Water Science and Technology: Water Supply **Vol 2 No 5–6 pp 419–426** © IWA Publishing 2002

Application of physico–chemical parameters and particle-bound biomarkers to indicate microbial contamination of aquifers

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Abstract Reliance on coliform monitoring of ground waters is slowly changing as is reflected in the proposed US EPA Ground Water Rule. In line with this we have investigated the use of an expanded range of faecal indicators and potential surrogate analytes within the Gwelup and Jandakot borefields in Perth, Western Australia. The aims of the study included comparing contamination in bores and surface waters in vulnerable locations, quantifying aquifer removal of microorganisms, trialing novel biochemical pollution indicators such as faecal sterols, assessing *Escherichia coli* as a measure of groundwater contamination and generating data for risk assessments. Sampling was undertaken of nine production bores, nine monitoring bores and four surface waters for 32 parameters comprising seven microbial indicators, 12 physico–chemical parameters and 13 biomarkers (including 8 faecal sterols and caffeine) at sampling stations potentially impacted by urban development. Concentrations of microbial indicators and biomarkers followed the pattern: basins >> monitoring bores >> production bores. Only one production bore sample contained bacterial indicators (0.1 enterococci.100 mL^{-1} on 1 occasion). Of the faecal biomarkers, coprostanol was generally at background levels. Cholesterol appeared to be a more sensitive measure of infiltration, but was also effectively removed. *E. coli* appeared to be a less sensitive indicator than enterococci. None of the physico–chemical parameters were useful surrogates. Overall apparent faecal microbial removal by aquifer filtration averaged >4-5 logs (not accounting for viruses). To maximise warning time and assay sensitivity it is suggested that enterococci be considered as the key bacterial indicator rather than *E. coli* and that different combinations of indicators and biomarkers be used to identify aquifer locations at risk, the presence of significant faecal material, and the likely presence of pathogens.

Keywords Biomarkers; groundwater; indicators; monitoring; pathogen; sterols

Introduction

The traditional reliance on coliform monitoring for groundwater quality protection is slowly changing. This is reflected in the proposed US EPA Ground Water Rule (USEPA, 2000), where faecal contamination may be assessed by *E. coli*, enterococci and coliphage analysis and the replacement of simple presence/absence or single number-based assessments of the contaminants by quantitative risk assessment (Regli *et al.*, 1991). Such developments should provide the basis for more rational management of the microbiological quality of drinking source waters. For example, risk-based quality assessments should provide a defensible and consistent basis for monitoring and auditing program design, triggering of remedial action when an unacceptable risk may develop, and assessing the value of such remedial action. For this more sophisticated management approach to work effectively, three dataset types will be needed:

- estimates of the environmental levels in drinking source waters of various indicators and pathogens;

- the relationship of pathogens to the behaviour of indicators, such as enterococci, coliphages and faecal biomarkers, throughout the source water-aquifer system;
- estimates of the likely infectivity (age) of pathogens.

Such datasets need to be complemented by estimates of mean analyte concentration and variance and identify the likely statistical distribution function. To fully measure pathogen risk, a range of parameters covering viral, bacterial and parasitic protozoan pathogens need to be measured and the risks summed or otherwise combined (e.g. USEPA, 2001). Analyte types and methods should be selected depending on what risks are most likely and the specific information required for source water management based on an extensive sanitary survey of the water supply system.

Our study aimed to collect information relating to the first two data sets above, as part of a broader Cooperative Research Centre for Water Quality and Treatment survey of source water pathogens. The current study was located within the metropolitan area of Perth, Western Australia, and aimed to:

- compare levels of contamination in surficial production bores with monitoring bores, aquifer linked surface water through a survey of the quality of water supplying, and within, two urban aquifers perceived to be vulnerable to faecal contamination;
- estimate the capacity of the aquifers to remove faecal contaminants;
- assess how satisfactory *Escherichia coli* is as a measure of microbiological contamination of groundwater and identify potential alternatives or surrogates;
- trial novel biochemical pollution indicators (e.g. faecal sterols) in groundwater;
- generate datasets suitable for risk assessments.

