

Groundwater impact of Danescourt Cemetery, Wolverhampton

National Groundwater And Contaminated Land Centre
December 2002

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Foreword

This report presents the results of a joint investigation by the British Geological Survey (BGS) and the Environment Agency (the Agency) into the impact cemeteries on groundwater quality at the Danescourt cemetery, Wolverhampton.

It has been carried out within the terms of the memorandum of understanding between the Environment Agency and the Natural Environment Research Council (British Geological Survey) which aligns research activities.

The report is a contribution to the Environment and Health Project of the British Geological Survey which is looking into the main pathways of human exposure to anthropogenic contamination.

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1 Introduction

In 1998, the Environment Agency initiated a literature review with the following objective and rationale:

“to provide detailed guidance which will enable Agency staff to adopt a consistent approach when assessing the risks associated with cemetery developments. The guidance is to be directed principally at potential risks to groundwater resources, but taking account also of surface waters, soil and air”.

The study, conducted by Young *et al.* (1998), identified incidences of groundwater pollution from burial grounds situated in regions where hydrogeological factors favour the development of anoxic ground conditions. Shallow water table and high burial rates were also cited as potential concerns. The prolonged presence of decay products under anaerobic conditions could also be found in areas of low permeability (non-aquifers) that could threaten local surface waters.

In 1999, the British Geological Survey in partnership with the Environment Agency undertook a site investigation of the 19th century Carter Gate Cemetery in Nottingham (Trick *et al.* 1999). Drilling three boreholes after the 1100 graves had been exhumed, allowed pore water geochemical profiles in the unsaturated zone and saturated zone to be determined.

The solute profiles are believed to provide evidence of migration of grave derived material. However, modelling showed major ion species such Na, Cl, SO₄, potentially released from the graves, would migrate through the unsaturated zone within a period of 20 years after the cemetery closed and hence the likelihood of detecting an impact after such a prolonged period since site closure is low. The report concluded that *“For any risk assessment to be undertaken from whatever potentially polluting activity, the actual source composition of contaminants and their fate in the subsurface need to be addressed. The source term defines the range of contaminants and how their flux into the natural environment varies in terms of time and concentration. Their fate in the subsurface depends on the nature of the contaminant and the hydrogeological environment in which they are released. In order to study these aspects a combination of laboratory and controlled field experiments could be considered in conjunction with field investigations of a range of cemeteries in hydrogeologically representative situations within the UK”.*

A recent review (West, *et al.* 1998) of microbiological contaminants in groundwater confirmed that little information on groundwater microbiology of both indigenous and introduced populations exists in the UK and neither are the implications for groundwater quality and human health understood. There is little information available on the survival and migration of pathogens in the unsaturated zone and in groundwater. This study gave an opportunity to look at those pathogens most closely associated with the decomposition of human corpses and their possible survival and transport to groundwater beneath the burial site.

Waterborne pathogens consist of several groups of enteric and aquatic bacteria, enteric viruses and enteric protozoa. Pathogenic organisms, when present in groundwater and surface water, may originate from a number of sources; human, animal or the environment itself; soil, water, and air. Most waterborne infectious agents are from the enteric tracts of humans and animals. The persistence of enteric organisms in the aquatic environment is dependant upon a number of environmental factors, e.g. inactivation (half-life) of microbes, the nature of the soil, temperature, availability of nutrients, pH and adsorption. A more detailed account of the survival and transport of microbiological contaminants in groundwater was provided by West *et al.* (1998). In terms of human decomposition a variety of organisms have been isolated from the human corpse the majority originating from the intestine and most are strict anaerobes.

A desk study of the pollution potential of cemeteries (Young *et al.* 1998) concluded there is very little information from the UK. Previous field studies in Brazil (Pacheo *et al.* 1991) looked at biological contamination of groundwater by micro-organisms associated with decomposition of human corpses and monitored the water quality at three cemeteries with different geological characteristics. Results indicated that contaminant transport to the water table was greatly influenced by lithology. In variable thickness Tertiary sediments of uniform grain size the lowest levels of micro-organisms were detected, possibly due to the sediment acting as a natural filter retaining micro-organisms and organic

material. Bacteria attributed to decomposition were detected at all three sites and the study concluded that cemeteries do indeed pose a risk to groundwater. The study also noted that an Australian cemetery investigation identified the bacterial pathogen *Pseudomonas aeruginosa* as being closely associated with graves.

Background to the investigation

In September 1999 a heavy rainfall event affected the Danescourt Cemetery in Wolverhampton causing widespread subsidence of graves, many collapsing by as much as 0.3 metres, Figure 1.1. The Wolverhampton City Engineer gave permission to the BGS to carry out a site investigation as a result of this event. During the course of the investigation boreholes were drilled into roadways to establish water levels. The site was considered to be suitable to investigate the impact of a modern working cemetery on a major aquifer, the Sherwood Sandstone Group, and the opportunity was taken to complete the investigation boreholes with screened casings and establish a groundwater monitoring network at the site.



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Figure 1.1 Collapsed grave at Danescourt Cemetery

A preliminary desk study was carried out to identify any existing sources of data relevant to the investigation. The succeeding geological investigation consisted of drilling two cored boreholes and construction of a drift map based on a hand auger and walk over survey.

The hydrogeological investigations consisted of:

- construction of a piezometric map based on water level data gathered from ten boreholes;
- determination of the hydraulic conductivity in the drilled holes using falling head slug tests;
- determination of the vertical hydraulic conductivity of the drift using infiltrometer tests;
- determination of a value of effective precipitation using MORECS data;
- determination of the chemical and microbiological quality of the groundwater beneath the site;
- modelling unsaturated zone contaminant transport from a grave.

Site description

Location, rainfall and drainage

The Danescourt Cemetery is situated off Wergs Road in the Wergs district of Wolverhampton, national grid reference SJ 881002 (Figure 1.2).

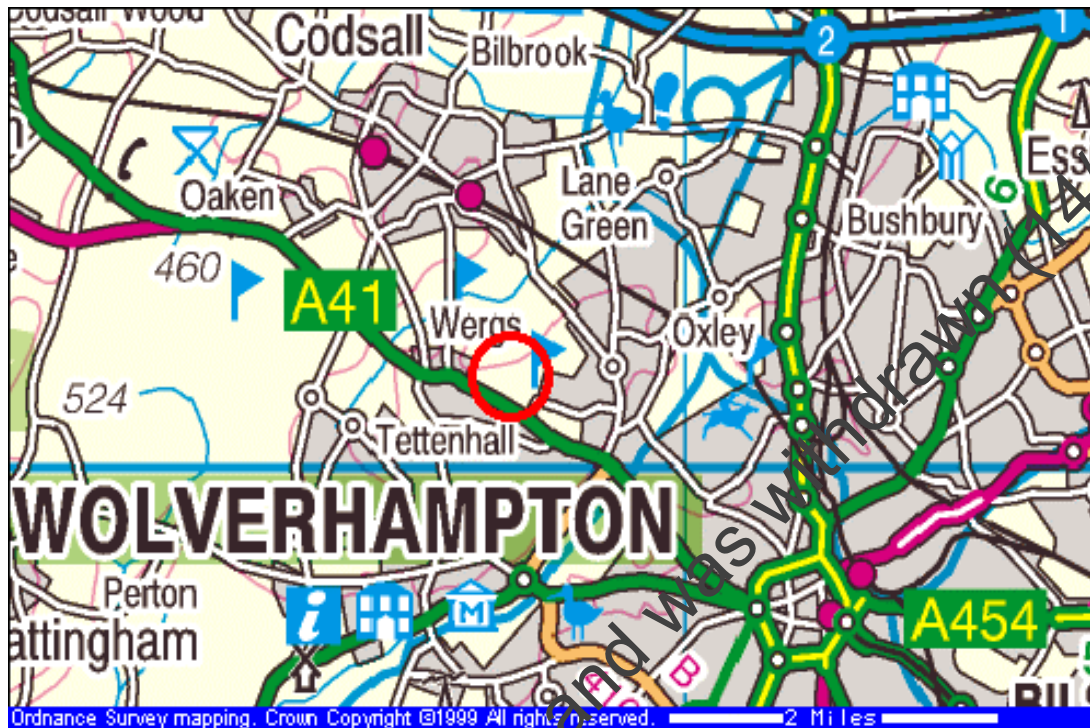


Figure 1.2 Location of Danescourt Cemetery

Records of rainfall, temperature, sun hours, humidity and wind speed data for Tettenhall Pumping Station (SJ 885001) have been measured daily since 1970. The average annual temperature is 10°C with temperatures less than 5°C in the winter to temperatures over 16°C in the summer. Daily rainfall records for the Barnhurst weather station (SJ901017) situated less than 2km from the cemetery show a yearly average rainfall of 74.28mm between 1979 and 1999. Any surface water drainage is towards the River Pen, which flows about 0.5km to the north of the cemetery.

Site history

Danescourt Cemetery opened on a 4.8ha site in 1959. Following established practice the first burials were placed at the bottom of the slope so that any water running into new graves is not polluted by up-gradient burials. By 1995 the site was nearing capacity and was expanded to the south although re-internments and some new burials are still placed in the old section.

The cemetery now occupies an area of 9ha and comprises two sections:

1. An older section that has an entrance via Coppice Lane at the northwest corner of the site and extends to the woods at the rear of the former Danescourt Territorial Army Centre. The older section of the site is situated on a north-west facing slope with a gradient of 1:20.
2. A newer section that is accessed via the main entrance to the cemetery from a vehicular access road off Wergs Road (Figure 1.2).

