2-Dibromoethane
Methylethyl ketone	Polychlorinated biphenyls
Chlorobenzene	Nitrobenzene
Dichlorobenzenes	Nitrotoluenes
1,2,4-Trichlorobenzene	Dinitrotoluenes
1,2,4,5-Tetrachlorobenzene	1,3-Dinitrobenzene
Chlorophenols	Nitrophenols
Pentachlorophenol	2,4-Dinitrophenol
Methylene chloride	Picric acid
1,2-Dichloroethane	Nitrobenzoic acids
Perchloroethylene	Nitroglycerin
Trichloroethylene	Pesticides
Dichloroethylene	Aniline

6.7 References

Adiraens . P. and Vogel, T.M. (1995) Biological treatment of chlorinated organics. In Young L.Y. and Cerniglia, C.E. (eds) Microbial Transformation and Degradation of Toxic Organic Chemicals. Wiley-Liss . pp 435-487.

Alvarez, P.J.J. and Vogel, T.M. (1994) Degradation of BTEX and their aerobic metabolites by indigenous microorganisms under nitrate reducing conditions. Abstracts of the 94th Annual Meeting, American Society for Microbiology, Q-339

Anon. (1995) How to evaluate alternative clean-up technologies for underground storage tank sites: A guide for corrective action plan reviewers. US EPA 510-B-95-007.

Atlas, R.M. (ed.) (1984) Petroleum Microbiology. New York: Macmillan Publishing.

Ball, H.A. Reinhard, M. and McCarty, P.L. (1991) Biotransformation of monoaromatic hydrocarbons under anoxic conditions. In: Hinchee R.E. and Olfenbuttel (eds) In Situ Bioreclamation: Applications and Investigations for Hydrocarbon and Contaminated Site Reclamation. Butterworth-Heinemann.

Ball, H.A., Hopkins, G.D., Orwin, E. and Reinhard, M. (1994) Anaerobic bioremediation of aromatic hydrocarbons at Seal Beach, CA: Laboratory and field investigations. Environmental Protection Agency Symposium on Intrinsic Bioremediation of Groundwater, August 30 - September 1, 1994, Denver, Colorado.

Barrett, M.H., Gikas, L. and Lerner, D.N. (1994) The effects of cosolutes and cosolvents on the adsorption of BTEX compounds from groundwater. In 'Transport and Reactive Processes in Aquifers' ed. Dracos, T.H. and Stauffer, F. pub. Balkema. p125-129.

Barrett, M.H., Yang, Y., Lerner, D.N., French, M.J. and Tellam, J.H. (1997) The impact of cities on the quantity and quality of their underlying groundwater. Final Project Report to the EPSRC, Environment Agency, Severn Trent Water plc, Stanton plc and MAFF.

Beller, H.R., Edwards, E.A., Grbic-Galic, D., Hutchins, S.R. and Reinhard, M. (1991) Microbial degradation of alkylbenzenes under sulphate reducing and methanogenic conditions. United States Environmental Protection Agency Project Summary, EPA/600/S2-91/027.

Bonaventura, C. and Johnson, F.M. (1997) Healthy Enviroments for healthy people: Bioremediation today and tomorrow. Environmental Health Perspectives, 105, 5-20.

Bossert, I.D. and Compeau, G.C. (1995). Cleanup of petroleum hydrocarbon contamination in soil. In: Young, L.Y. and Cerniglia, C.E. (eds) Microbial Transformation and Degradation of Toxic Organic Chemicals. Wiley-Liss. pp 77-125

Bouwer, E.J (1992) Bioremediation of Organic contaminants in the subsurface. In: Mitchell, R. Environmental Microbiology. Wiley Liss.

Bouwer, E.J., Rittmann, B.E. and McCarty, P.L. (1981) Anaerobic degradation of halogenated 1- and 2- carbon organic compounds. Environmental Science and Technology, 15, 596-599.

Bouwer, E.J. and McCarty, P.L. (1983) Transformation of 1- and 2- carbon halogenated aliphatic organic compounds under methanogenic conditions. Applied and Environmental Microbiology, 45, 1286-1294.

Bowlen, G.F. And Kosson, D.S. (1995) In Situ processes for bioremediation of BTEX and petroleum fuel products. In Young, L.Y., and Cerniglia, C.E. (Eds) Microbial Transformation and Degradation of Toxic Organic Chemicals. Wiley-Liss. Pp 515-542.

Braddock, J.F. and McCarthy, K.A. (1996) Hydrologic and microbiological factors affecting persistence and migration of petroleum hydrocarbons spilled in a continuous permafrost region. Environmental Science and Technology, 30, 2626-2633.

Brock, T.D., Smith, D.W. and Madigan, M.T. (1984) Biology of Microorganisms, 4th ed. Prentice-Hall, Englewood Cliffs, N.J.

Bumpus, J.A. and Tatarko, M. (1994) Biodegradation of 2,4,6-trinitortoluene by *Phanerochaete chrysosporium*: identification of initial degradation products and the discovery of a TNT metabolite that inhibits lignin peroxidase. Current Microbiology, 28, 185-190.

Burston, M.W., Mazari, M.M., Bishop, P.K., and Lerner, D.N. (1993) Pollution of groundwater in the Coventry region (UK) by chlorinated hydrocarbon solvents. Journal of Hydrology, 149, 137-161.

C.I.A. (1993) Contaminated land and land remediation – guidance on the issues and techniques. The Overview. Chemical Industries Association.

Chapelle, F.H. (1993) Ground-water microbiology and geochemistry. John Wiley and Sons, New York pp 424

Cozzarelli, I.M., Eganhouse, R.P. and Baedecker, M.J. (1990) Transformation of monoaromatic hydrocarbons to organic acids in anoxic groundwater environment. Environmental Geology and Water Science, 16, 135-141.

Cozzarelli, I.M., Baedecker, M.J. Eganhouse, R.P. and Goerlitz, D.F. (1994) Transformation of monoaromatic hydrocarbons to organic acids in anoxic groundwater environment. Environmental Geology and Water Science 16, 135-141.

Crawford, R.L. (1995) Biodegradation of nitrated munitions compounds and herbicides by obligately anaerobic bacteria. In: Spain, J.C. (Ed) Biodegradation of Nitroaromatic Compounds. Plenum Press, New York.

Christensen, T.H., Kjeldsen, P., Albrechtsen, H-J., Heron, G., Nielsen, P.H., Bjerg, P.L. and Holm, P.E. (1994) Attenuation of landfill leachate pollutants in aquifers. Critical Reviews in Environmental Science and Technology, 24, 119-202.

Dalton, H., Prior, S.D., Leak, D.J., and Stanley, S.H. (1984) Regulation and control of methane monoxygenase. In: Microbial Growth on Cl Compounds. . Proceedings of the Fourth International Symposium, R.L. Crawford and R.S. Hansen, eds. American Society for Microbiology, Washington DC, pp75-82.

Davis, J.B. (1967) Petroleum Microbiology. New York: Marcel Dekker.

De Stefano, T.D., Gossett, J.M. and Zinder, S.H. (1991) Reductive dechlorination of high concentrations of tetrachloroethene to ethene by an anaerobic enrichment culture in the absence of methanogenesis. Applied and Environmental Microbiology, 57, 2287-2292.

Dean, B.J. (1985) Recent findings on the genetic toxicology of benzene, toluene, xylenes and phenols. Mutant Research, 154, 153-181.

Dominco, P.A. and Schwarz, F.W. (1990) Physical and Chemical Hydrogeology, Wiley and Sons, New York. pp 824.

Edwards, D.A., Luthy, R.G. and Liu, Z. (1991) Solubility of polycyclic aromatic hydrocarbons in micellar nonionic surfactant solutions. Environmental Science and Technology, 25, 127-133.

Ember L. (1992) Toxic release inventory data show steady drop in emissions. Chemical Engineering News, 70, 13-15.

Energy Information Administration (1992) International Energy Annual. Washington D.C: Department of Energy.

Evans, W.C. and Fuchs, G. (1988) Anaerobic degradation of aromatic compounds. Annual Reviews of Microbiology, 42, 289-317.

Evans, P.J., Mang, D.T., and Young, L.Y. (1991) Degradation of toluene and m-xylene and the transformation of o-xylene by denitrifying enrichment cultures. Applied and Environmental Microbiology, 57, 450-454

Fan , S and Scow, K.M. (1993) Biodegradation of trichloroethene and toluene by indigenous microbial populations in soil. Applied and Environmental Microbiology, 59, 1911-1918.

Fernando, T., Bumpus, J.A. and Aust, S.D. (1990) Biodegradation of TNT (2,4,6-trinitrotolunene) by *Phanerochaete chrysosporium.* Applied and Environmental Microbiology, 56, 1666-1671

Fetter, C.W. (1993) Contaminant hydrogeology, Prentice Hall, New Jersey. pp 458.

Freedman, D.L. and Gossett, J.M. (1989) Biological reductive dechlorination of tetrachloroethene and trichloroethene under methanogenic conditions. Applied and Environmental Microbiology, 55, 2144-2151.

Fries, M.R., Hopkins, G.D., McCarty, P.L., Forney, L.J. and Tiedje, J.M. (1997a) Microbial succession during a field evaluation of phenol and toluene as the primary substrates for trichloroethene cometabolism. Applied and Environmental Microbiology, 63, 1515-1522.

Fries, M.R., Forney, L.J. and Tiedje, J.M. (1997b) Phenol and toluene degrading microbial populations from an aquifer in which successful trichloroethene cometabolism occurred. Applied and Environmental Microbiology, 63, 1523-1530.

Gorontzy, T., Kuver, J., and Blotevogel, K-H. (1993) Microbial transformation of nitroaromatic compounds under anaerobic conditions. Journal of General Microbiology, 139, 1331-1336.

Grbic-Galic, D. (1986) Methanogeic transformation of aromatic hydrocarbons and phenols in groundwater aquifers. Geomicrobiology Journal, 8, 167-200.

Herbert, B.N. (1985) Sulphate reducing bacteria in oil bearing reservoirs. Conference proceedings, World Biotechnology Report Vol I. Pinner UK Online Publishers pp 417-427

Higgins, I.J., Best, D.J. and Hammond, R.C. (1980) New findings in methane utilising bacteria highlight their importance in the biosphere and their commercial potential. Nature, 286, 561-564.

Hinchee and Olfenbuttel (1991) In situ bioreclamation. Applications and investigations for hydrocarbon and contaminated site remediation. Butterworth- Heinemann.

Hirata, T., Nagasugi, O., Yoshida, M. and Sumi, K. (1992) Groundwater pollution by volatile organochlorines in Japan and related phenomenon in the subsurface environment. Water Science and Technology, 25, 9-16.

Hopkins, G.D. and McCarty, P.L. (1995) Field evaluation of in situ aerobic cometabolism of trichloroethene and three dichloroethylene isomers using phenol and toluene as primary substrates. Environmental Science and Technology, 29, 1628-1637.

Hopkins, G.D., Semprini, L. ad McCarty, P.L. (1993) Microcosm and in situ field studies of enhanced biotransformation of trichloroethene by phenol utilising microorganisms. Applied and Environmental Microbiology, 59, 2277-2285.

Hutchins, S.R. (1993) Biotransformation and mineralisation of alkylbenzenes under denitrifying conditions. Environmental Toxicology and Chemistry, 12, 1413-1423.

Janssen, D.B. and Witholt, B. (1992) Aerobic and anaerobic degradation of halogenated aliphatics. In Sigel, H and Sigel, A (eds), Metal Ions in Biological Systems, vol 28. Degradation of Environmental Pollutants by Microorganisms and their metalloenzymes. Marcel Dekker, Inc., New York.

Kaplan, D.L. and Kaplan, A.M. (1982) Thermophilic biotransformation of 2,4,6-trinitrotoluene under simulated composting conditions. Applied and Environmental Microbiology, 44, 757-760.

Kile, D.E. and Chiou, C.T. (1989) Water solubility enhancement of DDT and trichlorobenzene by some surfactants below and above the critical micelle concentration. Environmental Science and Technology, 23, 832-838

Kile, D.E. and Chiou, C.T. (1990) Effect of some petroleum sulfonate surfactants on the apparent water solubility of organic compounds. Environmental Science and Technology, 24, 205-208.