Methods

Sampling sites

The Western Australian state capital of Perth (approximate population of one million) is unusual in that most of its metropolitan area is situated immediately above a series of interconnected, unconfined coastal sand dune aquifers (Davidson, 1995). This aquifer complex provides much of the city's baseload drinking water supply and sustains numerous freshwater wetlands and lakes. Significant recharge of the aquifers occurs from rainfall within the metropolitan area so it is essential that pollutants of all kinds are managed on a sustainable basis.

Of Perth's major borefields (Underground Water Pollution Control Areas – UWPCAs) Gwelup and Jandakot UWPCAs appeared to be most exposed to waterborne contaminants and in need of active management (Barber *et al.*, 1993, Otto *et al.*, 1994, WRC, 1998a, 1998b) and hence the most appropriate localities for investigating microbiological contaminant management. Possible contaminant sources included urban and agricultural impacts on run-off and direct recharge and wildlife inputs to lakes perched above the water table. Water samples were collected over 2-4 periods during 2000 (April, July, early October, late November) from 21 stations (Table 1). All stations, including production bores, were situated in close proximity (typically less than 100 m) to urban dwellings.

Analyses

The main analytes measured comprised:

- 7 microbial indicators (presumptive sulphite-reducing clostridia - SRCs, *Clostridium perfringens*, *E. coli*, faecal streptococci, enterococci, somatic and F-RNA coliphages) (undertaken by PathCentre and CSIRO);
- 13 particle bound biomarkers (8 faecal sterols, 4 hormones, caffeine) (based on GC-MS analysis of 10 L filtrates undertaken by CSIRO Marine Research, Hobart, as described by Leeming *et al.*, 1998);

Table 1 Source water sampling sites

Sampling location type	Sampling station location and number	Average bore depth/ depth from water table to screen (m)	Comments
Surficial production bore	Gwelup UWPCA (7), Jandakot UWPCA (2) lines.	32.8 ± 5.7/24.7 ± 6.3	Bores selected for proximity to end of flow
Shallow monitoring bore	Gwelup UWPCA (7), Jandakot UWPCA (2)	3.5 ± 4.9/0.3 ± 0.6	Bores selected for proximity to production bores.
Collection basins/lakes	Gwelup UWPCA (3 basins, 1 lake)	0	Collection basins were run-off fed and the lake received both groundwater and run-off.

- 12 physicochemical parameters (pH, DO, UV_{254nm} absorbance, filterable organic carbon (FOC), NH_4^+-N, NO_2—N, NO_3—N, SO_4^{2}—S, turbidity, colour (Pt–Co), conductivity, temperature (undertaken by SGS Scientific and Water Corporation staff).

Analytes were fitted to possible distributions, such as lognormal, Poisson and beta (Regli *et al.*, 1991, CRNYCWMS, 2000), using Excel V7 (Microsoft) and the solver function to fit probably density function (PDF) parameters.

Results and discussions

Estimation of mean source water concentrations

Microbiological risk assessment requires datasets of pathogen and indicator concentrations which identify the type of statistical distribution and include estimates of the key parameters describing that distribution, e.g. mean and standard deviation for lognormal probability density function (PDF).

Analysis of the datasets collected indicated that most analytes (with detects) correlated well with cumulative lognormal distributions. As a result means and standard deviations were estimated (Figures 1-3). In the case of the bacterial indicators and biomarkers standard deviations were between 0.5 and 2 $\log_{10}$ units, and 0.25 and 1 $\log_{10}$ units, respectively. R^2 values were generally > 0.9.