Burial practice

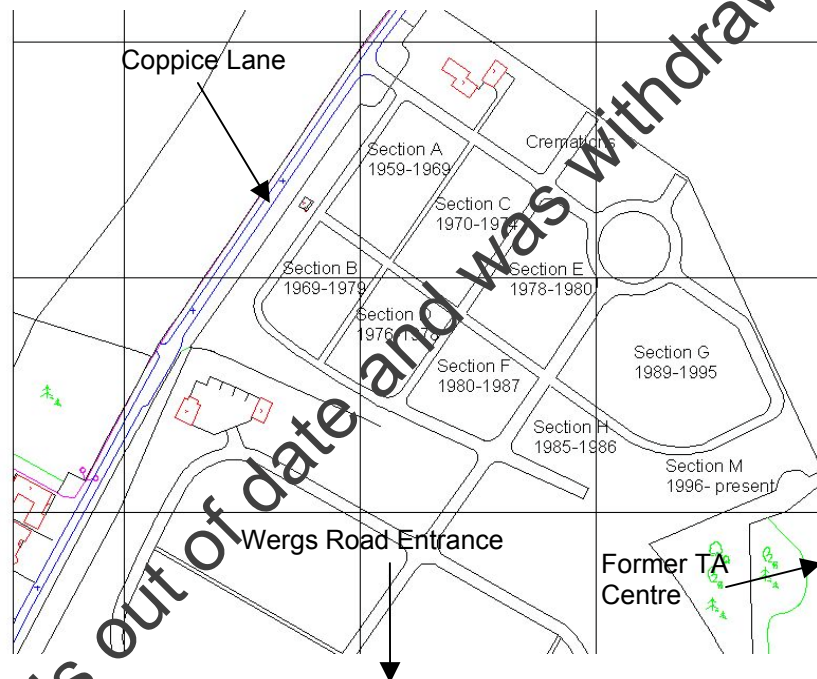
The depth of graves is dependent on the anticipated number of coffins as shown in Table 1.1

Table 1.1 Depths of burial

Number of coffins	Depth m bgl
1	1.40m
2	1.83m
4	2.74m

Coffins generally stay intact for up to five years depending on the materials used in their construction. However, coffin lids often break upon grave back filling. Approximately 90% of graves at the Danescourt Cemetery are dug to 1.8 metres depth. Figure 1.3 is a site plan showing the approximate date of the burials at the site and gives an indication of how the site was developed.

Figure 1.3 Site plan showing approximate date of burials



2 Detailed site investigation

Geophysical survey

Electrical resistivity tomography (ERT) is a method by which 2D or 3D models of subsurface resistivity distributions are generated. Using this method, features with electrical properties, which contrast with surrounding material, may be characterised in terms of their resistivity, geometry and depth of burial. ERT data are typically collected from surface or down-hole multi-electrode arrays using computer controlled measurement systems. The data are inverted to produce models of subsurface resistivity.

At Danescourt Cemetery, ERT was employed to investigate the lithological variation in the superficial deposits and underlying sandstone. The survey was carried out on 24th February 2000. The resistivity measurements were made using the prototype ABEM IPT Instrument. Data were collected from a single line, 195 m in length, extending from 388179 mE, 300820 mN to 388018 mE, 300930 mN. The line comprised 40 electrode positions at 5 metre intervals (Figure 3.2).

Drilling

Nine boreholes were drilled at the location shown in Figure 3.2. Two of these boreholes were fully cored to obtain continuous samples to approximately five metres below the water table. Borehole details are given in Table 2.1. To avoid contaminating the cores and aquifer during drilling, no fluids apart from air were introduced into the borehole unless unavoidable for progress. Boreholes BH1 and BH2 were intended to be up gradient of the graves to provide information on background conditions. Borehole BH3 - BH7 were positioned within the graveyard and boreholes BH8 and BH9 were positioned down gradient to intercept any contamination migrating off-site. Six metres of screened casing (50mm HDPE) were installed into the boreholes and then they were back filled with a 6.5m pack of clean 0.5-1.0 mm quartz sand. A bentonite seal was placed above the sand pack to at least one metre into the overlying till. The sandstone cores were collected in rigid plastic core liners, and between 80-100% core recovery was achieved on each core run. A shallow, small diameter borehole near to borehole 8, M1, was installed to a depth of three metres using a Marlow portable-drilling unit to complete the ten-borehole array.

In addition to the deep drilling, a shallow hand held auger survey was carried out to define the distribution of the superficial deposits (Figure 3.2).

Groundwater chemistry

Sampling Protocol

Groundwater samples were collected from the nine boreholes by using dedicated Wattera™ inertial pumps that were sterilised by autoclaving prior to placement in the borehole. Before a sample was taken at least 60 litres of water were purged from each borehole. Samples were taken for organic, inorganic and microbiological analysis. Precautions were taken to avoid exposure of the groundwater to air or foreign materials.

Temperature, pH, electrical conductivity (EC), redox potential (Eh) and dissolved oxygen concentration (DO₂) were determined on unfiltered bulk samples using calibrated electrodes.

Samples for inorganic laboratory determinations were filtered through 0.45µm cellulose acetate membrane filters prior to preservation. The samples were kept in a cold box in the field and transferred to cold storage in the evenings.

Blank samples were also collected. These included a field blank, consisting of a sample of laboratory de-ionised water collected through an inertial pump and preserved in exactly the same manner as the samples, and a de-ionised water blank which was preserved without any handling, or preservation. All inorganic chemical analysis was carried out under the BGS Analytical Geochemistry Laboratory's Quality System, compliant with the requirements of the International Standard BS EN ISO 9001: 1994. In addition, for certain tests, the laboratory holds accreditation (Testing Laboratory 1816) from the United Kingdom Accreditation Service (UKAS), as detailed in the following table, Table 2.1.

Table 2.1 British Geological Survey analytical geochemistry laboratory accreditation details

Determinands	Test method	Procedure
Ca, Mg, Na, Ni, Cu, Zn, Li, As, Pb,	ICP-AES	AGN 2.3.5
Cl, SO ₄ , NO ₃ , NO ₂	Ion chromatography	AGN 2.3.6
pH and alkalinity	Potentiometric titration	AGN 2.3.7
TOC, TIC	TOC analyser	AGN 2.3.8

All organic chemical analysis was carried out by gas chromatography-mass spectrometry (GC-MS) for a range of organic compounds by SAC Scientific to UKAS Standards. Samples were collected on two occasions (June 2000 and January 2001) and the results are given in Appendices 1 and 2.

Identification of microbial contaminants

Groundwater samples were extracted from all 10 boreholes and were processed on site and in the laboratory to identify a number of microbial contaminants. Tests were carried out to determine the presence of some of the dominant groups of micro-organisms most commonly associated with decomposition of the human corpse. These groups included *Staphylococcus* spp. in particular *Staphylococcus aureus*, a bacteria present in the nasal passage, throat and on the hair and skin of most humans. *Staphylococcus aureus* is often used as an indicator bacterium in water systems, e.g. in swimming pool or recreational waters, to assess the human contact and loading on a water system. *Bacillus cereus*, found in the intestinal tract and implicated in the processes of putrefaction, and *Clostridium perfringens*, which is widely distributed in the environment and frequently occurs in the intestine and is the predominant anaerobic bacteria in post-mortem microbial communities. The presence of faecal indicator bacteria, thermotolerant coliforms (TTC) and faecal streptococci (FS) which shows the water has been contaminated with bacteria of intestinal origin were also determined. Diagnostic tests for *Salmonella* spp., which are responsible for a number of gastrointestinal disorders, were also carried out.

The Robens Centre (University of Surrey) also carried out a series of analyses to determine the presence of enteric viruses such as *Rhodococcus* and *Bifidiobacteria*. Methods for detection of the micro-organisms mentioned above are detailed in Appendix 5.

3 Interpretation of results

Geophysics

The resistivity model generated from the Danescourt Cemetery survey data is shown in Figure 3.1. It shows a clear division between a more resistive and variable upper layer and the more homogeneous and conductive underlying material. The resistive surface layer thickens uphill to the southwest, and has a base that approximately coincides with the estimated groundwater level. The borehole records indicate that the drift deposits thicken from approximately 2 metres in the southeast to 6 metres in the northwest. It is therefore likely that the dominant structure shown in the model is primarily a function of water content, rather than lithology, and represents the change from unsaturated to saturated conditions.

Lithological variation is however represented within the unsaturated upper layer. The surface layer comprises resistivities of between approximately 80 and 600 Ωm . The variable nature of the model in this area is representative of the drift deposits, which are known to be complex and heterogeneous, and the transition from drift to weathered and competent sandstone bedrock. The model surface resistivities reflect the distribution of drift shown in Figure 3.2; the more conductive surface areas at each end of the model correspond approximately to the areas of till, whilst the more resistive zone in the centre of the model coincides with the area of sandy till. Resistivity values below the estimated groundwater level range from 50 to 150 Ωm , which is consistent with the presence of saturated Bromsgrove Sandstone. Resistivity variations in the lower part of the model may be due to physical differences within the sandstone, e.g. groundwater chemistry, pore space geometry, degree of weathering and clay mineralogy; alternatively, they may be a function of measurement noise and decreasing resolution with depth. In view of the very low model RMS error, and the good signal to noise properties of the Wenner array, the former explanation is perhaps the more likely.

No resistivity anomalies within the model can be attributed to contamination by cemetery leachate without being corroborated by further ground truth information. Contamination may be expressed as a bulk decrease in resistivity across the site, though this is not discernible from a single resistivity section within the site.