Komatsu, T., Momonoi, K., Matsuo, T., and Hanaki, K. (1994) Biotransformation of cis-1,2-Dichloroethylene to ethylene and ethene under anaerobic conditions. Water Science and Technology, 30, 75-84.

Krumholz, L.R., Caldwell, M.E. and Suflita, J.M. (1996) Biodegradation of BTEX hydrocarbons under anaerobic conditions. In Bioremediation: Principles and applications. Ed Crawford, R.L. and Crawford D.L. Cambridge University Press

Kuhn, E.P. and Sulfita, J.M. (1989) Dehalogenation of pesticides by anaerobic microorganisms in soil and groundwater – A review. In Sawhney B.L. and Brown, K. (eds) Reaction and movement of organic chemicals in soils. Madison, WI: Soil Science Society of America and American Society for Agronomy. Pp 111-180.

Leahy, J.G., Bryne, A.M. and Olsen, R.H. (1996) Comparison of factors influencing trichloroethene degradation by toluene-oxidising bacteria. Applied and Environmental Microbiology, 62, 825-833.

Longstaff, S.L., Aldous, P.J., Clark, L., Flavin, R.J. and Partington, J. (1992) Contamination of the chalk aquifer by chlorinated solvents: a case study in the Luton and Dunstable area. Journal of the Institute of Water and Environmental Management, 5, 541-550.

Mars, A.E., Prins, G.T., Wietzes, P, de Koning, W. and Janssen, dB (1998) Effect of trichloroethene on the competitive behavior of toluene degrading bacteria. Applied and Environmental Microbiology, 64, 208-215.

Mather, J. (1993) The impact of contaminated land on groundwater: A United Kingdom appraisal. Land Contamination and Reclamation, 1, 187-195

Michels, J. and Gottschalk, G. (1994) Inhibition of the lignin peroxidase of *Phanerochaete chrysosporium* by hydroxylamino-dinitrotoluene, an early intermediate in the degradation of 2,4,6-trinitrotoluene. Applied and Environmental Microbiology, 60, 187-194.

Morgan,P and Watkinson, R.J. (1992) Factors limiting the supply and efficiency of oxygen supplements for the in situ biotreatment of contaminated soil and groundwater. Water Research, 26, 73-78.

Mueller, J.G., Cerniglia, C.E. and Pritchard, P.H. (1996) Bioremediation of environments contaminated by polycyclic aromatic hydrocarbons. In: Bioremediation: Principals and Applications. Ed Crawford, R.L. and Crawford, D.L. Cambridge University Press.

Munakata-Marr, J., McCarty, P.L., Shields, M.S., Reagin, M., and Francesconi, S.C. (1996) Enhancement of trichloroethene degradation in aquifer microcosms bioaugmented with wild type and genetically altered *Burkholderia (Pseudomonas) cepacia* G4 and PR1. Environmental Science and Technology, 30, 2045-2052.

National Rivers Authority (1992) Policy and Practice for the protection of groundwater, NRA, Bristol pp51

Neilson, A.H. (1990) The biodegradation of halogenated organic compounds. Journal of Applied Bacteriology, 69, 445-470.

Nelson, M.J.K., Montgomery, E.J., O'Neill, E.J. and Pritchard, P.H. (1986) Aerobic metabolism of trichloroethene by a bacterial isolate. Applied and Environmental Microbiology, 52, 383-384.

Nelson, M.J.K., Montgomery, E.J., Mahaffey, W.R., and Pritchard, P.H. (1987) Biodegradation of trichloroethene and involvement of an aromatic biodegradative pathway. Applied and Environmental Microbiology, 53, 949-954.

Norris, R.D., Hinchee, R.E., Brown, R.A., McCarty, P.L., Semprini, L., Wilson, J.T., Kampbell, D.H., Reinhard, M., Bower, E.J., and Borden, R.C. (1994) Handbook of bioremediation . Boca Raton, FL: CRC Press.

Parsons, F., Lage, G. and Rice, R. (1983). Transformation of chlorinated organic solvents in groundwater environments in Southern Florida. Journal of the American Chemical Society, 23, 286-287.

Parsons, F., Wood, P.R. and DeMarco, J. (1984). Transformation of tetrachloroethene and trichloroethene in microcosms and groundwater. Journal of the American Water Works Association, 76, 56-59.

Patel, R.N. (1984) Methane Monooxygenase from *Methylobacterium* sp. Strain CRL-26. In: Microbial Growth on Cl Compounds. Proceedings of the Fourth International Symposium, R.L. Crawford and R.S. Hansen, eds. American Society for Microbiology, Washington DC, pp83-90.

Patel, R.N., Hou, C.T., Laskin, A.I., Felix, A. and Derelanko, P. (1979) Microbial oxidation of gaseous hydrocarbons. II. Hydroxylation of alkanes and epoxidation of alkenes by cell free particulate fractions of methane utilising bacteria. Journal of Bacteriology, 139, 675-679.

Patel, R.N., Hou, C.T., Laskin, A.I. and Felix, A. (1982) Microbial oxidation of hydrocarbons: Properties of a soluble methane monooxygenase from a facultative methane utilising organism *Methylobacterium* sp strain CRL-26. Applied and Environmental Microbiology, 44, 1130-1137.

Pfiffner, S.M., Palumbo, A.V., Phelps, T.J. and Hazen, T.C. (1997) Effects of nutrient dosing on subsurface methanotrophic populations and trichloroethene degradation. Journal of Industrial Microbiology and Biotechnology, 18, 204-212.

Piet, G.J. and Smeenk, J.G.M.M. (1985) Behaviour of organic pollutants in pretreated Rhine water during dune infiltration. In: Ward, C.H., Giger, W. and McCarty, P.L. (eds) Ground water Quality. John Wiley and Sons, New York.

Pritchard, P.H. (1992) Use of inoculation in bioremediation. Current Opinion in Biotechnology , 3, 232-243.

Rao, P.S.C., Davis, G.B. and Johnston, C.D. (1996) Technologies for the enhanced remediation of contaminated soils and aquifers: overview, analysis, and case studies. In Naidu, R., Kookana, R.S. Oliver, D.P. Rogers, S. and McLaughlin, M.J. Contaminants and the Soil Environment in the Australasia-Pacific Region. Kluwer Academic Publishers.

Rivett, M.O., Lerner, D.N., Lloyd, J.W. and Clark, L. (1990) Organic contamination of the Birmingham aquifer. Journal of Hydrology, 113, 307-323.

Roberts, P.V., Hopkins, D.M., Mackay, D.M. and Semprini, L. (1990) A field evaluation of in-situ biodegradation of chlorinated ethenes: Part I, methodology and field site characterisation. Groundwater, 28, 591-604

Rosenberg, E. and Ron, E.Z. (1996) Bioremediation of petroleum contamination. . In Bioremediation: Principles and applications. Ed Crawford, R.L. and Crawford D.L. Cambridge University Press. Pp 101-124.

Salanitro, J.P. (1993) The role of bioattenuation in the management of aromatic hydrocarbon plumes in aquifers. Ground Water Monitoring Review, 13, 150-161.

Salkinoja-Salonen, M., Uotila, J., Jokela, J., Laine, M. and Saski, E. (1995) Organic halogens in the environment: studies of the environmental biodegradability and human exposure. Environmental Health Perspectives, 103, Supplement 5, 63-69.

Semprini, L (1995) In situ bioremediation of chlorinated solvents. Environmental Health Perspectivies, 103, Supplement 5. 101-105.

Semprini, L., Roberts, P.V., Hopkins, G.D. and McCarty, P.L. (1990) A field evaluation of in situ biodegradation of chlorinated ethenes: Part 2, Results of biostimulation and biotransformation experiments. Groundwater, 28, 715-727.

Spain, J. (1997) Synthetic chemicals with potential for natural attenuation. Bioremediation Journal, 1, 1-9.

Stahl, J.D. and Aust, S.D. (1993) Metabolism and detoxification of TNT by Phanerochaete chrysosporium. Biochemical and Biophysical Research Communications, 192, 477-482.

Swoboda-Colberg, N.G. (1995) Chemical contamination of the environment: Sources types and fate of synthetic organic chemicals. In: Young, L.Y. and Cerniglia, C.E. (eds) Microbial transformation and degradation of toxic organic chemicals. Wiley-Liss. pp 217-243.

Thierrin, J., Davis, G.B. and Berber, C. (1995) A groundwater tracer test with deuterated compounds for monitoring in situ biodegradation and retardation of aromatic hydrocarbons. Ground Water, 33, 469-475.

Thierrin, J., Davis, G.B., Barber, C., Patterson, B.M., Pribac, F., Power, T.R. and Lambert, M. (1993) Natural degradation rates of BTEX compounds and naphthalene in a sulphate reducing groundwater environment. Hydrological Sciences, 38, 4, 8/1993. 309-322.

Toze, S. (1996) TNT Busters: Microbes that degrade munitions compounds in groundwater. Search, 27, 58-61.

UNEP (1992) Environmental Data Report, 2nd Edition 1989/90, Blackwell Reference, Oxford.

Vogel, T.M. and McCarty, P.L. (1985) Biotransformation of tertachloroethylene to trichloroethene , dichloroethylene , vinyl chloride and carbon dioxide under methanogenic conditions. Applied and Environmental Microbiology, 49, 1080-1083

Vogel, T.M., Criddle, C.S. and McCarty, P.L (1987) Transformations of halogenated aliphatic compounds. Environmental Science and Technology, 21, 722-736.

Vorbeck, C., Lenke, H., Fischer, P., Spain, J.C. and Knackmuss, H-J. (1998) Initial reductive reactions in aerobic microbial metabolism of 2,4,6-trinitrotoluene. Applied and Environmental Microbiology, 64, 246-252.

Wackett, L.P. (1995) Bacterial co-metabolism of halogenated organic compounds. In Young, L.Y. and Cerniglia, C.E. (eds) Microbial transformation and degradation of toxic organic chemicals. Wiley-Liss. Pp 217-243.

Williams, R.A., Shuttle, K.A., Kunkler, J.L. , Madsen, E.L and Hooper, S.W. (1997) Intrinsic bioremediation in a solvent-contaminated alluvial groundwater. Journal of Industrial Microbiology and Biotechnology, 18, 177-188.

Williams, R.T., Ziegenfuss, P.S. and Sisk, W.E. (1992) Composting of explosives and propellant contaminated soils under thermophilic and mesophilic conditions. Journal of Industrial Microbiology, 9, 137-144.

Wilson, J.T. and Wilson, B.H. (1985) Biotransformation of trichloroethene in soil. Applied and Environmental Microbiology, 49, 242-243.

Wilson, B.H., Wilson, J.T., Kampbell, D.H. Bledsoe, B.E. and Armstrong, J.M. (1990) Biotransformation of monoaromatic and chlorinated hydrocarbons at an aviation gasoline spill site. Geomicrobiology Journal, 8, 235-240

Wood, J.M. (1982) Chlorinated hydrocarbons: oxidation in the biosphere. Environmental Science and Technology, 16, 291A-297A

Young, L.Y. and Cerniglia, C.E. (1995) Microbial transformation and degradation of toxic organic chemicals. Wiley-Liss.

Zylstra, G.J. (1994) Molecular analysis of aromatic hydrocarbon degradation. In: Garte, S.J. (ed) Molecular Environmental Biology. Lewis.

7. ANTIBIOTIC RESISTANT AND GENETICALLY MODIFIED ORGANISMS IN AQUIFERS

7.1 Introduction

Until recently the use of molecular biology for the genetic modification of micro-organisms had been restricted to experimental investigations in contained laboratories. As the science of molecular biology developed the potential to apply genetically modified micro-organisms to solve a wide range of agricultural and environmental problems has emerged. Their potential use for the control of insect pests, wastewater treatment and the treatment of soil and water contaminated with toxic wastes has been well documented. In addition to planned releases there is always the risk of accidental release from containment facilities.

The presence of antibiotic resistant organisms in the environment, due to their release from a number of sources, is well known (Grabow and Prozesky 1973). Information has been gathered about their fate in the environment, and the stability of the genetic material that they carry. Some of the issues that have emerged from the study of antibiotic resistent bacteria in the environment may be relevant to GMO release.

