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BIOLOGICAL TRACERS OF POLLUTION PLUMES FROM SEPTIC SYSTEMS

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ABSTRACT

Chloride (Cl) and electrical conductivity (EC) as tracers of chemicals and indicator bacteria--total coliforms (TC), fecal coliforms (FC), and fecal streptococci (FS)--were determined for 7 months in septic tank effluent and ground water samples collected downgradient from 17 new or replacement septic systems. Indicator bacteria were also determined occasionally in ground water samples from wells near the systems for a continuing period of 2 years. Following 5 years of system operation a last round of analyses was conducted. Water table fluctuations in the vicinity of the septic systems were monitored for 2 years.

As a model for viruses, vaccine poliovirus in feces from babies who had received the trivalent Sabin oral poliovirus vaccine was introduced into one septic system as a single dose by flushing down the toilet. Effluent and ground water were assayed for poliovirus over a period of 6 months.

The data were evaluated statistically and a contaminant transport model was tested to simulate ground water contamination by the tracers of pathogens. No tracer bacteria reached ground water from any of the 17 septic systems. Presumably, the bacteria were removed by the soil under the seepage bed; mean transport was <1 count/100 mL for each bacterial type, in keeping with U.S. codes of public health drinking water regulations. Poliovirus entered and spread in ground water from the one septic system tested, even though this was functioning properly. Of 3.3×10^8 counts of poliovirus inoculated into the septic tank, a mean of 70 counts/100 mL escaped from the tank with the effluent and an overall mean of 62 counts/100 mL were transported to ground water. Septic systems had little influence on water table fluctuations in their vicinity compared to the seasonal climatic factors.

INTRODUCTION

Total and fecal coliforms (TC and FC) and fecal streptococci (FS) are three groups of bacteria used as tracers (indicators) of microbial contamination and possible occurrence of bacterial pathogens in ground water. These bacterial groups are present in high numbers in the human intestines and are shed naturally in feces (Ziebell *et al.*, 1974).

Viruses most likely to be encountered in septic systems are the "enteroviruses" that include polioviruses (PV), coxsackieviruses, and echoviruses. In contrast to indicator bacteria, enteroviruses are not normally found in septic tanks unless an individual served by the septic system is infected by the virus. A suitable virus indicator does not exist, and a quest for one continues. Since PV obtained from vaccinated individuals are nonpathogenic enteroviruses and relatively easy to assay, they were used as virus indicators in this study. Bacteria and viruses which cause intestinal or so-called gastro-enteric diseases such as typhoid and paratyphoid, food poisoning, and other diarrheas and dysenteries are of major public health concern. While most intestinal bacteria are usually harmless, when pathogenic bacteria or viruses are excreted with feces they are potential pollutants and must be contained in the septic tank or eliminated by treatment through soil.

Bacteria in septic tanks are capable of biodegrading numerous chemical compounds in the waste and are thus essential to the treatment process. In this respect, they differ from viruses, which are merely carried along without multiplication after leaving the human body (Wellings, 1980).

Ground water used as a potable water source may be microbially contaminated by improperly functioning septic systems. Although a high degree of purification of wastewater can be achieved by soil treatment, recent cases of disease resulting from ground water contaminantion are reported (Center for Disease Control, 1985). Craun *et al.* (1984) reported 320 waterborne outbreaks affecting 77,989 individuals in 43 states and Puerto Rico from 1971 to 1980, and 32 further outbreaks and 4,430 cases of illness in 1981. Almost half of the outbreaks of waterborne diseases in the USA are caused by contaminated ground water; overflow from malfunctioning septic systems is responsible for 43% of outbreaks and 63% of illnesses in non-municipal areas (Craun, 1984).

Indicator organisms (TC, FC, and FS) were reported to reach ground water and travel more than 15 m from a septic tank in an area of high water table (Viraraghavan, 1978). However, the unsaturated zone under that absorption field was not deep enough to comply with recommendations of the Small Scale Waste Management Project group at the University of Wisconsin-Madison for a successful septic system. Cech and Harrist (1984) also detected indicator bacteria in shallow and deep wells of Houston County, Texas, and claimed they came from septic systems. Brown *et al.* (1979) reported basically no transport of total and fecal coliforms to ground water from properly functioning septic systems.

Numerous studies have shown that many soils are effective at removing viruses from percolating wastewater, but most have involved only laboratory

experiments. Current knowledge about removal of viruses by soil filtration as well as sorption mechanisms has largely been derived from column studies (Lance *et al.*, 1976; Robeck *et al.*, 1962;) or laboratory batch experiments (Carlson *et al.*, 1968; Goyal and Gerba, 1979). These studies have been criticized because of the lack of standardization in experimental conditions and because column experiments do not always simulate natural field conditions (Bitton *et al.*, 1979). Much confirmatory field work is still needed.