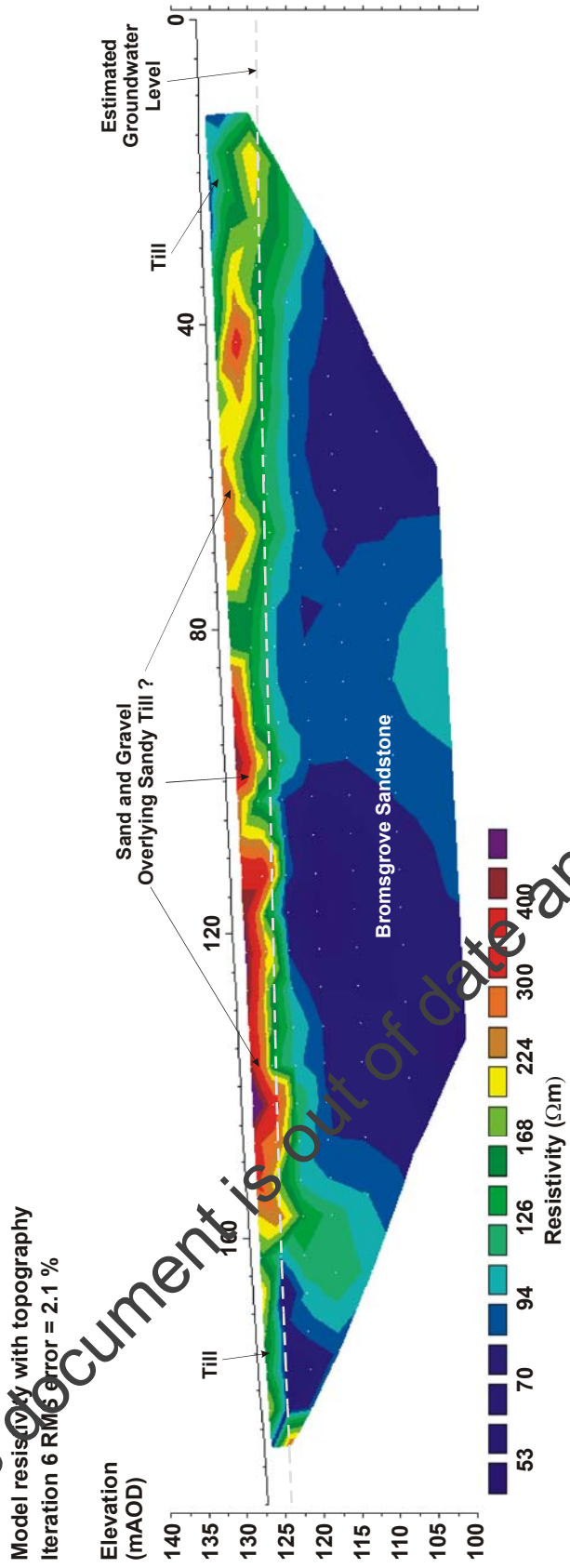
Geology

Superficial deposits

The district lies close to the maximum southerly limit of the Devensian glaciation, which reached as far south as the Wolverhampton area. Till and sandy till in the district overlay much of the bedrock and produce a gently undulating topography. Drift deposits cover about 70% of the area of the site consisting of till and sandy till (Boulder Clay) together with patches of glaciofluvial sand and gravel. The till and sandy till give rise to red, sandy clay and brown clay soils with abundant pebbles and cobbles. The sandy till includes thin lenses of red-brown, clayey gravel. The tills vary in thickness across the site, but in general are between 2 and 5 m thick. Figure 3.2 shows the varying sand content across the site that was constructed from the shallow auger profiling and a site walk over with the grave diggers.

The soil in the cemetery is medium grained sandy, reddish brown to a dusky red colour, and a sandy-clay of dark reddish brown and very fine grain size. Six trial pits were sunk by Geotechnics Ltd. (1994) adjacent to Coppice Lane, as part of the site investigations for construction of the cemetery reception building. Four pits were located in the lower part of the site and encountered glacial drift comprising sands and gravels over boulder clay to depths of about 2.4 m below which was encountered the weathered top of the sandstone. Further trial pits sunk at the main entrance to the site encountered sandstone immediately below a thin layer of topsoil. Groundwater seepages were recorded in two of the pits which intersected the till indicating the local presence of perched water tables.

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First electrode is located at 0 mNW
Last electrode is located at 195 mNW
Unit electrode spacing = 5 m

Figure 3.1 Resistivity model generated from the Danescourt Cemetery survey data

Solid geology

The geology of the area is shown on BGS one-inch sheet 153 (Wolverhampton) published in 1929 and described in an accompanying memoir. Powell (1991) gives a more recent description of the geology of the Penn District. The contact between the glacial drift deposits and the underlying Bromsgrove Sandstone Formation is shown on a current geological standard to run through the centre of the site.

The Bromsgrove Sandstone Formation of the Triassic, Sherwood Sandstone Group underlies the superficial deposits. The formation consists of dark red and brown, calcite cemented, locally micaceous, medium to coarse-grained sandstone with beds and lenses of pebbly, conglomeratic sandstone. As mentioned in the previous section the top of the sandstone is generally weathered and gives rise to silty fine sand with occasional pebbles. Well-rounded quartz granules and pebbles and intraformational red mudstone rip-up fragments are common clast components. Thin beds of red mudstone, siltstone and lenticular beds of calcite conglomerate are locally present. Large-scale trough cross-bedding in the sandstones commonly passes up to rippled siltstones.

Two of the boreholes drilled during the present investigation were cored and the sandstone is generally reddish brown in colour consisting of thin beds from 1cm up to 40cm with either fine or medium sized equigranular sand grains occasionally intercalated with thin mudstone layers.

Hydrogeology

The Sherwood Sandstone Group forms a major aquifer in the area and has been extensively developed for public and private water supply. The site lies within a Source Protection Zone (Zone 2), as it is less than 1 km from the Tettenhall public supply abstraction well. Groundwater quality is generally good but the upper aquifer has a tendency for high nitrate to be present, derived mostly from agricultural application of nitrate-based fertilisers (Powell *et al.* 1991).

The borehole (BH) water levels at the Danescourt site range from less than one metre in BH 8 to 9.4m below ground level (bgl) in BH 1 (Table 3.1). Figure 3.3 illustrates the sandstone groundwater flow direction at the time of monitoring. Flow is essentially from SE to NW and Borehole 1 should serve as a background control since it is up-gradient of any burials.

Table 3.1 Groundwater level variation from June 2000 to January 2001

	Top of BH's m AOD	Borehole Depth m bgl	Water level 12/6/00 m bgl	Water level 27/6/00 m bgl	Water level 27/7/00 m bgl	Water level 1/8/00 m bgl	Water level 23/01/01 m bgl
BH 1	132.7	13.50	9.4	7.6	7.7	7.7	6.5
BH 2	135.9	13.90	7.9	7.6	7.8	7.9	6.6
BH 3	128.6	10.50	2.6	2.9	3.9	3.1	2.3
BH 4	130.1	8.50	3.3	3.5	4.3	3.8	2.9
BH 5	131.6	10.50	8.7	3.9	5.7	5.2	3.1
BH 6	128.6	10.50	2.5	2.6	2.8	2.9	1.9
BH 7	124.7	10.50	1.8	2.0	2.2	2.2	1.4
BH 8	125.8	13.00	0.6	0.70	0.6	0.6	0.05
BH 9	124.5	11.50	2.2	-	2.5	2.6	1.7
M1	-	3.11	2.38	0.99	-	-	0.50

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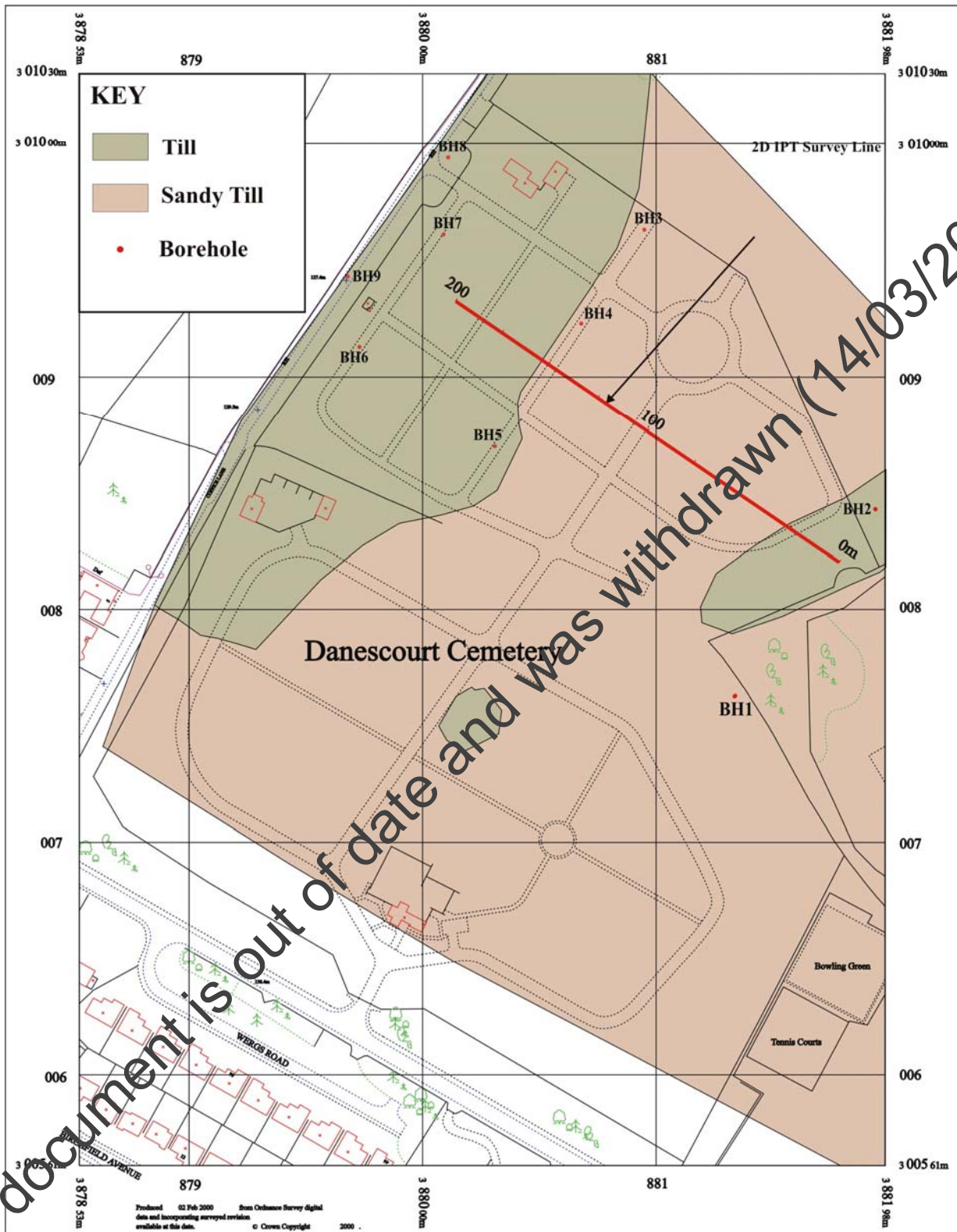