Unfortunately, a substantial number of datasets were characterised by many "not-detected" values. In particular, most microbial counts of production bores (and many monitoring bores) were near to, or below, the limit of detection (0.1 cfu per 100 mL). This was consistent with the findings of routine surveys and other studies of Perth's aquifers (Water Corporation, 1998; Otto *et al.*, 1994; Larsen *et al.*, 1998). A related problem was that high counts of SRCs and the limited number of confirmations practicable tended to restrict appropriate quantification of *C. perfringens*. Similarly, only trace amounts of specific faecal biomakers were detected and these proved not to be significantly higher, e.g. coprostanol, than the trace concentrations detected in blanks.

Truncated datasets are common in microbial surveys especially of groundwaters (e.g. Otto *et al.*, 1994; Larsen *et al.*, 1998) so we investigated how such datasets might be expressed in a form that could be more useful for risk analysis purposes. Two approaches were investigated and appeared to provide reasonable measures of microbial abundance beyond the normal reporting of detection limits, arithmetic means and detection frequency.

For the first approach, datasets were assumed to fit lognormal distributions, as identified for the three analytes described in Figures 1-3. An example of fitting the truncated data sets for enterococci is provided in Figure 4. Similarly, when the curves were fitted to artificially

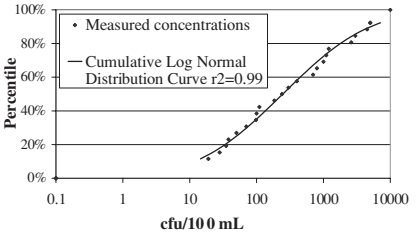

Figure 1 Distribution of SRC counts in monitoring bores

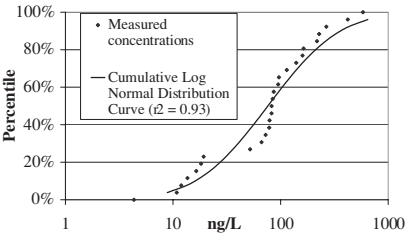

Figure 2 Distribution of cholesterol concentration in monitoring bores

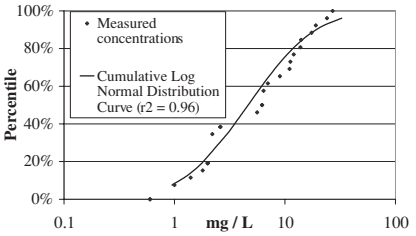

Figure 3 Distribution of dissolved organic carbon concentrations in monitoring bores

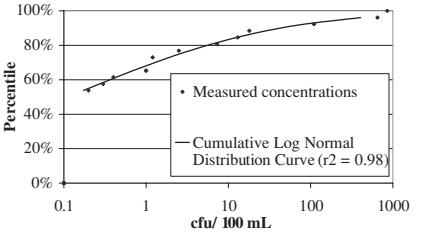

Figure 4 Distribution of enterococci counts for monitoring bores (note that 15 of the 27 samples had concentrations at or below the detection limit)

truncated datasets in which the lowest 50% of values were omitted to simulate the effect of a high detection limit (e.g. SRCs in monitoring wells), the lognormal distribution parameters did not change appreciably.

Where only one or two analyte values were available above the detection limit, or the only value was an upper limit of detection, use of the 'Solver'-based curve fitting was not possible. Hence, a second alternative method of approximating the distribution parameters was used. As well as assuming that lognormal distributions applied, the experimentally observed ranges of standard deviations (0.5-2.0 $\log_{10}$ units) were used to estimate the most likely value of the mean. This was done by generating lognormally distributed datasets with incrementally increasing geometric means and one of the selected SD values, using the RAND() and NORMSINV() functions in Excel. The upper limit value or upper value(s) were then substituted for the upper value(s) in these sets to form a series of hybrid datasets. The mean was taken as the value where the skew of the hybrid dataset converged to zero and the experimentally measured values were located in the top percentile(s) of the hybrid distribution. The resulting lognormal (geometric) means and standard deviations are shown in Table 2.