There is a serious lack of information regarding virus movement from septic systems in the field. Brown *et al.* (1979) found that approximately 1 m of any of three soil types in central Texas prevented ground water contamination by coliphages introduced into the soils with septic tank effluent. However, soils in this region do not experience seasonally perched water tables. Viruses often reach ground water from sewage effluent land disposal sites (Schaub and Sorber, 1977; Vaughn *et al.*, 1978; Wellings *et al.*, 1974). All of these sites were located on sandy soils similar to those of southcentral Wisconsin. The only study which addresses the issue of viral contamination of ground water from septic systems does not definitely indicate that enteroviruses enter the drainfields and eventually escape into lakes and streams (Vaughn and Landry, 1977). Only seven samples were obtained from lake water and only two samples in two areas gave a positive test for virus at times which corresponded with the enterovirus season (May through September). Septic tank leachate was considered the only identifiable source of human enterovirus.

In this investigation, indicator bacteria were counted in septic tank effluent and ground water samples from 17 septic systems at sites in southcentral Wisconsin. Three different types of contemporary septic systems were represented in the study; eight conventional, four pressurized dosing, and five mound systems. Viral contamination of ground water from one system was investigated using poliovirus as a tracer.

MATERIALS AND METHODS

Site Selection

Seventeen closely matched, new or replacement septic systems of approximately the same age, located in coarse-textured soils over shallow aquifers close to ground water discharge areas were selected in five counties in southcentral Wisconsin. Three different types of contemporary septic systems were represented in the study; conventional systems at Sites 4 to 8, 13, 16, and 17; pressurized dosing systems at Sites 3, 9, 11, and 12; and mound systems at Sites 1, 2, 10, 14, and 15.

Well Installation and Monitoring Network

The direction of local ground water movement at each site was determined roughly using a "Dowser" model 10 ground water flow meter (K-V Associates, Falmouth, MA), and confirmed by piezometric measurements.

Three interceptor wells for ground water monitoring were placed in the effluent plume downflow in the hydrologic gradient from the drainfields of each septic system. Wells labelled 1 were 30 cm from the edge of the drainfields and those labelled 2 and 3 were downgradient at further intervals of 3 m. Background (control) monitoring wells labelled 4 were 10 m or more upgradient from the edge of the drainfields at each site. Additional wells labelled 5 were located adjacent to the drainfield at Sites 8 and 10.

The wells were made of schedule 40 polyvinyl chloride pipe, 3.8 cm in diameter with 1 m points each with 4 rows of slits 0.15 mm broad and 25 mm long. They were installed by hand using a 76 mm diameter bucket auger to drill to or slighly below the watertable. The well points and attached casings were pounded with a sledgehammer approximately 1 m below the watertable using a steel driving cap inserted at the top of the casings. The casings were sealed with bentonite to 1 m from the ground surface to prevent intrusion of surface water. All wells were developed before sampling by pumping until no deposit of sediment was seen in 1-L samples on standing and no residue occurred on filtration.

Sampling and Monitoring

At sites with an effluent dosing pump (mounds and pressurized dosing systems), effluent samples were taken through the pump chamber manhole with a baling device. At conventional systems at Sites 4 to 8, 13, 16, and 17, a 1-cm diameter Plexiglass tube was cemented into the clean-out plug of the septic tank and inserted into the effluent beyond the outlet baffle within the tank. A Tygon tube was attached to the Plexiglass tube and coiled in a recessed receptacle in the ground. Samples were withdrawn using a manual bilge pump and vacuum flask flushed five times prior to sample collection. For 2 years depth to the water table from the land surface was measured for each well just before pumping to determine the seasonal fluctuation of water in the vicinity of the systems. The device used for this purpose was a "popper" (a plastic cylinder with a concave base which emits a popping sound when it impinges on a water surface) suspended at the end of a steel measuring tape. This device gave accurate measurements of water table depths without contaminating the well water. Records of air temperature and total precipitation were compiled from the monthly publications of the National Oceanic and Atmospheric Administration, National Climatic Data Center, Asheville, NC.

Wells were emptied three to five times before a sample was taken using the bilge pump and a clean vacuum flask. Wells were always sampled in the sequence 4, 3, 2, 1, and 5. For 7 months effluent samples for Cl, EC, TC, FC, and FS analyses from each septic tank and water from wells at each system were collected in clean plastic bottles; occasional samples were collected in sterilized bottles for a continuing period of 2 years and tested for indicator bacteria. Sterilized polypropylene bottles were used to collect effluent and ground water samples from the septic system at site 8; polypropylene bottles adsorb the least virus (Moore *et al.*, 1981). All samples were placed in ice for transport to the laboratory, where they were refrigerated at 4°C and processed within 24 hours for biological parameters.

Analytical Methods

The bacterial and viral analyses were performed at the Food Research Institute, and chloride (Cl) and electrical conductivity (EC) at the Soil Science Department, University of Wisconsin-Madison. The EC, TC, FC, and FS analyses were conducted in accordance with the standard methods for examination of water and wastewater (APHA, 1980). Chloride was analyzed by the potentiometric titration method (Cotlove *et al.*, 1958).