Figure 3.2 Varying sand content of superficial deposits over the site

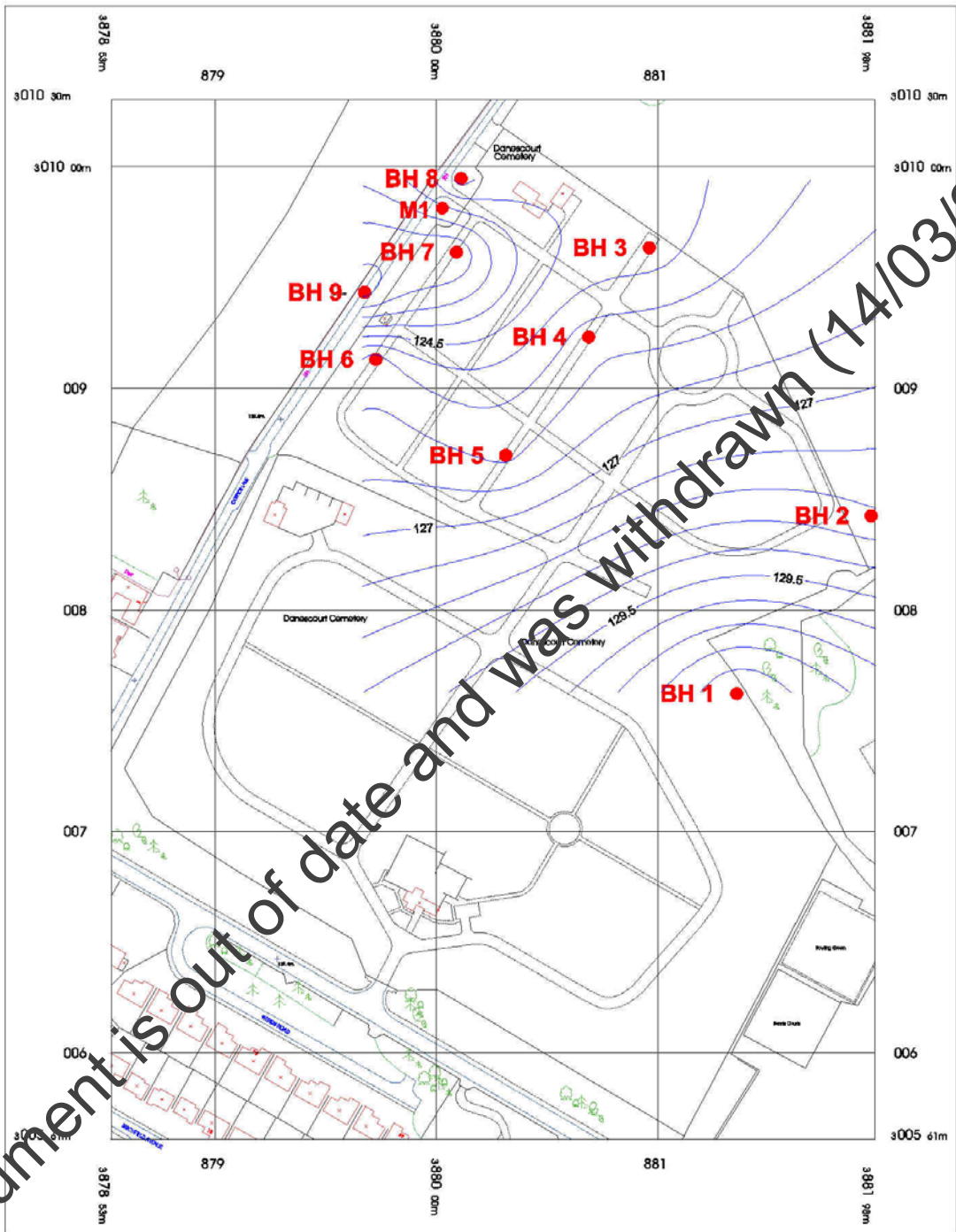


Figure 3.3 Groundwater flow direction.

Hydraulic testing

Most of the hydraulic conductivity values determined during the field-testing are within the range quoted for the Bromsgrove Sandstone by Allen *et al.* (1997) of 1.3 to 6 m/d for medium to coarse sandstones, Table 3.2. Falling head slug test analysis was performed in each borehole using a pressure transducer and a Fluke Hydrobucket data logger. Some of the very rapid recovery slug tests could not be analysed and BH9 with a conductivity of 29 m/d was the maximum credible value from a test. It is believed that these rapid recovery tests were due to the locally highly fractured nature of the sandstone.

Table 3.2 Hydraulic conductivity of the Bromsgrove Sandstone

Borehole	K m/d
BH3	7.3
BH4	4.3
BH5	5.0
BH6	9.9
BH8	9.7
BH9	29.0

Infiltration tests

The drift hydraulic conductivity was determined by dual ring infiltrometer and found to be typical of the lithologies present. Sandy clay lithologies gave values in the range 7.7e-2 to 5.1e-1 m/d and the more sandy drift values in the range 2.9e-1 to 1.8m/d. Table 3.3 summarises the results.

Table 3.3 Infiltrometer test results

Test #	Infil 1	Infil 2	Infil 3	Infil 4	Infil 5	Infil 6	Infil 7
I (m/d)	2.85	7.17e-2	13.8	4.32	4.49e-1	3.88	3.11
Kz (m/d)	1.96	7.81e-2	1.85	0.5	2.9e-2	4.14e-1	2.89e-1
Soil type	Sand	Sandy clay	Sand	Sandy clay	Sandy clay	Sand	sand

Groundwater chemistry

Analysis of groundwater chemistry from June 2000 sampling round

The results of the June 2000 inorganic and organic chemistry are given in Appendix 1. The microbiological analyses are presented in Appendix 3. Graphical comparison of the two datasets is given in Appendix 4.

Major components

Major cation and anion chemistry for the groundwater samples collected from the ten boreholes have been plotted on a Piper diagram, Figure 3.4, showing relative concentrations in milliequivalents per litre.

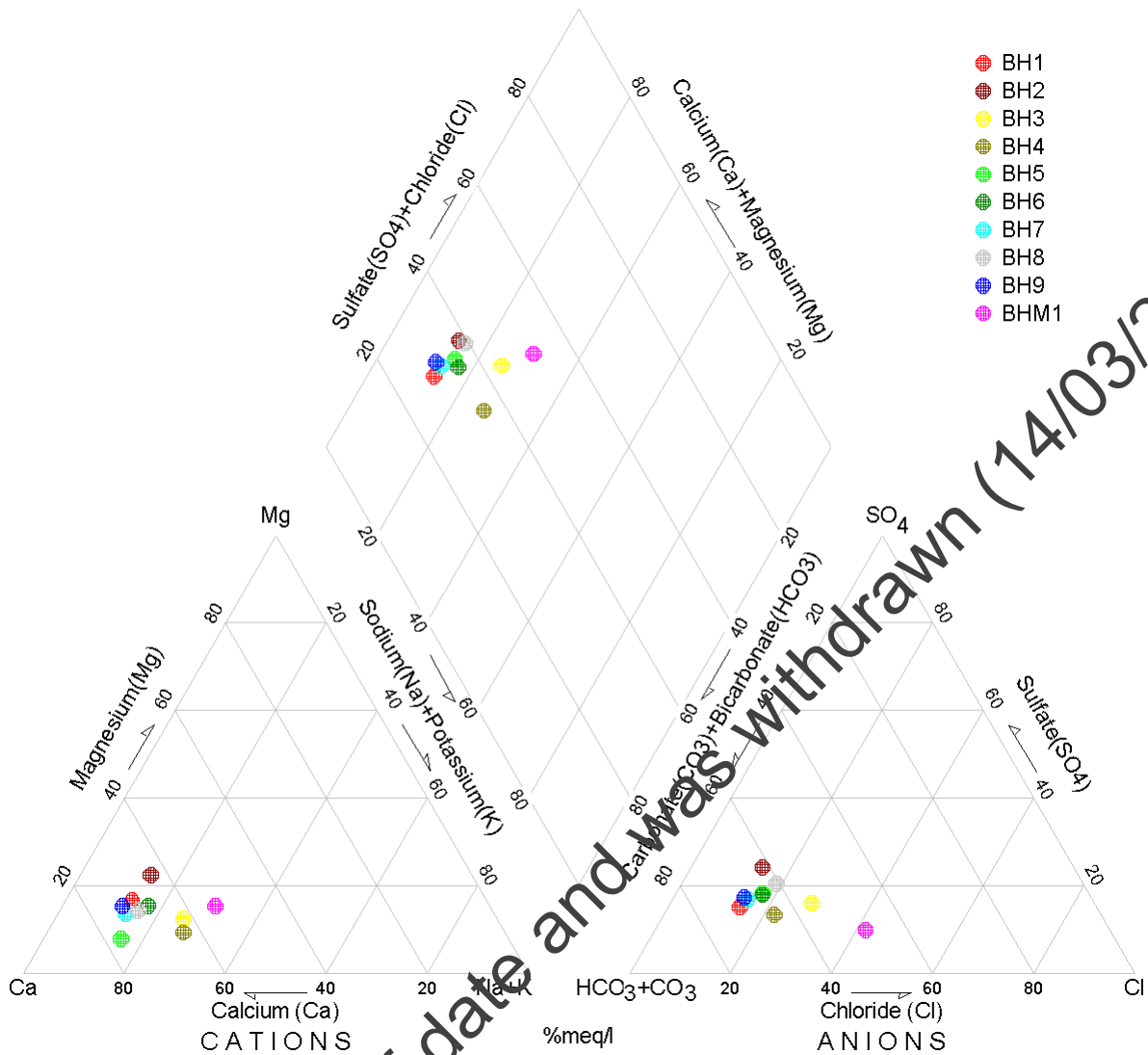


Figure 3.4 Major ion chemistry of all boreholes from June 2000 sampling round

The waters are calcium bicarbonate dominated, which is typical of a calcite cemented sandstone aquifer. When compared to uncontaminated groundwater from the Sherwood Sandstone aquifer (Bridge *et al.* 1997) it is evident that there are elevated concentrations of the major ions in all boreholes down-gradient of the burials.

Sodium concentrations in Boreholes 2, 3, 4 and M1 are two to three times higher than Borehole 1 and correlate closely with chloride concentrations in the same boreholes.

Potassium concentrations range from 3.6 mg/l to 15.5 mg/l and are generally higher in the upper part of the cemetery in boreholes 1, 2, 3, 4 and 6.

Sulfate concentrations show a slight increase in most boreholes down hydraulic gradient from BH1 but significant increases in Boreholes 2 (127 mg/l SO₄) and 8 (96 mg/l SO₄).