The second approach was, in principle, the same as has been used for estimating risk assessment input values for protozoa by the CRNYCWMS (2000). They also reported that pathogen numbers tended to follow a lognormal distribution and had to contend with truncated datasets. It is noteworthy that they found that the standard deviation of the distributions to be about 0.9 $\log_{10}$ units, midway between our extreme estimates (0.5 and 2.0) for SRCs, enterococci and *E. coli*.

Key findings

In general, the observed concentrations of microbial indicators and biomarkers followed the following pattern: surface water >> monitoring bores >> production bores. Based on the estimated mean concentrations of analytes (or lower limits) the protective effect of current management procedures and the physical filtration by the aquifer's sand medium could be calculated. Data from run-off basins and lake waters demonstrated the initial presence of

Table 2 Concentrations of selected analytes in source water

Analyte/parameter	Units	Detection limit	Geometric mean concentration [±1 SD](log_{10}) (number of detections/ number of samples analysed)		
			surface water	monitoring bores	production bores
Escherichia coli	cfu/ 100 mL	0.1	62[±1.22] (6/6)	0.4[±0.5] – 0.0003[±2.0] (1/27)[1] (0/27)[1]	<0.01[±0.5] – <0.000009 [±2.0]
Enterococci	"	"	140[±0.75] (6/6)	0.11[±2.02] (13/27)[1]	0.01[±0.5] – 0.000009 [±2.0] (1/27)[1]
Faecal streptococci	"	"	140[±0.75] (6/6)	0.47[±1.99] (15/27)	0.01[±0.5] – 0.000009 [±2.0] (1/27)[1]
SRCs	"	"	1300[±0.45] (6/6)	250[±1.02] (24/27)	<0.01[±0.5] – <0.000009 [±2.0] (0/27)[1]
C. perfringens	"	"	120[±0.5] – 0.1 [±2.0] (1/6)[1]	160[±0.5] – 0.2[±2.0] (2/27)[1]	<0.01[±0.5] – <0.000009 [±2.0] (0/27)[1]
Coprostanol	ng L^{-1}	0.2	6.6[±0.25] (4/6)	<0.25[±0.65] (12/27)[2]	<0.03[±1.13] (6/27)[2]
24 ethyl coprostanol	"	"	4.26[±0.53] (4/6)	<0.21[±0.97] (9/27)[2]	<0.3[±0.48] (7/27)[2]
Cholesterol	"	"	1300[±0.45] (6/6)	76[±0.52] (27/27)	<4.0[±0.33] (27/27)[2]
24ethyl cholesterol	"	"	790[±0.37] (6/6)	59[±0.56] (27/27)	<3.0[±0.31] (25/27)[2]
NO_3-N	mg L^{-1}	0.002	0.018[±0.65] (5/6)	1.1[±0.92] (27/27)	0.28[±1.22] (26/27)
NH_3-N	"	0.005	0.024[±0.86] (5/6)	0.023[±0.39] (26/27)	0.16[±0.48] (27/27)
FOC	"	0.2	8.6[±0.15] (6/6)	4.6[±0.48] (24/27)	5.3[±0.48] (26/27)
UV abs_{254nm}	OD units	0.001	0.22[±0.16] (6/6)	0.056[±0.63] (27/27)	0.13[±0.50] (27/27)

1. Number of positive samples low or zero. Means were estimated iteratively based on the number of samples analysed, the standard deviation indicated and assuming values are lognormally distributed. Where only one or two data points were available a range of estimation is shown based on the selected deviation values. A "<" sign indicates that the estimates were based on upper limit values.
2. Estimates of faecal sterol species were not significantly different from levels detected in blanks.

microbial indicators and other pollutants of concern in groundwater recharge cycle at concentrations comparable to those found in other Australian surface waters (unpublished data). The concentrations were reduced within the first few meters of the travel beneath the aquifers' surface by 1-3 orders of magnitude. By the time water was extracted from production bores (a depth of *ca*. 25 m), biomarkers and indicator bacteria were all but undetectable.