This section will review the occurrence of GMOs and antibiotic resistant organisms in the environment, and evaluate both their direct and indirect impacts on groundwater quality. It will deal principally with bacteria, as they are the organisms which are most likely to have an effect on groundwater.

7.2 Production Of Antibiotic Resistance

Antibacterial and antifungal compounds are produced naturally by a wide range of bacteria and fungi. In some cases these compounds are produced so that the organisms may compete effectively for an environmental niche; in some cases it may protect the cell from predation.

Antibiotics, have been widely available since the 1950s. Early studies with penicillin showed that bacteria could develop resistance to the antibiotic. As further antibiotics were developed resistant organisms were recorded. Today, two categories of antibiotic resistance are recognised:

- Intrinsic resistance, where inherent features of the bacterium render it naturally unsusceptible to an antibiotic, eg they may lack the target site for that antibiotic. Intrinsic resistance is usually carried by chromosomal genes;
- Acquired resistance, where resistant strains appear from a previously sensitive population. The genes for acquired antibiotic resistance arise by spontaneous mutation and it is postulated that many predate the antibiotic era. Acquired resistance is often plasmid borne.

The widespread use of antibiotics in medicine and in agriculture, has introduced selective pressure amongst microorganisms to develop widespread antibiotic resistance. Resistant organisms will arise wherever there are sufficient levels of an antibiotic to affect the population. Frequently, selection will occur in the host animal, in particular in circumstances where sub-lethal doses of antibiotic have been administered. It has generally been assumed that most antibiotic resistant micro-organisms do not survive well in the environment, either

because they are not adapted to life outside the host or because they are at a selective disadvantage in the absence of the antibiotic.

The occurrence of antibiotic resistant bacteria in water, both indicator organisms and pathogens, has been documented (Alcaide and Garay, 1984). They have been recovered in high numbers from remote upland tarns, away from sewage contamination (Jones, Gardener and Simon, 1986a), from the Baltic Sea (Niemi, Sibakov and Niemela, 1983) and from drinking water.

The presence of multiply antibiotic resistant (MAR) bacteria (chiefly coliforms) in drinking water has been well documented. The percentage of antibiotic resistant organisms has been shown to be consistently higher in treated water than in untreated water. This has been reported as an increase from 20.4% in the raw water to 36.4% in the distribution water (Calomiris, Armstrong and Seidler, 1984), 79% in raw sewage to 87.5% in regrowth samples (Murray, Tobin et al, 1984) and 15.8% of river water samples to 57.1% of treated water samples (Armstrong, Calomoris and Seidler, 1982). The increase has been attributed by some authors to chlorination. However, many antibiotic resistant bacteria can tolerate a range of metals. These metal resistance genes are generally on the same plasmids as the antibiotic resistance genes and it is possible that in a water distribution system, where metal ion concentrations may be raised due to corrosion, selection for antibiotic resistance occurs as a by-product of selection for metal resistance (Calomoris, Armstrong and Seidler, 1984).

7.3 Overview Of The Characteristics And Production Of GMOs.

Genetically modified organisms are defined under the Environmental Protection Act 1990 as "any micro-organism, be they bacteria, virus, fungi, algae of parasite that has been constructed or modified by the insertion of heritable material (DNA or RNA) which it would not normally contain, that has been prepared, by whatever means, outside of the cell or organism". The addition of a clause, within this definition, to include the deliberate insertion of genetic material from outside the cell is important. Its purpose is to provide the basis of a clear distinction between GMOs, and those organisms which have been genetically modified by selective breeding.

With one or two exceptions, all cells and viruses contain nucleic acid. Eukaryotic and prokaryotic cells contain both ribonucleic acid (RNA) and deoxyribonuleic acid (DNA). Viruses contain only one type of nuleic acid; either RNA or DNA. In prokaryotic (bacterial) cells the DNA molecules are found throughout the cytoplasm, while in higher animals and plants (eukaryotes) the DNA is confined to the nucleus and a few organelles.

Bacterial DNA can be present in three forms: chromosomal, plasmid or as a transposon. In general, chromosomal DNA contains the major, inheritable genetic material of the cell: the processes which control cell growth and division and the enzymes of the principal metabolic pathways. A plasmid is usually a circular DNA molecule, distinct from chromosomal DNA. It is typically much smaller than a chromosome, and a single bacterium may contain several plasmids. Plasmids vary in size from 1 kilobase (kb) - 1000 nucleotide base pairs or one or two genes - to over 300 kb. Plasmids may encode almost any feature, including antibiotic and metal resistance, toxins and ability to use different substrates.

Within the cell genetic information can be exchanged between plasmids, or between the chromosome and plasmids, by means of transposons, sometimes called jumping genes. These are important in the formation of multiple antibiotic resistant plasmids.

Unlike eukaryotic cells, bacterial cells do not retain significant amounts of redundant DNA sequences. This is important for maintaining the efficiency of the cell and to control space requirements. Excess DNA can slow down replication as well as increasing energy requirements, and this can have a serious effect on an organism's capacity to compete in the environment. Therefore genes, or DNA sequences, which do not confer an advantage on the host cell may be excised, or the host cell fails to thrive in the environment. Table 7.1 gives some examples of genera of bacteria capable of natural transformations.

Although humans have been selectively breeding animals and plants for thousands of years, the techniques of genetic engineering have been developed only recently. The first practical method for the cloning of specific DNA fragments was carried out in 1973 by Boyer and Cohen. The potential of these methods was recognised instantly and major advances soon followed. The first notable success occured with the insertion of the human insulin gene into Escherichia coli. Genetic engineering has since been widely used to produce a range of medical products, including growth hormones, vaccines and some blood products. In conrast, the application of this technology in the environment is still in its early stages. This is partly due to the amount of testing that is necessary to license a product, but also due to the generally lower priority that is attached to environmental research compared to medical research.

7.3.1 Production of Genetically Modified Organisms

Producing a genetically engineered organism involves several steps:

- Identification and isolation of the required gene(s), along with the relevant control sequences, from a donor organism.
- Insertion of the gene into a length of carrier DNA.
- Insertion of the carrier DNA into the host organism.

DNA may be taken up by the recipient organism using any of the following methods.

Conjugation is the direct transmission of DNA from one bacterial cell to another. It is controlled by the presence of a plasmid in the donor cell that carries the genetic information necessary to promote DNA transfer. These genes are called tra or transfer genes, and the bacterial strains that have them are called tra+ strains or F+ strains: the F standing for fertility factor. There are several different groups of tra genes each of which seems to be quite different. Conjugation is common in gram negative bacteria, such as *E. coli*, but appears to be less common in gram positive bacteria. Gram negative donor cells typically carry long thin appendages on their surfaces known as sex pili. These are coded for by the tra genes .

Transformation is the principal method of inserting genetically engineered DNA into bacteria. It is a process by which bacteria take up fragments of DNA from the surrounding medium. For the transfer of genetic material to be successful the DNA must eventually be expressed in the host cell, otherwise it will be excised and lost.

The process of taking up exogenous DNA is known as competence. Bacteria may be naturally competent or only capable of induced competence in the laboratory. Early opinion amongst microbiologists was that competence could only be induced in the laboratory. However, competence has now been observed in bacteria isolated from environmental sources (Table 7.1). Cells are competent for transformation under certain conditions of growth, usually towards the end of log phase growth, or during sporulation.(for example

Bacillus spp.) *Bacillus* spp also release competence factors into the surrounding medium to induce competence in neighbouring cells (see Table 7.2).

Transduction is the mechanism of gene transfer in which viruses act as the vehicle for the transmission of DNA from one cell to another. The process has been well characterised in bacteria using bacteriophage (viruses which infect bacteria).

Protoplast fusion is used naturally by fungi and one group of bacteria, *Streptomyces* spp. to transfer genetic material. The cell walls between the two cells breaks down and the two genomes fuse and form a diploid protoplast. When the cell walls reform two haploid daughter cells, with mixed genomes are formed. The importance of protoplast fusion in the environment is unknown, but it has been used in the laboratory to transfer antibiotic resistance between several groups of gram positive bacteria.

Identification of the genetically modified bacterial cells. Frequently, this is achieved by using marker genes, for example antibiotic or metal resistance, on the engineered DNA molecule.

The genetically engineered organism is then propagated.

7.4 Pathways Into The Environment

Section 4 in this review has examined the transport of microorganisms into, and through groundwater. Access to groundwater from the soil and surface water has been reviewed in the context of the transmission of pathogens. The pathways into groundwater that have been previously identified will be equally applicable to GMOs and antibiotic resistant bacteria. This section will briefly describe the routes for these organisms, or their genetic material, into the environment, from where they may be transported into groundwater.

GMOs have been used in a variety of applications that offer routes for the organisms to the environment. For example:

- Direct application to agricultural land for crop protection and soil improvement
- Use in the bioremediation of contaminated soil and water
- Effluent from wastewater treatment systems
- Accidental release

The direct use of GMOs in the environment has been restricted by the need for risk assessment. Even so, several countries have reported experiments with field releases (Mackenzie and Henry, 1990). Most of these have been designed to investigate the behaviour of the GMOs in the environment. Other examples of deliberate release include GMOs with potential commercial benefit to agriculture, including pest control (usually an environmental bacterial species containing genes from *Bacillus thuringiensis*), improved nitrogen fixation and improved crop yields due to control of soil borne plant pathogens.

Antibiotic resistant organisms may be released into the environment from several sources, including:

- Sewage treatment works
- Farmyard and fishfarm waste, including food residues and manure
- Clinical and veterinary waste including drug residues.

7.5 Fate of Antibiotic Resistant Bacteria And GMOs In The Environment.

There is very little evidence of antibiotic resistant bacteria or GMOs having any direct impact on groundwater microbiology, or any indirect impact by the transfer of genes to indigenous microbial populations.

Genetic transfer in the environment, either by conjugation, transformation or transduction occurs at higher rates than was once thought (Colwell and Grimes, 1986, McClure et al, 1990). It is believed to be a significant mechanism in the evolution of natural populations due to the introduction of new genetic elements (Miller, 1992).

Natural parameters that effect genetic transfer are pH, water content, temperature, and soil composition. Genetic transfer appears to be optimal at a pH of 6.8 - 7.2 (Stotzky and Babich, 1986, Richaume et al, 1989) and a moisture content of 24 -26% (Stotzky et al, 1990). The optimum temperature for conjugation varies from species to species. However, for most soil bacteria it appears to be between 20-30 °C (Richaume, 1989, Top et al, 1990).

While genetic transfer has been postulated as being important in the aquatic environment much of the evidence has been derived from experiments carried out in microcosms. Gene transfer has been observed in these systems, but the rate of transfer has been variable, ranging from 2.5×10^{-3} to 5×10^{-8} transconjugants per donor or recipient. Plasmid transfer experiments carried out in the river Taff (Fry and Day, 1990), using *Pseudomonas aeruginosa* PAO2002 containing plasmid pQM1 as the donor and *Ps aeruginosa* PU21 as the recipient, showed gene transfer rates in the biofilm layers only slightly lower than those in the laboratory microcosm experiments. Further evidence of gene transfer in nature comes from the discovery of antibiotic resistance genes, transposons and plasmids with similar DNA sequences in unrelated bacterial isolates. Toranzo et al (1984) characterised antibiotic resistance patterns and R-plasmids of bacteria isolated from the skin of Rainbow trout and from the water of their hatchery. A single identical plasmid coding for chloramphenicol resistance was found in *Vibrio spp, Citrobacter spp* and *Enterobacter spp*, which in the laboratory also readily transferred to *E. coli*. It has also been demonstrated in the laboratory that genetic transfer from GMOs to environmental strains does occur under low temperature and low nutrient conditions in drinking water.(Sandt and Herson, 1989).

Stotzky and coworkers (1990) have suggested that the presence of chemical pollution within an environment may stimulate gene transfer between bacterial cells. If pollution is shown to influence gene transfer rates, it will have clear implications for the use of GMOs for the remediation of polluted groundwater.