No ammonium was found in the ground water samples (except for the shallow M1 borehole) however nitrate is approximately 2-3 times the Sherwood Sandstone average of 11.3 mg/l in most boreholes. The highest concentrations are seen in Boreholes 4 and 5 in the middle of the cemetery.

The boreholes completed in the sandstone aquifer have less than 1 mg/l of TOC with the exception of the shallow M1 borehole, which has a concentration of 281 mg/l. and BH8 (down-gradient of M1) which has a concentration of 3.2 mg/l TOC.

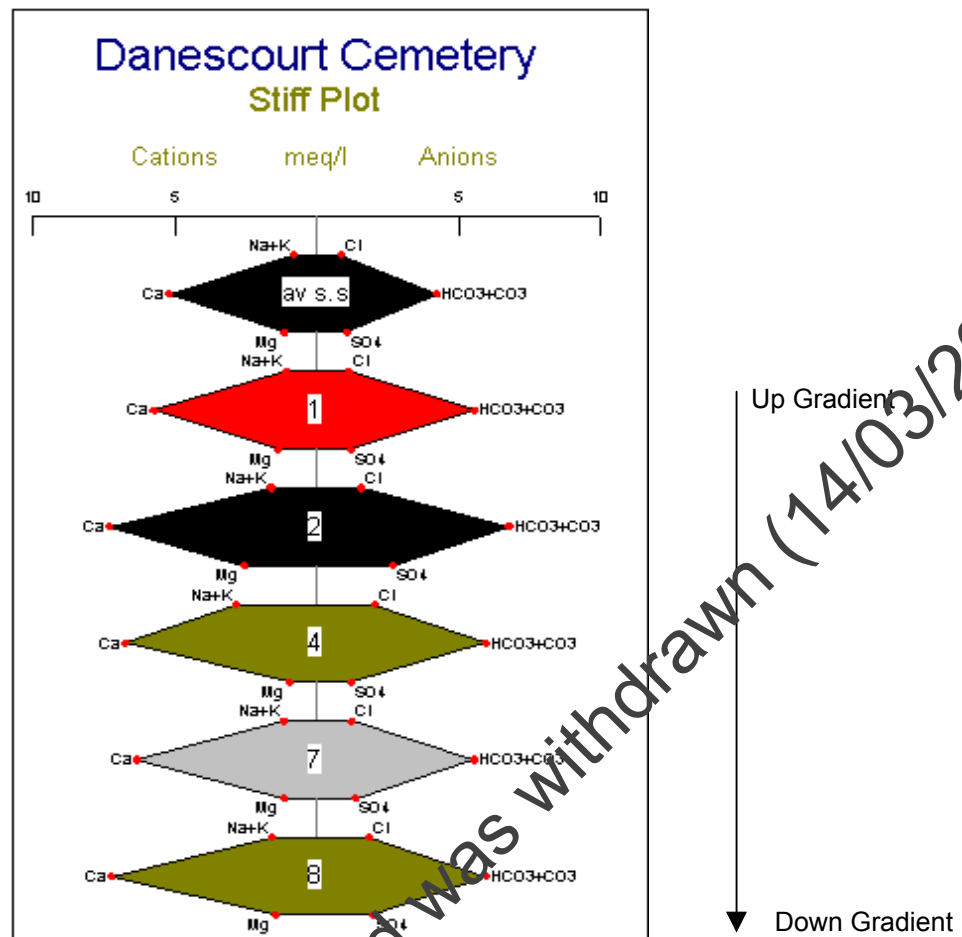


Figure 3.5 Stiff plot of groundwater chemistry

The Stiff Plot shown in Figure 3.5 demonstrates a slight increase in total dissolved solids with increasing distance down hydraulic gradient. This is indicated by increased area of the polygons representing BH1 to BH8. These results lend further support to the thesis of cemetery derived major ion components in the groundwater. An average Sherwood Sandstone groundwater (av. S.S.) is shown for comparison.

Trace components

The majority of trace elements in the ground water are below the analytical limit of detection the most notable exception being the shallow M1 hole that has 0.71 mg/l Cu, 0.15 mg/l Mn and 0.33 mg/l Zn, both ten times greater than the background sample.

Iron concentrations are low in all boreholes except Borehole 8, which has 1 mg/l compared to 0.04 mg/l seen in Borehole 1.

Borehole 1 has the highest concentration of Boron at 1.1 mg/l.

Arsenic concentrations are below the analytical limit of detection.

Organic compounds

Broadscan analysis by GC-MS showed Boreholes 1, 2, 3, 4, 5 and 9 to have varying concentrations of the chlorinated solvents trichloroethane and tetrachloroethane with Borehole 1 having the greatest concentrations in both cases. However, similar concentrations were detected in the method blank raising doubts over the origin of the contamination. Diethylhexylphthalate was also detected in all boreholes except Borehole 4. This compound is a commonly used plasticiser and environmental contaminant and may have originated by leaching from the sampling equipment. Volatile fatty acids

(VFAs), an expected product of putrefaction, were not detected in any boreholes with the exception of BH1, which had a concentration of 16 mg/l of acetic acid. The source of this contamination is unknown

Temporal variations in borehole chemistry

A second sampling round was undertaken in January 2001 (results provided in Appendix B) using the same sampling protocol and analytical suites as the previous round with the exception of the organic analyses where only VFAs were analysed for. Graphical comparison of the major inorganic chemistry for each borehole is presented in Appendix 4. The overall trend is a slight reduction in the concentrations of the major elements. Electrical Conductivity (EC) values are greatly reduced in a number of boreholes in the January sampling, principally those with high EC values in the June sampling, which may be an indication of dilution due to winter recharge. The shallow borehole M1 exhibits the greatest change in chemistry particularly the concentration of Na (49 mg/l in June 2000 to 6.5 mg/l January 2001), Cl (99 mg/l to 19 mg/l) and TOC (281 mg/l to 9 mg/l).

Microbiology

The results of microbial analyses have demonstrated microbial contaminants to be present in the groundwater from a number of boreholes and details are given in Figure 3.6 and Appendices 3 and 4. One of the most significant was *Staphylococcus aureus* that was detected in boreholes 2, 3, 4 and 6. Numbers were greater in the June sampling period when compared to those from the January sampling. This may be due to temporal changes with an increased rainfall contributing to attenuation of the contaminants in the months preceding January. Since *Staphylococcus aureus* is a rare environmental contaminant of groundwater systems and is most commonly associated with human origin its presence suggests that the groundwater is being contaminated with organisms derived from a human source possibly as a result of human decomposition. There is no available information (at the time of writing) on the persistence of this organism in the environment.

Results of analyses for faecal streptococci (FS) and thermotolerant coliforms (TTC) from the June sampling do show a significant correlation (ANOVA analysis shows a correlation at the 95% level). This is a common observation from water contaminated by a relatively 'uncomplicated' source. These faecal indicator bacteria suggest a common source which, given the high TTC to FS ratio in boreholes 3, 5, and 8 may be human in origin, but not necessarily as a result of decomposition. The contamination by faecal indicator bacteria may also be due to surface contamination finding a pathway to the groundwater.

The absence of *Salmonella* in any of the boreholes was not significant as this intestinal pathogen may be destroyed as a result of environmental stress. However, other studies in Brazil (Pacheco *et al*, 1991) detected *Salmonella* in groundwater from a cemetery site suggesting some degree of tolerance to environmental stresses.

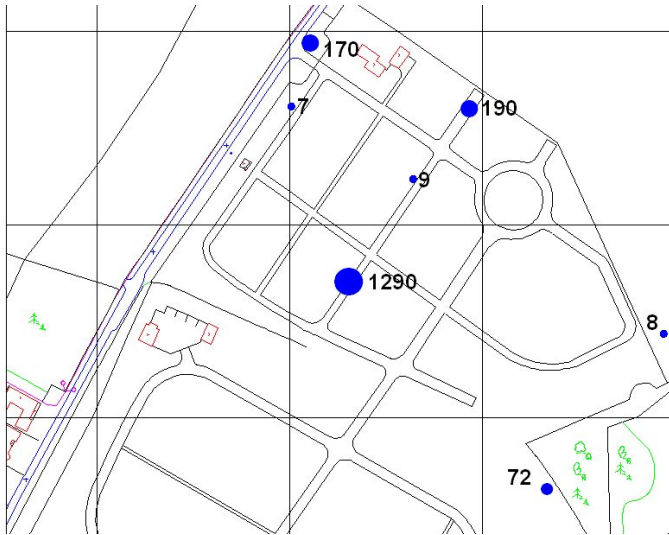
Results of the analyses for enteroviruses were negative. This may be due to the problem of concentrating and growing viruses from environmental samples. Since there is little information available on the fate of viruses in the body after death, it may be that they are at such low levels in the leachate or inactivated during the process of decomposition as to be undetectable in any of the boreholes. Like bacteria, the survival of enteric viruses in the aquatic environment depends on a number of factors and their impact and migration is limited by their survival time (Moe, 1996). *Bacillus cereus* was detected in boreholes 2-9 in the January 2001 samples but only in borehole 2 in the June 2000 sampling. This bacteria is widespread in nature and it is therefore unusual for it to have been absent in the earlier round of sampling. This may be due to a problem in the processing of earlier samples. Since *Bacillus cereus* are common environmental bacteria their number gives a useful indicator as to background levels at the site and if there is a significant increase in number detected at any particular borehole. The results do not appear to indicate a significant difference in numbers detected across the site and any seasonal variation cannot be determined due to the negative results for June and the absence of longer term monitoring results.

The negative results for analyses of *Clostridium perfringens* in June samples may have been as a result of laboratory processing, as it is widely distributed in the environment it is unlikely to be completely absent from all samples. Results from boreholes 2-9 in the January samples show large numbers of Clostridia which would suggest it may well have been present but gone undetected in the previous set of samples. *Clostridium perfringens* was detected in greatest numbers in boreholes 2, 3, 4 and 8, which follow the direction of groundwater flow at the site, borehole 2 being up-gradient of boreholes 3, 4, and 8.