Overall, reductions for bacterial indicators of at least 4-5 orders of magnitude were observed. In the case of biomarkers the reduction indicated by cholesterol removal was nearly 3 orders of magnitude. In contrast, no clear pattern of change was observed with any of the common physico–chemical parameters as exemplified by the abundance of nitrogen species (ammonium and nitrate) and organic matter (FOC and UV absorption).

Given that the survey was focused on those wells most at risk, the data indicates that under 'normal' conditions virtually no microbial indicators should be detected in the aquifers. This appeared to be the case and indicates from a microbiological perspective that the Perth aquifer system is in a good state of health. On one occasion, one production bore sample was found to contain 1 enterococci.L^{-1}. The implied overall mean concentration implied of between 0.001 and 0.000009 cfu 100 mL^{-1} is very small compared to values typical of surface waters (tens to thousands per 100 mL). Hence, there is probably a high degree of removal of larger pathogens (i.e. protozoa) prior to treatment and reticulation.

Relative strengths of microbiological indicators

Although faecal bacterial numbers followed the same general removal pattern, *E. coli* appeared to be a less sensitive indicator than enterococci with *E. coli* concentrations much lower in run-off and much less frequently detected in monitoring bores than enterococci or faecal streptococci (Table 2). Although not recommended by some as a groundwater

quality indicator (Regli *et al*., 1991) *C. perfringens* measurement was still seen as useful in the urban borefields as it is persistent in the environment and has been found to be useful in a previous study (Otto *et al*., 1994). It is also likely to be present in very high concentrations in urban run-off due to its common presence in dog, as well as human, faeces (9.8×10^8 and 1.7×10^5 cfu/g; Leeming *et al*., 1998*)*. Data from monitoring bores also suggested that there was a higher background load of SRCs/*C. perfringens,* however, *C. perfringens* proved difficult to detect in the presence of large numbers of SRCs using the local laboratory method.

Our findings compared well with previous analyses of the Jandakot aquifer (Larsen *et al*., 1998) and the work of Otto *et al*. (1994). In the former study from 43 stations, 1 positive faecal coliform, 3 positive faecal streptococci and 1 positive *C. perfringens* were detected in water samples in areas of low density development. Comparison with the results in Table 2 indicated that concentrations of equivalent indicators were the same or only marginally higher in our study sites.

Biomarkers versus physico–chemical parameters

One aim of this study was to assess the value and sensitivity of a range of potential biomakers as groundwater contamination indicators, in particular sterols (Leeming *et al*., 1996, 1998), caffeine and pharmaceuticals (Seiler *et al*., 1999). It had been hoped that biomarker assays would provide complementary and comparable information to microbial indicators and the results of analyses, in general, supported this view.

Overall the particle bound sterol biomarker species exhibited the same pattern as indicator bacteria with marked reductions between run-off, monitoring and production bores. For data from stations where biomarkers were clearly detected (principally cholesterol and ethyl cholesterol), correlation coefficients (R) with microbial indicators of *ca*. 0.7 were observed. Coprostanol concentrations in bores were generally at background levels indicating only limited faecal contamination, even in run-off. This was in line with indicator counts but contrasted with run-off water data from surface water in Eastern Australia where faecal sterol concentrations were 10-100 times higher (unpublished data). The coprostanol to 24-ethyl-coprostanol ratio of 1.2 ± 0.7 did not equivocally implicate either a human or herbivore source (Leeming *et al*., 1996, 1998). With the low observed concentrations in the starting material it was not possible to estimate the extent of coprostanol or ethyl-coprostanol levels observed. However, cholesterol, which is associated with biological material generally and is chemically very close to coprostanol, was reduced to undetectable levels (1,300 ng/L in basins compared to 76 ng/L in monitoring bores, and < 4 ng/L in production bores).