The potential for genetic transfer in the environment introduces a further complication to any method for predicting the impacts of releasing genetically modified organisms of any sort into the environment. Simply assessing the fitness of the organism to survive in the environment may underestimate the overall effect that the organism can exert upon the indigenous microbial populations by ignoring the contribution it makes to the gene pool. For many genetic modifications, for example antibiotic resistance, similar genes may already be present in the indigenous population, so that any new introduction may be assumed to have no serious long-term consequences. The introduction of multiple drug resistance into the gene pool of indigenous groundwater bacteria may have more serious implications if it can be shown to maintain a cycle of multiple drug resistant organisms in the food chain. None of the literature reviewed for this study included data that would suggest that such a cycle has been created. Even if a groundwater to livestock cycle is demonstrated, while the main selective

pressures for multiple drug resistant organisms in the food chain are being exerted by the widespread use of antibiotics in agriculture, the role of groundwater bacteria in maintaining the cycle may assumed to be low.

Other genetic modifications have more serious implications if introduced into the environment. The release of GMOs has generated widespread concern amongst the public, and a framework of legislation and risk assessment methodologies has been created to minimise their potential impact on the environment. Both legislation and risk assessment methodologies are discussed below. Although the main purpose of these instruments is to control the release of GMOs, some of the principles contained in the risk assessment methodologies could be applied to antibiotic resistant organisms.

7.6 Legislation Governing The Release Of GMOs Into The Environment

The release of genetically modified organisms into the environment initially generated considerable public concern that was dealt with, in part, by convening a Royal Commission investigation into the release of GMOs. This was published in July 1989 as the " Release of Genetically Engineered Organisms to the Environment." , the 13th Report of the Royal Commission on Environmental Protection, Cm 720. Prior to this report, the Organisation for Economic Co-operation and Development (OECD) produced the first "Blue book" on Recombinant DNA Safety Considerations (1986), which formed the basis of most of the European regulations. Many of their recommendations have been incorporated into current legislation.

Current UK legislation is as follows:

The Genetically Modified Organisms (Contained Use) Regulations 1992 (SI 1992 No 3217) as amended by the Genetically Modified Organisms (Contained Use) (Amendment) Regulations 1996 (SI 1996 No 967) implements the Contained Use Directive; EU directive 90/219/EEC on the contained use of genetically modified organisms. These regulations are monitored by the HSE.

The Genetically Modified Organisms (Deliberate Release) Regulations 1992 (SI 1992 No 3280) implements the Deliberate release Directive - 90/220/EEC on the deliberate release to the environment of GMOs. These regulations are administered by the DoE.

7.7 Risk Assessment Methodologies

The Genetically Modified Organisms (Risk Assessment)(Records and Exemptions) Regulations 1996 (SI 1996 No 1106) requires that a risk assessment is made for each GMO. The advisory Committee on Genetic Modification has produced guidance on risk assessment, although the HSE which administers the Regulations has not formerly approved this. There are four main elements to the risk assessment:

- hazard identification
- assessment of exposure to the hazard and the consequences of that exposure
- assessment of the level of risk, that is consider the scale of any harmful consequences and the chance of them being realised.
- managing the risk by appropriate control measures.

Most risk assessment schemes, such as Control of Substances Hazardous to Health (COSHH), were originally designed to evaluate chemical or physical risks. Others, such as Hazard Analyses of Critical Control Points (HACCP), have been designed to account for microbiological risk as well. The problem with all risk assessment schemes is that they are not good at assessing the risk from GMOs. Several differences exist in GMO and chemical risk assessment.

- GMOs may increase in concentration after release.
- Sensitive detection methodologies exist for many toxic substances but those for GMOs are still being developed.
- There is insufficient data for measuring the size or probability of risk from GMOs.
- Toxic chemicals are evaluated using defined and observable end points, such as LD50s and NOELs. These are not possible to apply to GMOs because of the lack of observable effects.

Risk assessment schemes already in use such as Environmental Impact Assessment have been used to evaluate GMO release. A pilot scheme, GENHAZ, developed by the Royal Commission on Environmental Pollution (RCEP) aims to help users to anticipate potential environmental impacts associated with GMO release. GENHAZ was derived from HAZOP and incorporates its essential features. It provides a systematic structure for assessment while still encouraging a creative approach and involves the setting up of a multidisiplinary committee around every planned release who have to consider a series of questions before the release goes ahead. GENHAZ has been used (DoE 1994) and problems with the questionnaire identified. Like many HAZOP based schemes it is more of a hazard identification procedure than a true risk assessment as it fails to encourage the identification of potential ultimate environmental impacts. This has been put down to problems with the questionnaire and the team make up. Further research has been recommended.

Risk assessments require that fundamental ecological and survival data is available on the GMO and an accurate assessment requires considerable anticipation of their behaviour following release. This includes knowledge of the survival time, the potential for genetic exchange with indigenous flora, the potential for dispersal from site, the possible effects on non-target organisms and on the ecosystem. Although experimentation is not specifically requested by the regulations, most of this data required for an assessment is not currently available. The only practical way to collect the initial data is to use contained systems or microcosms. Two approaches have been used in the application of microcosms to GMO risk assessment (Seidler, 1994). They are:

- Evaluation of general principles using non engineered microbes in order to investigate characters such survival, dispersal and gene transfer. It would be normal to conduct these studies on the host microbe.
- The GMO is then added to the test system in order to observe the consequences of specific metabolic alterations on ecological processes and study the effect on non-target organisms.

Both are important for providing information regarding regulation, but it must be remembered that the development of these methodologies is on going.

7.7.1 Survival

Organisms have the potential to increase in numbers in the environment, either at the site of application or following dispersal. The way an organism behaves in the laboratory, or even in microcosms, does not always accurately predict its behaviour after release. Survival of an organism under laboratory conditions does not ensure survival in natural ecosystems. Conversely, the ability to contain and control species under laboratory situations does not equate to containment and control in the environment. In order to affect the environment a GMO must do the following:

- DNA may be transferred to or from the GMO, thus altering the genetic composition of the GMO or the indigenous population.
- The GMO must survive in the community.
- The GMO may affect the microbial ecology including functional processes such as the geochemical cycling of nutrients.

Most of the information regarding the survival of introduced bacteria in the environment concerns *Rhizobium* spp., which are root colonisers that do not appear to move far from site of introduction. Introduced rhizobia can colonize and become major players in a local ecosystem but it is most unlikely that they would have any direct effect on an aquifer. Introduced species will always face an uphill struggle to become established, but the effects of many adverse factors can be reduced by increasing the number of bacteria being applied. This however will also increase the risk of groundwater contamination through run off.

Not much information exists for the release of GMOs into aquatic systems, although it is well known that introduced bacteria decline sharply in numbers when released into aquatic systems. Scanferlato et al (1989) found that engineered *Erwinia carotovora* declined at the same rate as its non-engineered parent and similar results were seen with engineered *E. coli* and *Ps putida*. (Awong et al, 1990). Although the numbers of introduced bacteria usually decrease sharply, it has been seen for many organisms in water that low numbers can survive for many weeks. The recommended way to initially evaluate survival is by the use of microcosms. These can be designed to mimic a variety of environmental conditions as well as being used to obtain basic information in a controlled environment. They do not always accurately predict behaviour upon release, but they have been shown to be reasonably reliable for most studies in the short term (Cairns and Orvos 1992).

7.7.2 Gene transfer

The potential for genetic transfer in the environment has been discussed in the preceding sections. It has already been noted that there is no information regarding gene transfer in aquifers. However, as gene transfer has been observed in water, soil and sediments, it may be anticipated that it will occur in groundwater.

The impact of the engineered DNA will also depend on what the gene codes for. For instance, if a gene is already present in that environment, albeit in a different species, then it is less likely that it will have any serious, long term consequences than if the DNA was completely new to that environment. Each time a GMO is evaluated, an assessment should be made of the sequence of any DNA inserted into the genome, the origin of the DNA and its' intended function. If DNA is inserted into a plasmid, then it is more likely that the DNA will be transferred than if the gene is inserted into the chromosome. Selection of a non-transferrable plasmid can reduce the likelihood of transfer. For instance the plasmid pBR322

is used extensively as a vector for inserting genes into bacteria because it is non conjugative and poorly mobilizable. However, transfer of this plasmid has been achieved in the laboratory (Stotsky and Babich, 1986). In addition, McPherson and Gealt (1986) have shown that members of the family Enterobacteriaceae, which had been isolated from wastewater, were able to mobilize pBR325 from a laboratory strain of E. coli into other recipients (McPherson and Gealt, 1986). This result indicates that while the introduced organism may not survive long, local inhabitants may be able to transfer even poorly mobilizable plasmids into other bacteria.

The potential for gene transfer is an important part of the risk assessment. In order for this to be assessed, it must be confirmed that the genes have been inserted into a host bacterium. If the gene is being expressed in the host cell, then the effects of this expression on the environment must be assessed. This will include transfer frequency to indigenous species.

7.7.3 Effects on the Ecosystem

The impact of GMOs on ecosystems is difficult to predict and assess. However, if the organism is unable to survive and/or transfer it's DNA, then the ecological effects are likely to be very low. There have been many small scale releases and only one or two have involved a disruption of the ecosystem. However, although the chance of ecological disruption is low, if it does occur the consequences could be potentially serious. For example, Wang and Crawford (1988) showed that a recombinant strain of *Streptomyces lividans* significantly altered soil organic carbon degradation rates by breaking down the organic carbon faster than the unengineered parent.

It is important to monitor any introduced GMO carefully after release. This includes any metabolic end points that may be altered as a result the DNA insert. It is however impossible to recall a GMO once it has been released.

7.8 Methods For The Detection Of GMOs In Groundwater

In order that a GMO can be traced in the environment it is necessary that there is an accurate method available for its' detection, although the release regulations do not specifically request that there is an available method. Any test protocol will have to consider the following points:

- What does the test detect, the organism, its modified DNA, or the effect of the organism eg antibiotic resistance?
- Sample size; how big a sample is necessary in order to detect the organism?
- How cheap/easy is the test to perform?
- Could the test be done in an ordinary water laboratory?
- How reliable is the test?
- Has it been validated?

Any monitoring method must be sensitive, specific, cost effective and capable of in situ analysis.

7.8.1 Plate count

The traditional technique for detecting viable bacteria, the plate count, is an inexpensive and reliable method for enumerating GMOs as well as indigenous bacteria. The type of bacteria must be known so that the correct selective media can be used. If the GMO has been manufactured containing an antibiotic or metal resistance gene, then these product can be included in the agar to select for the engineered organism. Care must be taken with this approach, however, as some antibiotic resistance genes have to be induced. This means that a process of resuscitation is necessary before attempting isolation on the selective media. This has been known to give false negatives . Sample size is important, small numbers of bacteria are going to require a larger sample and it must also be recognised that bacteria are not homogeneously distributed in water systems.

7.8.2. Direct or total counting systems

These usually involve staining the organisms, either using simple stains or fluorescent stains such as acridine orange. The problem with this approach is that it is not possible to differentiate the organism from the others in the sample. Tests using fluorescent antibodies, (specific for the organism) may be used but difficulties with background contamination and low sensitivity can be a problem. In water however, where large volumes of water may be filtered to extract the required organisms, better results have been seen. These tests however do not indicate whether or not an organism is replicating.

7.8.3 Molecular techniques

These techniques show promise not least because once the segment of DNA has been isolated for insertion, it can also be developed into a gene probe to detect its presence in environmental samples. However these techniques are always costly to develop and require well equipped laboratories and highly trained staff to perform them. DNA hybridisation methods have been developed which allows the detection of the GMO whether culturable or not (Barkay and Sayler (1988). While some authors have reported sensitivities as good as 10cells/g soil most others have reported only low sensitivity. The polymerase chain reaction (PCR) has been used for GMO detection where it shows promise with detection limits of 1 cell/g sediment being quoted in one study. In this study however (Steffan and Atlas, 1988), the gene was present as 15-20 copies. Where only one copy of a gene is present, the results have not been as good. There are also contamination problems with PCR.

7.8.4 Immunological methods

Immunological methods for the detection of microorganisms have been used for many years, although their application in microbial ecology is more recent. Basically these methods measure an antigen - antibody interaction. The antibody may be either polyclonal (raised in an experimental animal as a response to the antigen - they characteristically contain a mixture of different antibodies, able to bind to a number of sites on the antigen) or monoclonal (produced by a hybridoma cell line - this is a pure antibody, able to bind to one site only on the antigen)

Polyclonal antibodies have the advantage of being easier to produce. However, there can be problems with cross reactivity, background contamination and poor sensitivity. Monoclonal antibodies have a high specificity which means that false positives can be virtually eliminated. However, sometimes the organisms may lose the active site for that antibody due to growth stages or nutrient availability.