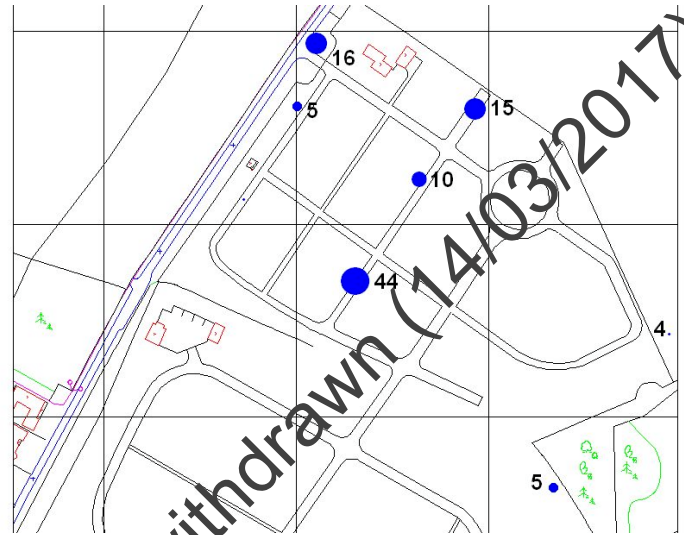
There is wide variation of survival times of bacteria but it is broadly accepted that most enteric pathogens die-off within 2-3 months once outside the human gut. Rapid transit to the water table means that the potential exists for microbial contamination of groundwater depending upon the survival and transport of the differing groups of bacteria. However, this study does suggest that pathogenic organisms are capable of reaching the water table underlying a cemetery site. More sampling would provide a greater indicator as to the effects that temporal changes have on the survival of microbial contaminants and transport mechanisms.

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Figure 3.6 Microbiological distribution plots



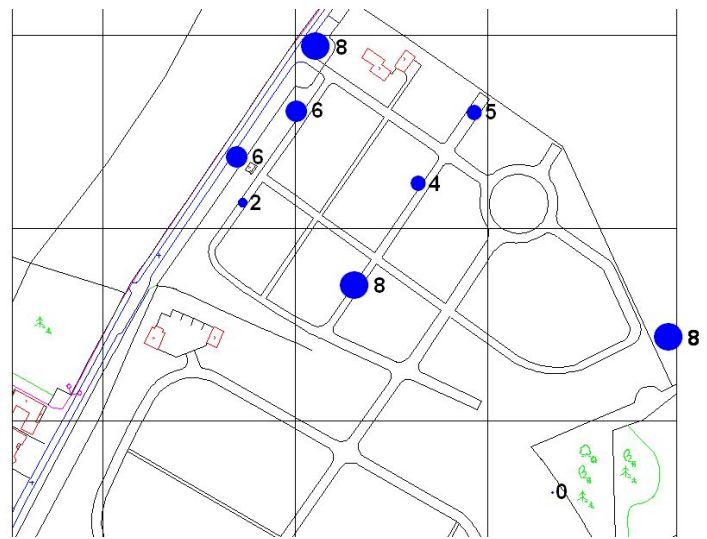
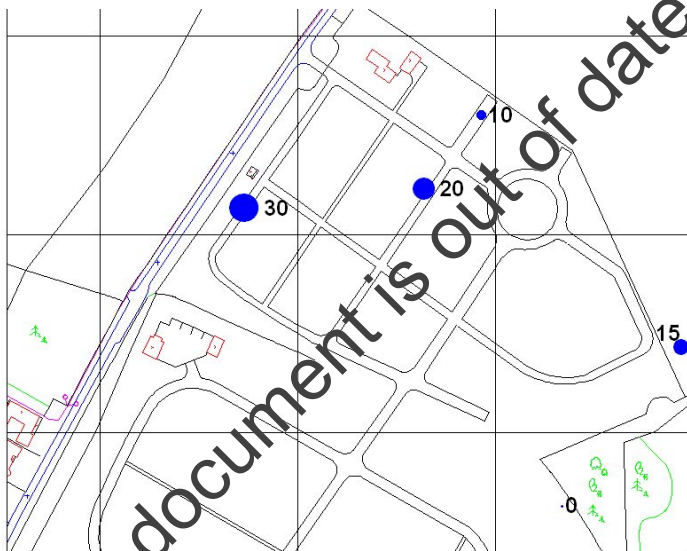
Thermotolerant coliforms



Faecal streptococci

Staphylococcus aureus

Bacillus cereus



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4 Modelling

The normal procedure for interment at the Danescourt site is for the grave slot to be dug to about 1.8 metre depth. Depending on the location of the bottom of the grave there will be drift or weathered sandstone. Figure 4.1 provides a conceptual model on which to base modelling solute transport.

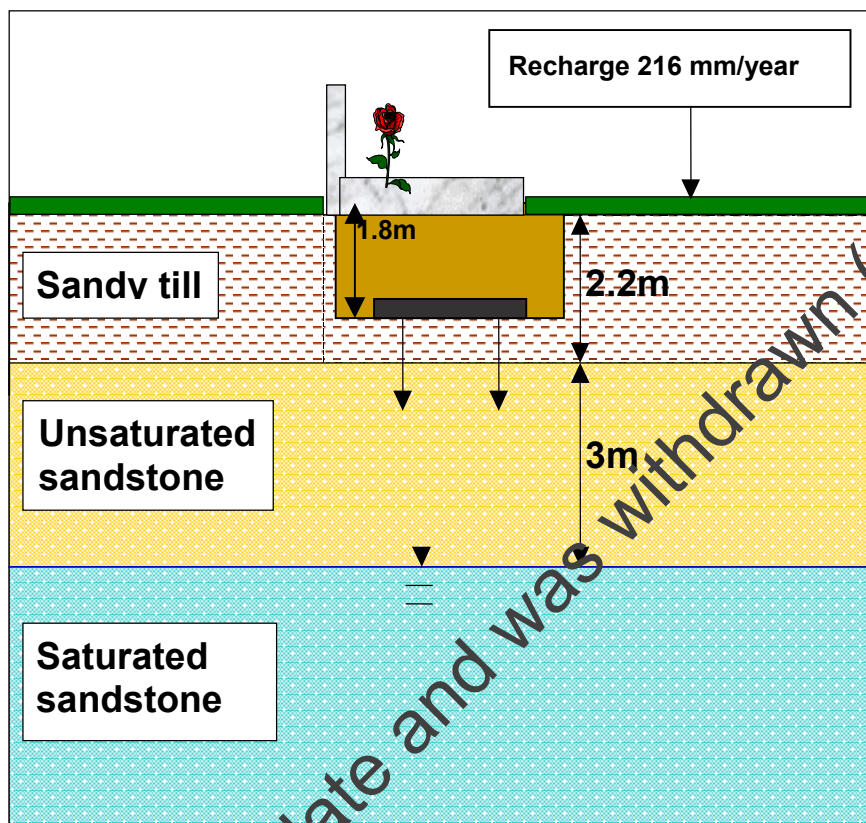


Figure 4.1 Conceptual model of a single burial at the Danescourt Cemetery.

It is assumed that the sandy till is 2.2 m thick, which appears to be an average value for the early phase area of the cemetery. Over the period of investigation the water level fluctuated between 3.86 and 5.74 mbgl in BH5 and 3 metres is taken as a representative unsaturated zone thickness. This lends some conservatism to the modelling.

The normal decay period of a buried, human corpse in a coffin is 10-12 years with more than half the loading leached in the first year (Young *et al.*, 1998). The rate of decay is dependent on a number of factors, e.g. temperature, lithology, presence or absence of a well-drained soil, the latter accelerating decomposition, coffin construction and depth of burial. For the purposes of the current modelling exercise the 12-year decay period is equated to a half-life of 1.5 years and assumes first order exponential decay.

Contaminant transport to the saturated zone is very much governed by leachate flux, which is coupled with recharge rate. Clearly lithology will play a major role in attenuation and rock fabric will determine the nature of any bypass flow mechanisms. Using MORECS data an effective rainfall of 216.03 mm/a was calculated. Based on the infiltration tests and assuming no run off, all of this should potentially go to recharge.

For the purpose of the present model it is assumed that the unsaturated sandy till has similar hydraulic properties to the unsaturated sandstone and that the flow is through a porous homogeneous medium. A numerical model was set up for the above conceptual model using the Femwaste code, (Yeh and Ward, 1981). Table 4.1 details the values used to parameterise the hydraulic model.

Table 4.1 Parameters for hydraulic model

Parameter	Till layer	Unsaturated Sandstone
K m/s	1e-5	7e-5
porosity	0.35	0.27
Residual moisture content	0.1	0.1
Van Genuchten α	0.027	0.145
Van Genuchten n	1.23	2.68
Dispersivity	0.04	0.30

The modelling indicates that peak breakthrough of contaminants can be expected within about 20 years and first breakthrough should occur about eight years after burial assuming a source term half life of one and a half years, shown as curve 1 on Figure 4.2. A second simulation was run to examine the role of a reduced unsaturated zone thickness during high water table level. As can be seen, curve 2, peak breakthrough occurs after about 16 years and initial arrival is at about five years.

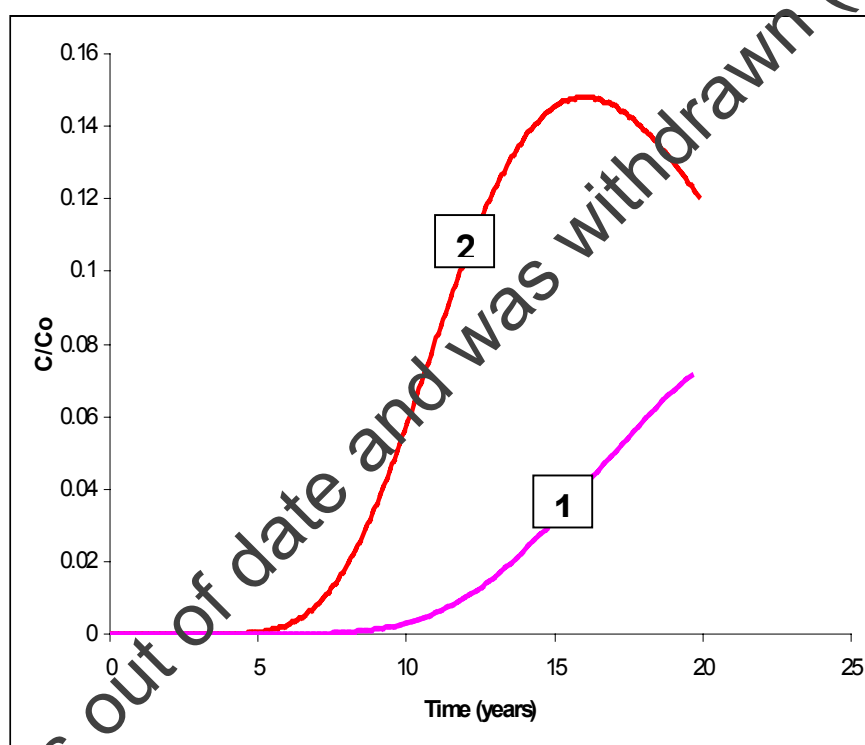


Figure 4.2 Numerical model breakthrough curves

The model shows that concentrations should be low at peak breakthrough and that essentially the sandstone-sandy till sequence is adequate to ensure that natural attenuation of contaminants will occur. This result is clearly at odds with the evidence of groundwater contamination both from dissolved major ions and bacteria and the notion of porous medium contaminant transport needs to be revised. Only in this way can the survival of bacteria to the water table be explained. The hydraulic testing suggests that fractures play an important role in contaminant transport at the site and perhaps a discrete fracture model would provide a more realistic assessment of the flow conditions.