Trace amounts of particle bound caffeine (0.2-2 ng/L) were detected in seven (six monitoring bores and one surface water) samples. Consistent with removal by the aquifer, none was detected in production bore water samples. The finding of caffeine at all was noteworthy as it is highly water soluble, easily metabolised and is predominantly expected in the aqueous phase. Hormonal compounds were detected in one sample on one occasion at trace levels (around 1 ng/L in a production bore), but were not subsequently detected in a follow-up sample from the same station, so no clear conclusion could be made.

In contrast to the biomarkers, physico–chemical parameters did not show any pattern of removal corresponding to faecal microorganisms. Colour, turbidity, UV absorbance and FOC were significantly correlated but did not show any relationship to faecal indicators. Gwelup aquifer was similarly shown to contain high levels of nitrogen but previous work on this aquifer has shown the likely source to be agriculture (Larsen *et al*., 1998).

Quality control issues

Extensive use of quality control samples (blanks, replicates, spikes) was used in the study

for all analytes amounting to *ca.* 30% of all samples analysed. Disappointingly, this work showed that, although bacteriophages may have been present at low concentrations (<1 pfu/ 100 mL) the enrichment technique applied was unacceptably sensitive to contamination, hence they have not been reported here. Similarly analysis of blank biomarker samples indicated the potential for contamination at the limits of detection (*ca.* 1-10 ng/L). Carryover from spiked samples and in the case of cholesterol extraneous dust, were two likely sources of contamination identified.

In addition to highlighting the need for reporting the quality control samples as part of any sampling project, the above problems emphasised the need for managers to better appreciate the importance of such data. Analysts are well aware of the uncertainties in data obtained by analytical methods, which often push current technologies to their limit in an effort to generate data for risk assessment. This must be a concern for groundwater managers if datasets are to be more than sets of 'non-detects'.

Conclusions

Overall, the results indicated that in the two Perth ground water systems, aquifer filtration provides a very effective barrier to bacterial-sized or larger organisms. In order to maximise warning time that such assays provide it is suggested that enterococci should replace *E. coli* as the key bacterial indicator and that assay sensitivity be maximised (e.g. 1 litre volumes be analysed rather than the normal 100 mL). Assayed *Clostridium perfringens* must be confirmed routinely, due to high surface numbers of background SRCs. None of the physico–chemical parameters appeared to be appropriate surrogates of microbial parameters. Human faecal biomarkers appear to provide complementary information and be worthy of further study.

Furthermore, the optimal approach to measuring for microbial contamination was not seen as the use of a single 'magic' analyte to provide all the information required. Rather a suite of analytes should be used to gain different information in a tiered fashion as follows.

- Use general biomarkers of small particle infiltration such as cholesterol and SRCs to indicate to what extent surface water is actually impinging on, and travelling into aquifers. These measure would not identify contamination *per se* but rather identify where infiltration might happen in the event of mishap or poor land management.
- Use enterococci and probably *C. perfringens* and faecal sterols, to provide evidence of run-off contaminated with faecal material (animal and human) rather than *E. coli*.
- Use human specific assays, e.g. high levels of any contaminant or the presence of pathogens or human specific markers (high coprostanol ratio, caffeine, hormones).

Two key limitations in the dataset were evident. Firstly we were unable to obtain information on the levels of bacteriophage and hence the potential for enteric virus infiltration. Secondly the study did not indicate absolutely how rapidly the different analytes were removed or the extent to which removal was due to death/decomposition or immobilisation within the sand matrix. These issues are being addressed in a follow-up study focusing on a marginally contaminated zone where all these analytes are likely to be present in more measurable concentrations.

Acknowledgements

Financial support was provided by the CRC for Water Quality and Treatment and the Water Corporation of Western Australia. PathCentre undertook the microbiological analyses and CSIRO undertook the biomarker analyses. In particular, thanks is due to Darryl Thompson, Ray Mogorosy (Pathcentre), Greg Burkett and Phil Hawkins (Water Corporation).

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