These techniques have been used to detect GMOs in water and soil samples where they compare favourably with nucleic acid techniques (Carter and Lynch, 1996).

7.9 Assessment Of The Impact Of GMOs And Antibiotic Resistant Bacteria

A review of introductions of non indigenous species into the UK over the last century (Williamson and Brown, 1986) concluded that about ten percent of the organisms had become established while only 10% had become established as pest species. The same authors listed the general characteristics of a invasive species as follows:

1. Origin in a geographically remote area
2. Climatic match
3. Possession of high reproductive rate and/or low mortality.
4. Easy dispersal over significant distances
5. Expression of low competition with indigenous species.
6. Lack of natural enemies
7. Possession of a capability to inbreed
8. Possession of the ability to reproduce from a single parent
9. The ability to find an empty niche.

The potential threat posed by introduced animals and plants has been summarized into six main impacts (Ebenhard, 1988):

1. Damage to the habitat
2. Imbalance in the prey population
3. Competition for resources
4. Spread of disease
5. Genetic exchange with native species
6. Provision of an additional source of food for native predators.

Most of these points are just as applicable to microorganisms, although the tendency to compare the introduction of GMOs with the introduction of macroorganisms has been criticised. Generalisations developed for certain groups cannot be automatically extended to other groups which may have very different genetic, demographic, dispersal and reproductive characteristics. Release of GMOs, if it respects high genetic diversity, is less likely to interfere with natural biological evolution than the use of a pesticide or fertiliser. Most biologists agree that there is no special risk associated with GMOs per se. The majority of GMOs are not novel genotypes and are not designed for release into the environment. GMOs do not therefore differ significantly from traditionally selected microbes with modified

properties such as vaccine strains of polio and typhoid. Usually the introduced DNA is already present in another organism in that environment. However, the problems that are most likely to occur are those that are unforseen. Many impacts will make their presence felt during testing in microcosms, although in most cases there appears to be no effects. Examples of effects are as follows:

1. The survival of a genetically engineered strain of *Erwinia carotovora* in a microcosm after heat treatment of the microcosm has been studied. After ten days and for the remainder of the study, the heat treated microcosms inoculated with the GMO, and the untreated, uninoculated microcosm had greater numbers of total and cellulitic bacteria than the untreated microcosms inoculated with the GMO. It was posulated that this was due to competitive exclusion by the GMO Also after treatment, the activity of the electron transport system (ETS) was lower in the microcosm uninoculated with the GMO than in the innoculated one. This decreased alongside the numbers of GMOs. The presence of the GMO therefore appeared to be the sole factor responsible for changes in the community dynamics in these experiments and that it appeared to be influenced by environmental factors. (Scanferlato et al, 1990).

2. Evaluation of GMOs has shown that predatory protozoa are affected by the genotype of their prey. The effects of a engineered strain of *Pseudomonas solanacearum* on numbers of Protozoa in soils was examined by Austin et al (1990). Five strains of Ps. solanacearum were examined; two of which were engineered. With all three non engineered strains and one of the GMOs, the numbers of flagellated Protozoa increased. However, with the other engineered strain, the numbers decreased. These results indicate that protozoa are affected by the genotype of their food source and that a GMO, if it is not consumed by predators may survive for longer in that environment.

3. A strain of *Pseudomonas cepacia*, engineered to degrade the herbicide 2,4,5-trichlorophenoxyacetate (2,4,5-T) was added to soil amended or not amended with 2,4,5-T and planted with radish seeds. In this test both the phenotypic and the genotypic diversity of the soil increased slightly and transiently over that seen in the uninoculated and unamended soil. The addition of the herbicide alone caused a decrease in diversity (Bej et al, 1991).

Assessments of the impacts of releasing large quantities of a specific microorganism are complicated by the lack of information regarding the natural genetic structure and gene transfer of the natural microbial population. Micro and mesocosms can provide a baseline and there have been many studies on gene transfer in these. The evidence so far points to most transfer events being evolutionary dead ends. However, the penetration of the gene pools of a species by a particular gene or combination of genes is likely to be significant if they give the organism an advantage.

7.10 Summary

1. There appears to be no research regarding the vertical escape of GMOs into groundwater.
2. GMOs may survive and become part of the natural ecosystem, although usually they appear to die out.
3. The transfer of genetic material between bacteria in the environment has been demonstrated. Antibiotic resistant organisms have been identified from surface waters, and the organisms have been shown to transfer antibiotic resistance to other bacteria under laboratory conditions..
4. While there appears to be no data regarding the levels of antibiotic resistant organisms in groundwater, there is plenty of evidence of their existence in other water systems and they are considered an important potential health problem. Antibiotic resistance in pathogens causes treatment difficulties and sometimes fatalities while antibiotic resistance in non pathogens is also of concern because of the possibility of transferance. Antibiotic resistant Pseudomonads from food and water have been shown to be a source of infection in hospitals, especially amongst the immunocompromised (Shooter et al 1971, Remington and Schimpff 1981). However, most of the problems seem to be caused by gram negative bacteria such as the Enterobacteriaceae and the Pseudomonads.

7.11 Recommendations For Future Work

- Genetically modified organisms have been produced for the control of agricultural insect pests. Trials with these organisms have been very successful. The application of GMOs' for pest control may have an indirect environmental benefit by reducing the level of pesticides in groundwaters below the treated areas. Considering the present concerns about pesticides in dirty water it would be expedient to promote further research into the stability of GMOs' in the environment.
- Research into the microbiology of aquifers including genetic transfer
- Research into the transport of introduced organisms through soil and into aquifers.
- The influence of groundwater pollutants on the rate of genetic transfer between bacterial cells.

• **Table 7.1. Genera of bacteria capable of natural transformation (after Stewart 1989)**

Genus	Reference
Achromobacter	Juni and Heym, 1980
Acinetobacter	Juni, 1972
Anacystis	Golden and Sherman, 1984
Azotobacter	Page and Sadoff, 1976
Bacillus	Bott and Wilson, 1967
Haemophilus	Smieth et al, 1981
Halobacterium	Mevarech and Werczberger, 1985
Methylobacterium	O'Connor et al, 1977
Micrococcus	Tigari and Moseley, 1980
Mycobacterium	Nogard and Imaeda, 1978
Moraxella	Juni and Heym, 1980
Neisseria	Sparling, 1966
Pseudomonas	Carlson et al 1983
Streptococcus	Chauvat et al, 1983
Thiobacillus	Yankovsky et al, 1983
Vibrio	Stewart et al, 1989

Table 7.2. Control of competence in Transformable bacteria (after Stewart 1989)

Mechanism	Representative species	Reference
Constitutive	*Neisseria gonorrhoeae*	Sparling, 1966
Soluble competence factor	*Bacillus subtilis*	Ayad and Shimmin, 1976
(External control)	*Streptococcus sp*	Tomasz and Hotchkiss, 1964
Shifts in growth state	*Haemophilus* sp	Smith et al, 1981
(Internal control)	*Azotobacter vinelandii*	Page, 1982
	Pseudomonas stutzer	Carlson et al, 1981

7.12 References

Alcaide and Garay (1984) R-plasmid transfer in *Salmonella sp*, isolated from Wastewater and sewage contaminated surface waters. Appl.ied and Environmental Microbiology, 48, 435-438.

Angles M L, Marshall K C, Goodman A E. (1993) Plasmid transfer between marine bacteria in the aqueous phase and biofilms in the reactor microcosm. Appl.ied and Environmental Microbiology , 59, 843-850.

Armstrong J L, Calomiris J J, Seidler R J. (1982) Selection of antibiotic resistant standard plate count bacteria during water treatment. Applied and Environmental Microbiology, 44, 308-316.

Austin HK, Hartel PG, Coleman DC (1990) Soil Biology and Biochemistry 22, 115-117.

Awong J, Bitton G, Chaydgyt GR. (1990) Microcosm for assessing survival of genetically engineered microorganisms in aquatic environments. Applied and Environmental Microbiology, 56, 977-983.

Barkay T, Sayler GS (1988) Gene probes as a tool for the detection of specific genomes in the environment. In Aquatic toxicology and hazard assessment, STP971. Eds Adams WJ, Chapman GA, Landis WG. ASTM. Philadelphia .

Bej AK, Perlin M, Atlas RM (1991) FEMS Microbiology and Ecology 86, 169-176.

Bowman JP, Sayler GS (1996) Nucleic acid techniques in the environmental detection of microorganisms in Molecular approaches to environmental microbiology. Eds Pickup RW, Saunders JR. Ellis Horwood.

Cairns J, Orvos D R. (1992) Establishing environmental hazards of genetically engineered microorganisms. Reviews of Environmental Containment and Toxicology, 124, 19-39.

Calomiris J J. Armstrong J L, Seidler R J. (1984) Association of metal tolerance with multiple antibiotic resistance isolated from drinking water. Applied and Environmental Microbiology, 47, 1238-1242.

Carter JP, Lynch JM (1996) Immunological approaches to the analysis of microbial populations" in Molecular approaches to environmental microbiology. Eds Pickup RW, Saunders JR. Ellis Horwood.

Chapelle F H. (1993) Groundwater Microbiology and Geochemistry John Wiley and sons New York.

Colwell and Grimes (1986) Evidence of Genetic modification of micro-organisms occurring in natural aquatic habitats, pp 222-230, in *Aquatic Toxicology and Environmental Fate*, Vol. 9 Eds Poston and Purdy. ASTM Pub. No 921 ASTM Philadelphia.

Department of the Environment, An evaluation of GENHAZ as a risk assessment system for proposals to release GMOs into the environment, Research Report 3, 1994.

Doyle JD, Stotzky G (1995) Effects of genetically engineered microorganisms on microbial populations and processes in natural habitats. Advances in Applied Microbiology, 40, 237-291.

Ebenhard T (1988) Introduced birds and mammals and their ecological effects. Swedish Wildlife Research 13(4) 5-107.

Fry J C, Day MJ. (1990) "Plasmid transfer in the epilithon" in *Bacterial genetics in natural environments*. Eds Fry J C and Day M J. Chapman and Hall. London.

Grabow and Prozesky (1973) Drug resistance of coliform bacteria in hospital and city sewage. Antimicrobial Agents and Chemotherapy, 3, 175-180.

Jones J G, Gardener S. Simon B M, Pickup R W (1986). Factors affecting the measurement of antibiotic resistance in bacteria isolated from lake water. Journal of Applied Bacteriology, 60. 455-462.

Jones J G, Gardener S, Simon B M, Pickup R W. (1986) Antibiotic resistant bacteria in Windermere and two remote upland tarns in the English lake district. Journal of Applied Bacteriology, 60, 443-453 .

Lorenz MG , Wackernagel W. (1991) High frequency of natural genetic transformation of *Pseudomonas stutzeri* in soil extract supplemented with carbon/energy and phosphorus source. Applied and Environmental Microbiology, 57, 1246-1251.

Mackenzie D R, Henry S C. (1990) *Biological monitoring of genetically engineered plants and microbes*. Agricultural Research Institute, Bethesda.

Madsen E L, Alexander M. (1982) Transport of *Rhizobium* and *Pseudomonas* through soil. 1982. Journal of the American Soil Science Society, 46, 557-560.

McClure et al (1990) Gene transfer in activated sludge pp111-129, *Bacterial genetics in Natural environments*, Eds Fry and Day. Chapman and Hall, London.

Miller R.V (1992). An Overview: Methods for the evaluation of genetic transport and stability in the environment. In *Microbial Ecology: Principles, methods and applications*. Eds Levin, Seidler, Rogul. McGraw-Hill, New York.

Murray GE, Tobin R S, Junkins B, Kushner D J. (1984) Effect of chlorination on antibiotic resistance profiles of sewage related bacteria. Applied and Environmental Microbiology, 48, 73-77

Niemi M, Sibakov M, Niemla S. (1983) Antibiotic resistance among different species of faecal coliforms isolated from water samples. Applied and Environmental Microbiology, 45, 79-83.

Postma J, Hok-a Hin C, van Veen J A. (1990) Role of microniches in protecting introduced *Rhizobium leguminosarum* biovar *trifolii* against competition and predation in soil. Applied and Environmental Microbiology, 56, 495-502

Remington JS, Schimpff SC (1981) Please don't eat the salads. New England Journal of Medicine, 304, 433-435.