5 Summary and conclusions

Cemeteries are in effect landfill operations but on a much smaller scale. In both cases, biodegradation of organic material produces a leachate, which has the potential to contaminate groundwater. The volume of leachate produced at a cemetery is much less than at a landfill site and the major ion chemistry of the groundwater at Danescourt reflects this. Generally most solutes are released in the first two years after burial but may well be held in the coffin for longer periods particularly if the coffin has been lined to prevent the release of fluids in the mortuary. In this case the leachate will only be released into the sub-surface once percolating rainwater has filled the coffin causing it to overflow or the coffin itself gives way.

Increased concentrations of most of the major elements particularly sodium, chloride and sulphate do indeed suggest that leachate from the graves is reaching the water table. Contrary to this is the lack of ammonium, an expected breakdown product from putrefaction. Nitrogen is the second most abundant element in the body and therefore release of nitrogen, as ammonium under the anaerobic conditions created by the bacteria involved in decomposition would be expected. Nitrogen is present as nitrate in greater concentrations than found in the background sample and ammonia oxidation might account for this. Furthermore the sandy tills are expected to have a certain amount of cation exchange capacity which would also account for a reduction in ammonia beneath graves.

Microbial induced reactions that breakdown fat and protein deposits in the body result in short chain water-soluble volatile fatty acids (VFAs) that leach from earlier in corpse decomposition than the long chain hydrocarbons (Vass *et al.* 1992). No evidence of VFAs was found in the groundwater, however analytical detection limits were quite high at 5mg/l in the June and 1mg/l in the January sampling rounds.

The principal results of the investigation can be summarised as follows:

- The 2D ERT technique has provided an indication of where the change from unsaturated to saturated conditions occurs. The resistivity model also reflects the complexity of the near surface geology across the site, and is consistent with the known distribution of superficial till and sandy till deposits. No information regarding leachate contamination of the aquifer was evident from the resistivity model.
- A slight reduction in major ion concentrations occurred over the two sampling rounds with the exception of borehole M1 in which a significant decrease was apparent.
- Major ion chemistry shows high concentrations of chloride and sulphate compared to background levels.
- Ammonium was only detected in the shallow borehole M1 completed in the till.
- Nitrate concentrations were generally higher than background.
- Total organic carbon concentrations were low in all boreholes except M1.
- Trace elements (with the exception of Fe) were generally below the analytical limit of detection in all boreholes except M1 which had detectable concentrations of iron, manganese, copper and zinc.
- Volatile fatty acids were not detected with the exception of acetic acid found in BH1.
- Faecal indicator bacteria were detected in the majority of boreholes in both sampling rounds.
- *Strepococcus aureus* a bacteria used as an indicator of human contact and loading on a water systems was detected.
- Bacteria implicated in the putrefaction process were detected in a number of boreholes
- Enteroviruses were not detected
- In contrast to actual monitoring data porous medium modelling suggested that natural attenuation should mitigate against contamination arising from graves.

Microbiological analysis was targeted at bacteria and viruses known to have a significant role in the process of decomposition or to be present in the human gut. In general, higher numbers of colony forming units were detected in the first sampling round in June 2000 particularly the thermotolerant coliforms which are a good indicator of human waste entering the groundwater. However, *Bacillus cereus* and *Clostridium perfringens*, both involved in post-mortem decay, were more prevalent in the January 2001 sampling round. The discrepancies between sampling rounds may be due to a number of factors:

- Heavy rainfall and an increase in water levels prior to the January sampling may have diluted the number of bacteria present
- Recharge induced pulses may lead to infrequent releases of leachate from individual graves.
- Reduction in groundwater temperature may affect the viability of certain bacteria
- Lower total dissolved solids may affect bacterial populations
- The increase in *Bacillus cereus* and *Clostridium perfringens* populations in the second round of sampling may have been due to a problem in the processing of the earlier samples, i.e. a false negative on the first round.

Thermotolerant coliforms and faecal streptococci were detected in a number of boreholes and indicate a human source but not necessarily as a result of decomposition. However the possibility of a leaking sewer being the source of these bacteria is ruled out because:

1. the inorganic chemistry results do not substantiate this and,
2. there are no sewer lines running across the site and,
3. in addition the presence of *Clostridium perfringens* is strong evidence of a human source.

The discovery of the bacteria detailed in this report is interesting not only because it is the first documented study of its type in the UK, but also because it offers an excellent site to monitor the survival and migration of pathogenic bacteria in the unsaturated zone and groundwater. Numerical modelling of unsaturated zone contaminant transport indicates that initial breakthrough occurs after about five years and peak break through of contaminants can be expected within about 16 years of burial assuming a source term half life of 1.5 years. Such a transit time is well in excess of the life expectancy of the bacteria detected. The conclusion to be drawn, and supported by hydraulic test results, is that by-pass flow is operating on fractures allowing rapid transit of contaminants to the water table.

The study has demonstrated that there is an impact of the burials on groundwater quality at the Danescourt site, but there are a number of outstanding issues that still need to be addressed by further work. The following list highlights some of the main areas of uncertainty identified in the current investigation that merit further study.

1. The role of recharge flushing on bacterial transport to the water table and the temporal composition of the bacterial community.
2. The possible role of bacteriophage in removing viruses.
3. Temporal changes in groundwater chemistry in response to recharge events.
4. The role of fractures in bypass flow through the unsaturated zone and the need for fracture flow modelling.
5. The role of the till in attenuating ammonia and the general issue of the transport and fate of ammonia.

Some of these issues could be addressed by setting up a routine monitoring programme of groundwater quality and microbiological testing. This would need to be coupled with the acquisition of detailed climate records and groundwater level monitoring at selected locations on the site. The role of recharge flushing could be possibly addressed using a buried tracer in a similar setting as a grave.

Model parameterisation would benefit from some unsaturated column experiments to examine flow in the unsaturated sandstone and silty sands. Materials retained from the site investigation work could be used initially in these studies.

6 References

Allen, D. J., Brewerton, L.J., Coleby, L.M., Gibbs, B.R., Lewis, M.A., MacDonald, A.M., Wagstaff, S.J., Williams, A.T. (1997). The physical properties of major aquifers in England and Wales. British Geological Survey Technical Report WD/97/34 (Environment Agency R&D Publication 8).

Bridge, DMcC, Brown MJ and Hooker, PJ. 1997. Wolverhampton urban environmental survey: An integrated geoscientific case study. British Geological Survey Technical Report WE/95/49.

Geotechnics Ltd (1994) Geotechnical Investigation of Danescourt Cemetery.

Moe, C.L.(1996).Waterborne transmission of infectious agents. In manual of environmental microbiology. Ed.C.J.Hurst, G.R.Hudson, M.J. McInery, L.D. Stezenbach, M.V.Walter. American Society for Microbiology

Pacheco, A.J.M., Mendes,T., Martins, S.Hassuda and A.A. Kimmelmann (1991) Cemeteries - a potential risk to groundwater. Water Science Technology, Vol. 24, No.11, pp97-104

Powell J H. (1991) Geology of the Penn district. *British Geological Survey Technical Report*. W/91/76.

Trick J.K., Williams G.M., Noy D. J., Moore Y. and Reeder S (1999). Pollution Potential of Cemeteries: Impact of the 19th century Carter Gate Cemetery, Nottingham. *British Geological Survey Technical Report* WE/99/4.

Vass, A. A., Bass, W. M., Wolt, J. D., Foss, J. E., and Ammons, J. T., (1992) Time since death determinations of human cadavers using soil solution. *Journal of Forensic Sciences*, **37**, 1236-1253.

West J.M., S. Pedley, S.J. Baker, L. Barrott, B Morris, A Storey, R.S. Ward and M Barrett (1998) A review of the impact of microbiological contaminants in groundwater. *British Geological Survey Technical Report* WE98/14C

Yeh, G.T. and Ward, D.S. 1981 FEMWAS 1E: A finite element model of contaminant transport through saturated - unsaturated media. Report Number ORNL – 5601, Oak Ridge National Laboratory, USA.

Young C P, Blackmore K M, Heavens A and Reynolds P J (1998). Pollution Potential of Cemeteries, Water Research Centre R&D Project record P2-024 for the Environment Agency. (Draft Report Only).

Appendix 1 Danescourt Cemetery June 2000 chemistry results

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Appendix 2 Danescourt Cemetery January 2001 Chemistry Results

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Appendix 3 Danescourt Cemetery summary of microbiology results (mean values)

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Appendix 4 Graphical comparison of June 2000 and January 2001 datasets

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Appendix 5 Microbiological methods

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Summary of methods for the isolation and enumeration of bacteria from groundwater

Coliform bacteria

The analysis of water samples for Thermotolerant (faecal) bacteria was carried out on site immediately after collection of the sample using a portable water testing kit, Oxfam Delagua. Following the standard procedure samples were incubated at 37°C and 44°C to determine numbers of total and Thermotolerant coliform bacteria.

Staphylococcus spp.

A groundwater sample of 100ml volume was filtered through a cellulose nitrate filter and then placed onto a previously prepared plate of Baird Parker medium (Oxoid). After 24 hours incubation at 35°C (Collins and Lyne, 1998) the plates were examined. *Staphylococcus aureus* appeared as black, shiny, convex colonies 1-1.5mm in diameter with a zone of clearing 2-5mm in diameter after a further 12 hours incubation. Further confirmation of identification was made using a Staphylase Test Kit, which detects the presence of the clumping factor, a characteristic of *Staphylococcus aureus*.

Faecal Streptococci

A groundwater sample of 100ml volume was filtered through a cellulose nitrate and placed onto the surface of a well-dried plate of Salnetz and Bartley medium (Oxoid). The plate was incubated at 37°C for four hours followed by 44°C for 44 hours. After incubation, all red, maroon or pink colonies that were smooth and convex in shape were counted. These were presumptive faecal streptococci.

Isolation of *Salmonella* spp from water

The medium was prepared according to the manufacturer's instructions. 100ml volumes of sterile Buffered Peptone Water (Oxoid) were dispensed into the specimen containers, and 10ml volumes of sterile Rappaport Broth (Oxoid) into 25ml universal bottles. Using a filter apparatus, a groundwater sample of 1 litre volume was filtered through a cellulose nitrate filter, placed into 100ml of buffered peptone water and incubated for 24 hours at 37°C. 0.1ml from the buffered peptone water was subcultured into 10ml of Rappaport broth and the broth incubated at 41°C. After 24 hours incubation, a loopful of the Rappaport broth was subcultured onto a plate of XLD agar and incubated at 37°C for 24 hours. The Rappaport broth was returned to the 41°C incubator. After incubation, the plate was examined for characteristic red colonies, usually with a black centre. Where no colonies were evident the Rappaport broth was, after a further 48 hours incubation, inoculated onto a plate of Xylose Lysine Deoxycholate Agar (Oxoid) and incubated at 37°C for 24 hours and re-examined for characteristic colonies. No characteristic colonies were observed.

Clostridia and *Clostridia perfringens* from water

Groundwater samples were heated to 75°C in a water bath and the temperature maintained for 10 minutes to destroy vegetative bacteria. After treatment, the water samples were allowed to cool and a 100ml volume filtered through a 0.45µm pore size filter using a filter apparatus. The membrane was placed onto a plate of supplemented Perfringens agar (Oxoid) and incubated in an anaerobic jar at 37°C. After 24 and 48 hours the plates were examined for characteristic black colonies.

Bacillus cereus

A groundwater sample of 100ml volume was filtered through a cellulose nitrate filter, placed into 0.1% Peptone Water and incubated for 24 hours at 30°C. 0.1ml, was then inoculated onto a previously

prepared plate of Bacillus Cereus Selective Agar (Oxoid) medium and incubated at 37°C for 24 hours and then at 30°C for a further 24 hours. Colonies of presumptive B. cereus were counted.

Enumeration of total numbers of bacteria

The technique used was epifluorescence microscopy, which gives direct total cell counts (Hobbie *et al.*, 1977). This method is based upon a light source transmitting short wavelength radiation onto a specimen that has been filtered and retained on a membrane. The specimen is first stained with a fluorochrome solution, acridine orange, which interacts with the nuclear material of the bacteria and emits longwave radiation. The bacteria appear as bright green and red fluorescing cells against a dark background which enables them to be counted. By examining 20 randomly selected fields of view individual numbers can be counted and the total number of bacteria per ml of sample determined.

Assay of viruses from groundwater (Robens Centre)

Cell culture

(CAMR, Porton Down, Wiltshire) were maintained by passage in 25cm³ sterile tissue culture flasks. Additional stocks of both cell lines were stored under liquid nitrogen.

Buffalo Green Monkey kidney (BGM) and foetal Rhesus monkey (MA-104) continuous cell lines for the assay of enteroviruses, BGM cells were passaged into six-well tissue culture plates and incubated until they produced confluent monolayers. For the assay of rotavirus MA-104 cells were grown to confluence in 96-well tissue culture plates.

Concentration of enteric viruses from groundwater

Standard filtration method

(APHA, AWWA, WEF 1999)

Groundwater samples were collected in sterile plastic 10L containers and transported immediately to the laboratory. Samples were stored at 4°C until analysis was conducted.

Groundwater samples were first adjusted to pH 3.5 with 1M HCl, and to 0.0015N AlCl₃. The sample was mixed vigorously during addition. Samples were filtered through 142mm diameter cellulose nitrate filters (0.45µm porosity) with an acrylic resin fibreglass filter (porosity > 0.45µm), at a rate not exceeding 4L per minute. After filtration excess fluid was purged from the filter holder. The filter was then washed with 0.14N NaCl to remove excess Al³⁺ (1.5ml NaCl solution/cm² filter area). Viruses were eluted from the filter using approximately 100ml of sterilised 3% beef extract (pH 9). To the volume of eluate collected, 1/10th of the volume each of penicillin-streptomycin 10X (5000 IU penicillin/ml and 5000µg streptomycin/ml) and Hanks balanced salt solution was added. The eluate was adjusted to pH 7.4 with glycine-HCl. Final eluates were stored at 4°C until virus assay could be conducted.

Glass wool traps

Disposable polyethylene tubes (67mm length, 20mm diameter) were used for housing the glass wool used to trap viruses. Oiled sodocalcic glass wool (rantigny 725) was supplied by Isover Saint Gobain, France. Before use the glass wool was first washed in one volume of 1m HCl, followed by one volume of 1m NaOH, and rinsed in sterile distilled water until the pH returned to approximately 7 (Vilagnes *et al.* 1993). the columns were then packed with the glass wool to a density of approximately 0.5g/ml.

The method used for the concentration of viruses from groundwater has been described previously (Environment Agency Report No. NC/99/40). Measured volumes of groundwater were pumped through the glass wool traps using a Watson Marlow peristaltic pump. Adsorbed viruses were eluted from the trap using 50ml glycine (0.05M) with 3% beef extract, pH 9.5. The elution fluid was passed

into the glass wool trap using a sterile syringe. The elution fluid was left in the column for 15 minutes. After this time the eluate was collected in a sterile container using the syringe to pass air through the column. The pH of the eluate was brought quickly to pH 7.0 and stored at 4°C.

Reconcentration of beef extract (APHA, AWWA, WEF 1999)

Reconcentration of the final eluate was necessary due to the potentially low number of infective virus particles. Reconcentration was carried out using a standard organic flocculation procedure. After elution from the glass wool trap or standard filtration apparatus, the pH of the eluate was reduced to 3.5 with 1M HCl while mixing vigorously. The eluate was continually mixed at a slow speed for 30 minutes before being centrifuged at 3000xg for 10 minutes. The supernatant was decanted and discarded, and the pellet resuspended in 1/20th of the initial sample volume of di-sodium hydrogen phosphate (Na₂HPO₄, 0.15M). A final addition of 1/20th the sample volume of penicillin-streptomycin 10X (5000 IU penicillin/ml and 5000µg streptomycin/ml) was made, and the pH brought to 7.0 with 1M NaOH and 1M HCl.

Enterovirus quantification by plaque assay

Confluent monolayers of BGM cells, grown in six-well tissue culture plates, were used for the assay of enteroviruses. The media was removed from each six-well plate and the cell monolayer was washed with sterile PBS. Each well was inoculated with a unit volume (0.5ml) of sample. Viruses were allowed to infect the cells for 2 hours at 37°C and with a 5% CO₂ atmosphere. Every 15 minutes the inoculum was redistributed over the cell monolayer manually, to ensure an even distribution. After the adsorption period, the inoculum was removed and the cell sheet covered with an agar overlay medium. The plates were left at room temperature for approximately 30 minutes to allow the agar to set, before being inverted and incubated at 37°C and 5% CO₂. The well plates were left to incubate for 4-14 days to allow plaques to develop.

Staining of cell monolayers

The agar overlay was removed without disturbing the cell monolayer. Each well was then washed with 2ml PBS to remove any remaining agar. To each well, 2ml of 99% ethanol was added before being incubated for 10 minutes at room temperature. After 10 minutes the ethanol was discarded to waste and each well was then rinsed twice with water. The cell monolayers were stained by adding 2ml of polychrome methylene blue to each well and incubating for 5 minutes at room temperature. Each well plate was then rinsed gently with tap water to remove excess stain. Enterovirus plaques were counted.

Rotavirus quantification by plaque assay method

Growth media was tipped off from a 96-well plate, and the cell monolayer was washed once with PBSaT (Phosphate Buffered Saline with Tween). Ten-fold dilutions were made of the virus inoculum, and 0.1ml of this inoculum was applied to between 4 and 8 wells of the microtitre plate. Negative and positive controls were included on each well plate. The plates were then incubated overnight at 37°C and 5% CO₂ overnight.

Immunoperoxidase staining of microtitre plates

The following day the inoculum was discarded and the plate blotted. Each well was immersed in 80% acetone and left at room temperature for 10mins. The acetone was then also discarded and the plate left until the acetone had evaporated. A 1:80 dilution of S7B (bovine stain – supplied by NIBSC) was added to each well (25µL), and placed within an incubator for 1 hour at 37°C. The well plate was shaken frequently to ensure distribution of the S7B. After 1 hour the plate was washed 3 times with PBSaT. A 1:400 dilution of rabbit anti-bovine (RAB) horseradish peroxidase (HRP) was added to each well (25µL), shaken, and left for 1 hour. The well plate was then washed twice with PBSaT, and then once with PBS before addition of 0.2ml of Sigma DAB (3,3'-Diaminobenzidine) tablets

(peroxidase substrate tablet set). The stain was left to develop over an hour, before the plate was finally rinsed with PBS, to prevent any further stain development.

REFERENCES

APHA, AWWA, WEF (1998). Standard methods for the examination of water and wastewater 20th edition (Eds. L.S. Clesceri, A.E. Greenberg, A.D. Eaton), APHA, Washington, DC.

Collins, C.H., Lyne, P.M. and Grange, J.M.(1998) Microbiological Methods 7th Edition Butterworth Heineman

Robens Centre for Public and Environmental Health, University of Surrey, Guildford, Surrey

Vilaginès, Ph., Sarrette, B., Husson, G., Vilaginès, R. (1993). Glass wool for virus concentration at ambient pH level. *Water Science and Technology*, **27**, 3-4, 299-306.

This document is out of date and was withdrawn (14/03/2017)