Sandt and Herson (1989). Plasmid mobilisation from a genetically engineered micro-organism to an environmentally isolated strain of *Enterobacter cloacae* in sterile drinking water. Abstr. Annu. Meet. Am. Soc. Microbiol. New Orleans.

Saye D J. Miller R V. (1989) "The aquatic environment: consideration of horizontal gene transmission in a diversified habitat" in *Gene transfer in the environment*. Eds Levy S B and Miller R V. McGraw Hill. New York.

Scanferlato VS, Orvos DR, Cairns J, Lacy GH (1989). Genetically engineered *Erwinia carotova* in aquatic microcosms. Applied and Environmental Microbiology, 55, 1477-1482.

Seidler R.J. (1994) Evaluation of methods for detecting ecological effects from genetically engineered microorganisms and microbial pest agents in Terrestrial systems.. In *Environmental Gene Release* Eds Bazin and Lynch. Chapman and Hall

Shooter RA, Faiers MC, Cooke EM, (1971) Isolation of *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella* from food in hospitals, canteens and Schools. Lancet 2 390-392.

Steffan RJ, Atlas RM. (1988) DNA amplification to enhance detection of genetically engineered bacteria in environmental samples. Applied and Environmental Microbiology, 54, 2185-2191.

Stewart G J (1989) "The mechanism of natural Transformation" in *Gene transfer in The Environment* eds Levy S B and Miller R V. McGrawHill. New York

Stotzky G, Babich H (1986) Engineered Bacteria in Natural Environments. Advances in Applied Microbiology, 31, 93-139.

Top et al (1990) Gene escape model: Transfer of heavy metal resistance genes from *E. coli* to *Alcaligenes eutrophus* on agar plates and in soil samples. Applied and Environmental Microbiology, 56, 2471 - 2479.

Trevors J T, Barkay T, Bouquin A W. (1987) Gene Transfer among bacteria in soil and aquatic environments. Canadian Journal of Microbiology, 33, 191 - 197.

van Elsas J D, Dijkstra A F, Govaert, van Veen J A. (1986) Survival of *Pseudomonas fluorescens* and *Bacillus subtilis* introduced into two soils of different texture in field microplots. FEMS Microbial Ecology, 38, 151-160.

Van Veen J A, Kuikman P J, van Elsas J D. (1994) " Modelling microbial interactions in soil" in *Environmental Gene Release*. Eds Bazin M J and Lynch J M. Chapman and Hall. London.

Walter M V, Vennes J W. (1985) Occurrence of multiple antibiotic resistant enteric bacteria in domestic sewage and oxidation lagoons. Applied and Environmental Microbiology, 50, 930-933.

Wilkins BM (1990) "Factors influencing the dissemination of DNA by bacterial conjugation" in *Bacterial genetics in Natural environments* eds Fry JC and Day MJ. Chapman and Hall. London.

Williamson MH and Brown KC (1986) The analysis and modelling of British invasions. Phil. Trans. Roy. Soc London B 314 505-22.

8. USING THE POLICY AND PRACTICE FOR THE PROTECTION OF GROUNDWATER (PPPG) TO ASSESS MICROBIOLOGICAL RISK TO GROUNDWATER

8.1 PPPG Principles And Their Relevance To Risk From Microbes

Like other threats to groundwater resources, hazard from microbiological agents can be assessed using the strategies of the national groundwater protection programme or PPPG (see Policy and Practice for the Protection of Groundwater, 1st Edition 1992: 2nd Edition 1998). The programme uses the twin tools of groundwater vulnerability classification/mapping and source protection zone definition in conjunction with matrices of policy statements covering a wide range of human activities. Used together, these land-surface zoning tools and policy statements provide guidance to applicants and to regulators on the acceptability or otherwise of various activities which are potentially or actually prejudicial to groundwater. Not all these activities have the same significance in terms of microbiological impact. Table 8.1 therefore provides a qualitative guide which can be used with the policy statements in the PPPG. The table focuses primarily but not exclusively on potential microbiological impact from pathogens.

Implicit in the concept of intrinsic aquifer vulnerability employed in the PPPG is recognition of the ability of the subsurface to attenuate pollutants and thereby mitigate their effect on the groundwater resource. There are a number of factors which favour pollutant attenuation (nature of the soil cover, presence of drift, depth of unsaturated zone etc) but for microbes perhaps the key element is that of travel time. Any process which extends the residence time of a water before it is abstracted from an aquifer reduces risk from pathogens. The corollary is that any structure constructed to tap groundwater whose design inadvertently or otherwise draws from shallow flow cycles likely to contain short transit-time recharge has a higher pollution risk than one drawing on long residence-time waters. Figure 8.1 shows the conceptual relationship.

8.2 Risk To Groundwater Sources; Assessment Using Source To Pathway To Receptor Principles

When assessing risk to groundwater sources from pathogens, the phrase 'short travel times' may need clearer definition. In terms of bacteria and many viruses, a residence time measured in terms of a few tens of days is usually sufficient to eliminate significant hazard. The Zone I of inner protection in the PPPG is defined by the 50-day travel time isochron on just such a consideration, and is the zone of most stringent land-use control. However, some protozoan parasites, once encysted, are more resilient outside their hosts than most bacterial and viral pathogens. It is known for instance that *Cryptosporidium* sp can survive dormant in cool moist soil for months (Anon, 1990). In circumstances where such parasites may pose a threat, 'short travel times' would be an order of magnitude larger.

Using short travel times/pathways as a prime criterion, many spring systems and abandoned mine workings, most infiltration galleries and catchpit systems and some shallow wells would be more likely to encounter microbially-related pollution problems than deep

wells/boreholes. In addition, some adited wells can inadvertently induce and favour rapid downward leakage of recent, potentially contaminated, water.

In view of the prime role of productive adited Chalk sources in public groundwater supplies in southern and eastern England, risk assessment for this special type of well would be of higher priority than that for deep boreholes. Such wells tend to be located close to valley axes, where experience has shown that the highest transmissivities (greatest productivity and most well-developed fracture systems) are encountered (Figure 8.2). Some underlie rivers and river alluvium. Pumping from such systems is inherently higher risk because:

- an adit is more likely than a shaft/bore to intersect vertical fractures which act as flow conduits
- pumping from an adit imposes a steep vertical gradient along its length instead of just in the vicinity of the well.

When pumping, the water level in the major vertical fractures is the same as in both the adit and the shaft, and the significant vertical gradient between gravels and large vertical fissures could drive rapid downward movement. Furthermore, if the river bed is permeable, the draining of the alluvium may induce leakage. This could be problematic if highly transmissive coarse gravels dominate, as they provide opportunity for rapid downward flow of surface water to the vertical fissure systems in the underlying Chalk.

This example demonstrates that realistic risk assessment would place equal importance on understanding:

- the activity which may generate the microbiological load,
- the likely behaviour of the microbes once in the subsurface,
- the hydrogeological setting and
- the vulnerability of the structure tapping the groundwater

This classic source->pathway->receptor approach to microbiological impact is consistent with the guidelines on risk assessment and management for environmental protection published by the Dept of the Environment (Anon, 1995). So, when trying to assess likely microbiological impact on groundwater in vulnerable aquifer situations, the following factors would be paramount:

1) What inherent features of the groundwater setting might render a particular aquifer or area of aquifer prone to receive and rapidly transmit microbes to the source of abstraction ie what *hydrogeological predisposing factors* are at work?

2) What activities are taking place on the catchment which might generate a load ie what *catchment predisposing factors* may be present?

3) What aspects of the groundwater supply source as a hydraulic structure might increase the chances of intercepting contaminated raw water which has already entered the subsurface ie what *pumping station/well design predisposing factors* may be operating.

Although in some respects these factors are complementary, for a given groundwater source those relating to the hydrogeology are inherent and cannot be altered short of relocating the source to a less vulnerable site. In contrast, land/activity control measures could drastically reduce likely contaminant loadings to the subsurface, while a critical appraisal of potentially

hazardous well design characteristics could further reduce the likelihood of encountering pathogens in sufficient numbers to trigger a disease outbreak.

An alternative strategy in the case of wells/springs providing potable water or for other sensitive use would be to employ the risk factors to identify those sources where additional precautionary raw water treatment measures would be advisable.

A flow-chart to assist the risk assessment process is shown in Figure 8.3 (from Lawrence et al, 1996).

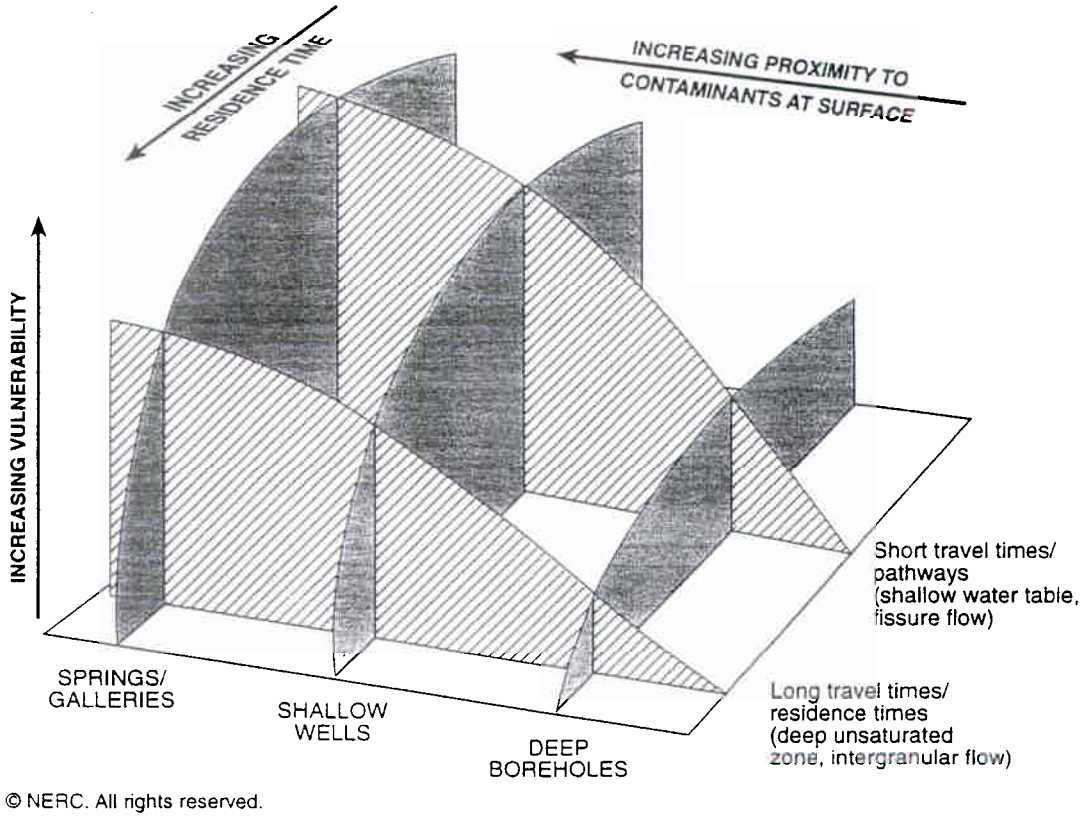


Figure 8.1. Relationship of vulnerability, residence time and type of abstraction to microbial risk (from Ball et al, 1997)

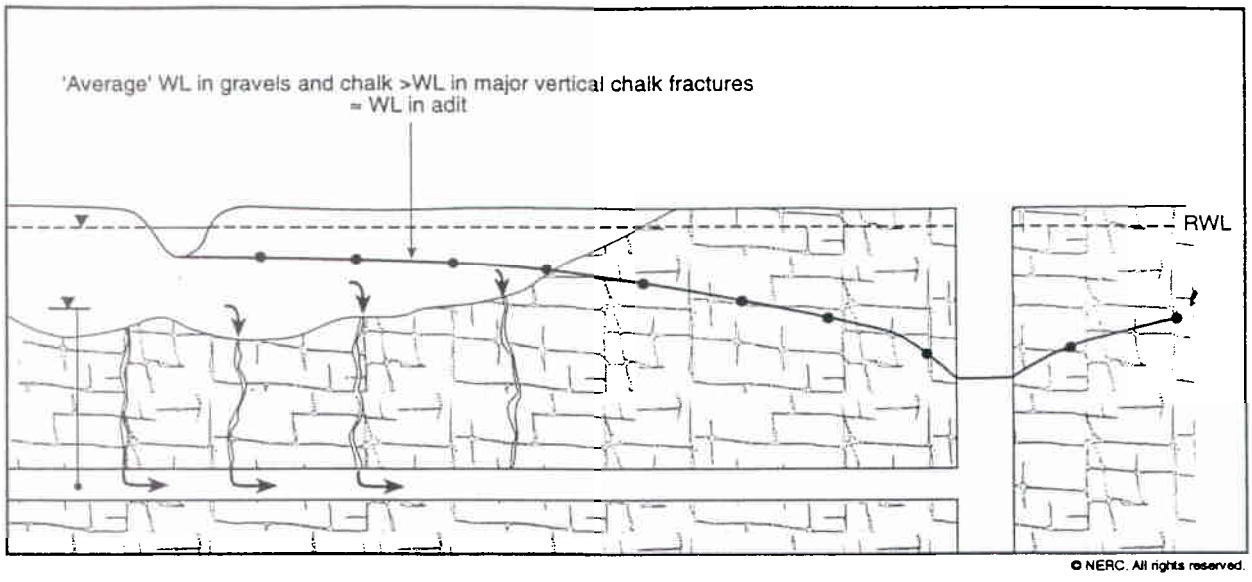


Figure 8.2. Adited Chalk systems can induce downward leakage from overlying river gravels, greatly reducing time of travel through the aquifer

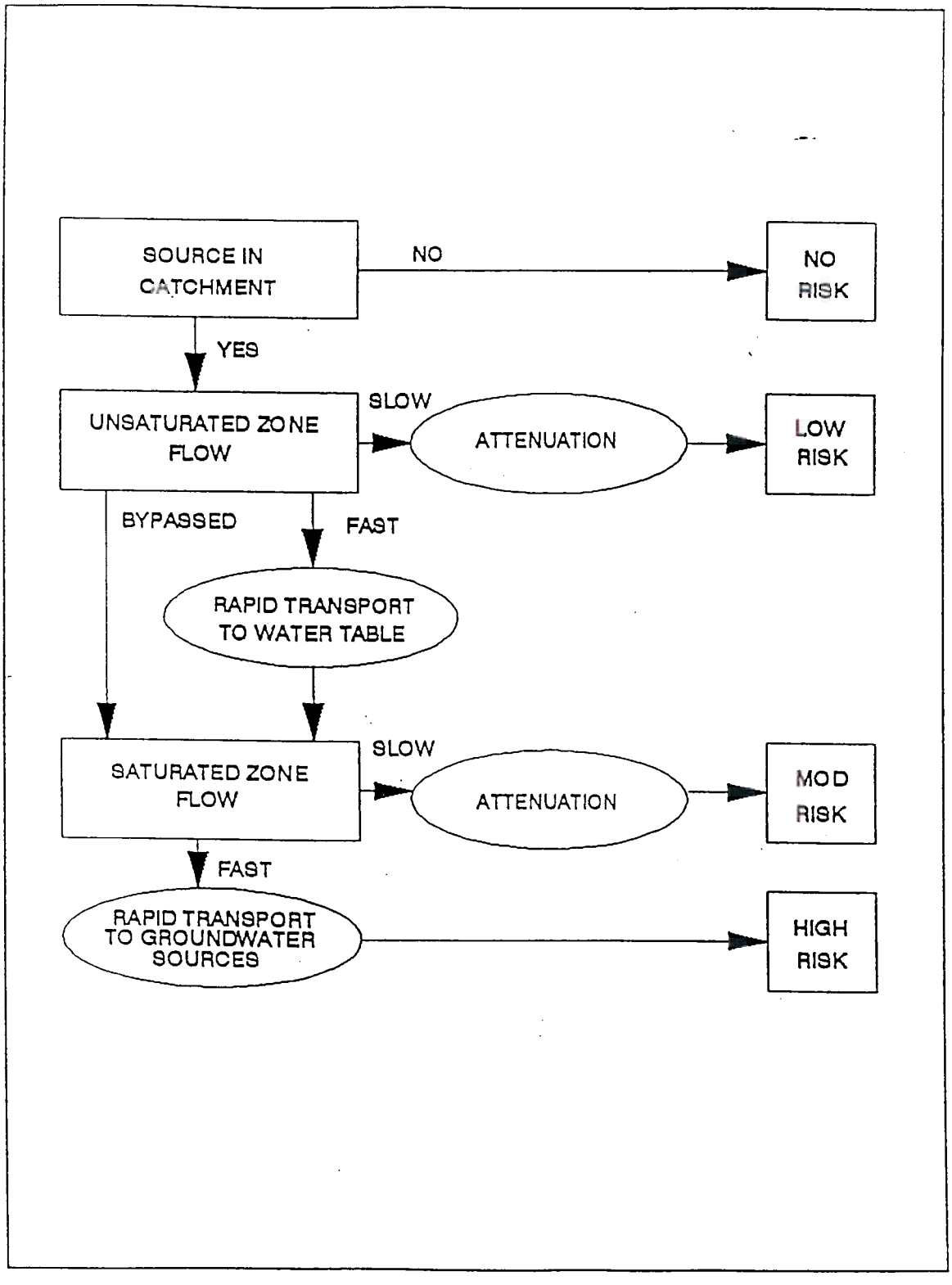


Figure 8.3. Conceptual model for assessment of risk to groundwater source from microbiological contamination

Table 8.1. Guide for use with PPPG policy statements to assess likely microbiological significance of activities potentially contaminating groundwater

Key

√ slight pathogen risk or limited to special situations

√√ pathogen risk likely to be present

√√√ Pathogens certain to be present; high significance

X Unlikely to be of pathogen significance

mainly bioremediation significance

	Groundwater resource threat category	Sub-category	Likely micro-bio l.significance	Comments
A	Control of groundwater abstractions	-	X to √	risk mainly from induced rapid infiltration eg through river beds due to aquifer dewatering
B	Physical disturbance of aquifers and gw flow	mining	X to √	associated with active drift mines; now much reduced with decline of coal industry
		civil construction	√	local temporary risk may be associated with dewatering for foundations, tunnels
		borehole construction/ abandonment	√√√	High risk to dual porosity aquifers from abandoned shafts, wells, boreholes, adit ventilation etc
		field drainage intercepting recharge	√	Risk mainly from reduced residence times in soil zone and increased hydraulic loadings around drains .
C	Waste disposal to land	high pollution potential landfills	√√	Risk greatest from still operational older un-engineered sites. Newer likely to be engineered to be low risk with respect to pathogen escape.
		medium and low pollution potential landfills	√	Loadings generally low due to nature of waste, but many sites unlined. Waste type crucial
D	Contaminated land	waste lagoons	√√ #	pathogen risk dependent on nature of effluent and vulnerability setting
		chemical manufacture	#	site cleanup options may include bioremediation. Potential hazard from pharmaceutical industry
		heavy industry	#	site cleanup options may include bioremediation
		mining	#	site cleanup options may include bioremediation
		sewage treatment works	√√	risk from pathogens where outfall enters losing river reach
		metal refining	#	site cleanup may include bioremediation option
		oil refining, hydrocarbon storage	##	site cleanup options likely to include bioremediation option

E	Disposal of liquid effluent, sludges, slurries to land	inorganic or non-biodegradable	X	-
		strong organic and biodegradable	√√√	Controlled wastes like cesspool contents, sewage sludges and agricultural wastes all significant
		Low pollution potential, high dilution	√	Pathogen transmission risk greatest in fissure flow- dominant and dual porosity aquifers where dilution effect impaired
F	Discharges to underground strata	Septic tank or treated sewage effluent to underground strata	√ to √√√	Significance depends on loadings (strength and total volume) and aquifer or discharge source vulnerability
		Trade effluent	√ to √√√	Controlled by effluent type; hazard could range form negligible (cooling water) to significant (some food/agricultural processing plants)
		Pluvial drainage to soakaways	√	Generally low significance unless transit time from soakaway to saturated zone very rapid (days -months) or bypass flow significant. Treat combined stormwater/foul sewers as category G.
G	Diffuse pollution of groundwater	Sewer leakage	√√√	Specially significant in settings where travel time to saturated aquifer is very short (days -months)
H	Additional activities or developments which pose a threat to gw quality	Intensive livestock rearing indoor or outdoor	X to √√√	Controlled by presence in catchments of rapid transport pathways eg through karstic features
		Graveyards/animal burial sites	√ to √√√	As category F . Specially significant in settings where travel time to saturated aquifer is very short (days -months)
		Oil/petroleum storage and transport	√	Any spillage cleanup options likely to include bioremediation
		Transport infrastructure	X to √√√	Highly dependent on pluvial and accidental spillage drainage arrangements and aquifer vulnerability setting

8.3 References

Anon.(1990) *Cryptosporidium* in water supplies. Department of the Environment, Department of Health. HMSO London

Anon. (1995) A guide to risk assessment and risk management for environmental protection. Department of th Environment. HMSO London

Ball DF, MacDonald AM, Morris BL and Lilly A (1997) Scotland's minor aquifers: a scoping study to assess groundwater source protection policy. BGS Technical Report WD/97/63 Edinburgh

Environment Agency (1998). Policy and practice for the Protection of Groundwater. 1st Edition 1992, 2nd Edition 1998. Environment Agency, Bristol. Stationery Office, London.

Lawrence AR, Williams AT, Brewerton LJ, Barker JA, Bird MJ and Ward RS (1996) Pathogen transport in the Chalk aquifer; an assessment of the risk to Chalk groundwater sources. BGS Technical Report WD/96/48C

National Rivers Authority (1992) Policy and practice for the protection of groundwater. NRA Bristol

9. CONCLUSIONS AND RECOMMENDATIONS

This review shows that there is little information on groundwater microbiology (both indigenous and introduced populations) in the UK and even less on the role of indigenous microbiology on groundwater quality and health. Most of the information obtained for this review has come from North American studies. US work focusses on aquifers with different hydrogeological properties to those in the UK, the majority being sandy. Therefore the data may not be directly relevant to the UK situation.

9.1. Groundwater Microbiology

It is recommended that a pilot study in selected areas be undertaken to establish a baseline of indigenous and pathogenic microbiological presence and activity. This could include monitoring in urban and rural areas to establish differences and trends in microbiological populations and would use existing protocols. Samples could be taken monthly to establish seasonal variations. Without this baseline study it is impossible to establish how introduced organisms from any source would disturb the aquifer ecosystems and is particularly valuable when considering the suitability of bioremediation in a particular area. The proposed pilot-scale monitoring programme is consistent with the national strategy for groundwater quality assessment adopted in 1995 (Chilton and Milne, 1994).

A separate study should also be performed to examine the effects of microbiology on mobilisation of elements damaging to health in certain aquifers. This could use existing databases (eg the British Geological Survey's baseline groundwater quality, HYCHEM and GBASE databases) linked to biogeochemical modelling of aquifer microbiology.

There is little information in the UK on the survival and migration of pathogens in the unsaturated zone. Little data are available on attenuation and transport of pathogens in groundwater. The World Health Organisation (WHO) has recognised this as a priority area for research as part of the rolling revision of Guidelines for Drinking Water Quality (minutes from WHO meeting, Medmenham, 17-21 March 1998). This review recommends a UK meeting to identify ongoing research and define priorities for research investment.

9.2 Subsurface Microbiology Protocols

The study has shown that there are no clear protocols for sampling subsurface materials or for examining microbial population diversity and activity. Faecal and total coliform tests have limited value as pathogen indictors. It is recommended that new protocols for both sampling and analysis of organisms in the subsurface be drawn up (they are unable, for instance, to provide warning of outbreaks caused by some viral or protozoan pathogens). These protocols would cover both indigenous populations and contaminant organisms hazardous to health.

These new protocols could then be implemented in the same pilot study suggested above. A useful comparison between the new protocols and former methods could then be made. This would prove important when advising on the implementation of the new protocols as real differences in their relative sensitivities could be seen.

9.3 Septic Tanks and Package Treatment Plants (PTPs)

The study has shown that there is little information on the effectiveness of removal of microbiological contaminants across septic tanks and PTPs. There is no consideration of biological quality of effluent nor of the capacity of the subsurface to treat effluent. This is a serious omission because, unlike sewage treatment works, both septic tanks and PTPs are designed to dispose of their effluent to the subsurface, without consideration of the ability of the receiving body to attenuate/absorb/dilute the effluent loading. The only concern is if a soakaway can physically cope with the volume of effluent. Urgent consideration must be given to the biological quality of effluents from septic tanks and PTPs.

The contamination of groundwater by septic tanks in the U.S.A. is well documented but there is very little data on the microbiological contamination of groundwater by septic tanks in the U.K. It is important to establish the extent of this contamination by studying existing septic tank and PTP systems throughout the country, in a range of geological conditions, and especially the microbiology of the groundwaters in the vicinity of the systems. This survey would provide essential background information and a basis for prioritisung further work.

The area of soakaway design and optimisation is of concern on water protection grounds. The existing percolation test may not be adequate and needs to assessed. There are no recommendations concerning minimum depth to water table for a soakaway. The main risk factors are: correct sizing of the plant; proper operation including control over what is discharged into the septic tank or PTP; proper maintenance including appropriate desludging and maintenance of plant; appropriate sizing and siting of the soakaway for the septic tanks or PTPs; and education of the users of septic tanks and PTPs. Correct operation of all the equipment is vital.

The publication in February 1998 of user and regulator guides to septic tank system operation by CIRIA has provided excellent good practice literature. However, the paucity of information from UK field studies on the microbial and chemical nature of typical septic and PTP effluent mean that the impact of these on-site sanitation systems on particularly fragile aquifer settings has not been assessed. This is an obvious area for further investment.

9.4 Bioremediation

The review has shown that there are very few studies on the impacts of bioremediation in groundwater microbiology. However, from the available work it is clear that there is a need to examine different remediation technologies and their effects on groundwater quality in terms of changes in microbiology and chemistry. For instance, oxygen and nutrient injection can change redox status, and this could lead to mobilisation of metals occuring naturally in the matrix. This process has already been observed below leaking sewers in Germany (Eiswith and Hotz, 1997) and below districts served by on-site sanitation in Bolivia and Thailand (Gooddy et al, 1997). Indeed some technologies may not be appropriate for certain sites. It is recommended that risk assessments on sites being considered for bioremediation should include inter alia possible effects on, for example, redox and consequent effects on mobilisation of elements. This information would be useful to the Environment Agency in assessing urban remediated water quality. Increasing demands on rural water supplies could mean increasing use of these waters and their quality must be assessed.

It is recommended that the Environment Agency maintains its awareness of bioremediation protocols so that it can advise enquirers on the suitability of the appropriate technique for a

particular site. Much of the information from case studies on bioremediation in the UK is 'commercial in confidence' and unavailable. It is recommended that the Environment Agency coordinates a series of meetings to scope the current status of bioremediation technology in the UK with the main aim to establish best practice and research priorities.

9.5 Antibiotic Resistant and Genetically Modified Organisms

No information is available on the antibiotic transfer or genetic stability of organisms in the subsurface or in groundwaters.

The Environment Agency is advised to keep a 'watching brief' on this new and rapidly evolving topic.

9.6 Microbiology and Policy and Practice For The Protection of Groundwater (PPPG)

This review has shown that the PPPG can be used as a framework to assess potential hazard from microbiological agents. PPPG policy statements guidelines, for instance, can assist in identifying and prioritising those activities which may impact adversely on groundwater resources and Figure 8.3 provides a flow-chart to help assess risk by the source -> pathway -> receptor method.

However, one weakness of the PPPG is that it does not easily allow for curtailment of potentially prejudicial **existing** activities/practices on the land surface rather than proposed ones.

This review strongly recommends a more proactive stance in assessing microbial risk to existing groundwater public supplies, in collaboration with water supply companies. Risk assessment should start with springs, mine workings, infiltration galleries and adited well systems. In addition, the significance of leaking sewers in urban areas must be considered as a potential source of microbial contamination.

9.7 Future Concerns

In the face of such scant knowledge of the microbiological effectiveness of on-site sanitation systems and of high-intensity sludge disposal to land, this report has shown that there are major uncertainties about the capability of the subsurface to accept and attenuate such waste loadings. An assessment of these future routes must be carried out.

9.8 Training

If the recommendations of this report are adopted then training of users in Microbiological Sampling and Analysis protocols is needed. This could be achieved by the production of leaflets and videos.

9.9 References

Chilton PJ and Milne CJ (1994) Groundwater quality assessment: a national strategy for the NRA. Final Report, November 1994. BGS Technical Report WD/94/40C BGS Keyworth Notts

Eiswirth M and Hotzl H (1997) The impact of leaking sewers on urban groundwater. In Procs of the XXVII IAH Congress on Groundwater in the Urban Environment, Nottingham UK, 21-27 September 1997 pp 399-404

Gooddy DC, Lawrence AR, Morris BL & Chilton PJ (1997) Chemical transformations of groundwater beneath unsewered cities. In Procs of the XXVII IAH Congress on Groundwater in the Urban Environment, Nottingham UK, 21-27 September 1997 pp 405-410

10. ACKNOWLEDGEMENTS

*We gratefully acknowledge Clearwater Polcon plc (formally Polcon (UK) Ltd) for permission to use Figure 5.4. Please note new details for this company are as follows:

Clearwater Polcon plc
Clearwater House
Bristol Road
Bridgwater
Somerset
TA6 4AW
Tel: 01278 433443
Fax: 01278453653

We would like to thank everyone at the Environment Agency for their support during this review.

GLOSSARY & ABBREVIATIONS

Glossary

Activated sludge	A flocculent microbial mass produced when sewage is continuously aerated
Aerobic action	A biological process promoted by action of bacteria in the presence of dissolved oxygen
Aerobe	An organism which grows in the presence of oxygen; may be facultative or obligate.
Anaerobic action	A biological process promoted by action of bacteria in the absence of dissolved oxygen
Anabolism	The biochemical processes involved in the synthesis of cell constituents from simpler molecules, usually requiring energy
Anaerobe	An organism which grows in the absence of oxygen
Autotroph	Organisms able to utilise carbon dioxide as a sole source of carbon
Biodegradable	Capable of being broken-down by living organisms
BOD	Biochemical Oxygen Demand. The amount of dissolved oxygen consumed by microbial action when a sample is incubated, usually for 5 days at 20 deg °C
Biological filter:	A bed of relatively inert material (such as slag, moulded particles, clinker etc.) to promote or assist natural aerobic digestion of sewage.
Catabolism	The biochemical processes involved in the breakdown of organic compounds, usually leading to the generation of energy
Chemoautotroph	An autotrophic organism obtaining energy from the oxidation of inorganic compounds
Chemolithotroph	An organism that uses an inorganic compound as an energy source
Chlorination	The practise of adding small amounts of chlorine to drinking water to ensure microbiological safety
Coliforms	Gram-negative, nonsporing facultative rods that ferment lactose with gas formation within 48 hours
Cyanobacteria	Blue-green bacteria which perform oxygenic photosynthesis
Cyst	A resting stage formed by some bacteria and protozoa in which the whole cell is surrounded by a protective layer
Denitrification	Conversion of nitrate to nitrogen gases under anaerobic conditions, resulting in loss of nitrogen from ecosystems
Distributor	A device for spreading settled sewage over the surface of a biological filter
Electron acceptor	A substance which accepts electrons in an oxidation-reduction reaction. An electron acceptor is an oxidant
Electron donor	A substance which donates electrons in an oxidation-reduction reaction. An electron acceptor is an reductant
Endotoxin	A toxin released from the cell; bond to the cell surface or intracellular

Enteric	Intestinal
Entrotoxin	A toxin affecting the intestine
Eucaryote	A cell or organism having a true nucleus
Eutrophication	Nutrient enrichment of natural waters, usually from artificial sources.
Exotoxin	A toxin released extracellulary
Facultative	A qualifying adjective indicating that an organism is capable of growth either in the presence or absence of an environmental factor
Filter medium	The material of which the biological filter is formed and on which a biological film containing bacteria and fungi develops
Final effluent	The effluent discharged from a sewage treatment works
Heterotroph	Organism obtaining carbon from organic compounds
Humus tank:	See secondary settlement tank
Host	Am organism capable of supporting the growth of a virus or parasite
Mixed liquor	A mixture of sewage and activated sludge undergoing circulation and aeration the aeration tank or channel of an activated sludge plant
Mixotroph	An organism able to assimilate organic compounds as carbon sources while using inorganic compounds as electron acceptors
Most probable	A statistical expression providing a measure of cell number in a Number (MPN) population
Nitrification	The conversion of ammonium to nitrate
Nitrogen fixing	Reduction of nitrogen gas to ammonium
Nutrient	A substance taken into a cell from its environment and used in catabolic and anabolic reactions
Obligate	A qualifying adjective referring to an environmental factor always required for growth
Oligotrophic	Describing a body of water in which nutrients are in low supply
Pathogen	An organism capable of inflicting damage on a host it infects.pH. An expression indicating the hydrogen-ion concentration of a solution
Plaque	A localised area of virus lysis on a lawn of bacteria
Protozoa	Eukaryotic microorganisms
Sewage	The water-borne wastes of a community
Sludge	A mixture of solids and water produced during the treatment of waste
SS	Suspended solids - solids in suspension in sewage liquors as measured by filtration through a glass fibre paper or centrifugation
Species	A collection of closely related strains
Spore	A general term for resistant resting structures formed by many bacteria and fungi
Toxigenicity	The degree to which an organism is able to elicit toxic symptoms
Toxin	A microbial produced substance capable of inducing damage to a host
Vector	An agent, usually an insect or other animal, able to carry pathogens from one host to another

Viable count	Measurement of the concentration of live cells in a microbial population
Virus	A genetic element that is able to alternate between intracellular and extracellular states, the latter being the infective state
Water table	The level below which the ground is saturated with water.

Abbreviations

ATP	Adenosine Triphoshate
BGS	British Geological Survey
BOD	Bio-Chemical Oxygen Demand
BSE	Bovine Spongiform Encephalopathy
BS	British Standard
BTEX	Benzene, Toluene, Ethylbenzene And Xylene
CFU	Colony Forming Unit
CIRIA	Construction Industry Research And Information Association
COSHH	Control Of Substances Hazardous To Health
CTC	Carbon Tetrachloride
CSIRO	Central Scientific Investigation And Research Organisation
DCO	Dissolved Organic Carbon
DMR	Department Of Mineral Resources, Thailand
DCE	Dichloroethene
DNA	Deoxyribonuleic Acid
DNT	Dinitrotoluene
EA	Environment Agency
EPA	Environment Protection Agency
EPS	Extracellular Polysaccharides
ETS	Electron Transport System
GENHAZ	The Principals Of HAZOP Applied To The Planned Release Of Gmos
GMO	Genetically Modified Organism
HACCP	Hazard Analyses Of Critical Control Points
HAV	Hepatitus A Virus
HAZOP	Hazards Opperability Study
HOC	Halogenated Organic Compound
HSE	Health And Safety Executive
LPP	Low Presure Pipeline System
NOELS	No Observerable Effect Levels
NRA	National Rivers Authority
MAR	Multiple Antibiotic Resistant
MMO	Methane Monooxygenase
OECD	Organisation For Economic Co-Operation And Development
OTA	Office Of Technology Assessment

PAH	Polycyclic Aromatic Hydrocarbons
PCR	Polymerase Chain Reaction
Pr P	Prion Protein
PPPG	Policy And Practice For The Protection Of Groundwater
PSU	Prince Of Songkhla University, Thailand
PTPS	Package Treatment Plants
RBCS	Rotating Biological Contractors
RNA	Ribonucleic Acid
SAFS	Submerged Aerated Filters
SEAC	Spongiform Encephalopathy Advisory Committee
2,4,5-T	Trichlorophenoxyacetate
TCA	Trichloroethane
TCE	Trichloroethene
TECE	Tetrachloroethene
TCM	Tetrachloroform
TNT	Trinitrotoluene
TOC	Total Organic Carbon
TSE	Transmissible Spongiform Encephalopathies
UNEP	United Nations Environment Programme
WHO	World Health Organisation