Virus Assays

Gamma globulin-free (GG-free) bovine serum (10 mL) was added to 100 mL of effluent and ground water samples immediately upon arrival at the laboratory. The pH of the samples containing calf serum was adjusted to 9.0 with 3 N NaOH, followed by treating the sample on ice in a sonic bath (Hurst *et al.*, 1978). After sonication, samples were clarified by centrifugation and the supernatant containing the viruses was collected, readjusted to pH 7.0 with 3 N HCl, and filtered through a decreasing-porosity series of cellulose triacetate membrane filters to remove all bacteria (Cliver, 1968).

Monolayers of BGM, "Buffalo" African green monkey kidney (Dahling *et al.*, 1974) or HeLa (human cervical carcinoma) cells were grown in 25 and 150 cm^2 polystyrene cell culture flasks in Eagle's minimum essential medium (MEM) containing standard Earle's balanced salt solution (EBSS), antibiotics (fungizone, penicillin G, and dihydrostreptomycin sulfate), and additional sodium bicarbonate as a buffer to adjust the pH between 7.2 and 7.4 (Kuchler, 1977), but was supplemented by 10% GG-free bovine serum for the BGM cell line, and by 1:1 mixture of fetal and newborn calf serum for the HeLa cells.

Two methods were used for quantifying virus in the samples. Cytopathic effects (CPE) assay was initially done to screen for the presence or absence of virus in paired 25 cm^2 flasks of cells containing fresh culture medium, each inoculated with 0.55 mL of sample; ten-fold dilutions were prepared using phosphate buffered saline (PBS) at pH 7.2 plus 2% calf serum ($PBS\text{-}Ca_2$), 100 units/mL penicillin, and 100 µg/mL dihydrostreptomycin. Another pair of flasks that did not receive a virus inoculum, but instead each received 0.55 mL $PBS\text{-}Ca_2$, served as a virus-negative control. Cell cultures were incubated at 37°C for 4 to 7 days, and flasks were examined for the presence of CPE.

Plaque assays were done on the basis of the positive results obtained from CPE analyses. The growth medium was decanted from paired flasks containing confluent cell sheets of BGM or HeLa cells, and each flask was inoculated with 0.55 mL of virus suspension and rocked on a platform rocker for 2 hours at room temperature. The inoculum was discarded and 5.0 mL overlay medium (maintenance medium plus 0.002% neutral red and 1% Noble agar) added. Once the agar added to the inoculated cells solidified, the flasks were inverted and incubated for 5 days at 37°C and the clear areas were counted as plaques. Plaques are seen because neutral red is a vital dye that stains only living cells and is not retained by the cells in areas killed by virus. Thereafter, the agar overlay was removed from each flask

by adding 10 to 20 mL distilled water and the cells were stained with an aqueous solution of 1.85% formaldehyde, 25% ethanol, 0.85% sodium chloride, and 0.5% crystal violet to guarantee that all plaques were scored.

Plaque counts from duplicate flasks of each cell line were summed and recorded as plaque-forming units (PFU)/mL and reported as counts/100 mL. An analysis of variance utilizing a randomized complete block design (Snedecor and Cochran, 1967) was used to compare the poliovirus sensitivities of the two cell lines. No significant difference ($p > 0.05$) was found between the duplicates of each sample tested in one cell line, and between the mean number of plaques on BGM and HeLa cells using samples of significantly different ($p < 0.001$) virus titers. Therefore, plaques on both BGM and HeLa cultures were statistically valid to be used as counts for virus in this study.

Viral Experiment

Stools containing various combinations of the three poliovirus serotypes were obtained from the diapers of infants who received the trivalent oral poliovirus vaccine (Sabin). Virus-containing stools were obtained (saved by the infants' mothers, who had frozen the stool-containing diapers until they were collected) from days 2 to 50 after vaccination. The stools from each diaper were removed and weighed. PBS-Ca_2 was added to a 0.25 g sample of each stool and mixed, making a 25 mL, 1:100 dilution that was used for virus titrations. Virus was eluted from the solids in the resulting slurries followed by solids removal, filter sterilization and assay in cell culture. Poliovirus-containing stools were stored at -10°C and thawed only 1 hour before dosing the septic tank. They were transported on ice to site 8. Site 8 was inoculated with a total poliovirus dose of 3.3×10^8 count introduced as a single dose or slug by three flushes of the toilet to carry the inoculum all the way to the septic tank. Thirteen effluent samples for viral analysis were collected, on days 3, 6, 9, 13, 16, 21, 29, 35, 50, 64, 78, 91, and 109. Nineteen sets of ground water samples were collected for viral analysis from individual wells on day 1, 2, 6 through 108, 131, 145, 159, and 173.

DATA ANALYSIS

Exploratory Data Analysis

Because of the large scatter in results, the effluent and ground water raw data were statistically evaluated using the Exploratory Data Analysis (EDA) techniques of Tukey (1977) and computer algorithms of Velleman and Hoaglin (1981). Compound smoothing techniques were used to smooth curves and examine the time series of counts of indicator bacteria in the various locations, i.e., effluent and wells 1 to 5. Based on the best regression model between the smoothed and original values and lowest random error, the best smoothers were 3RSSH, twice and 4253H, twice. The smoother 4253H, twice consisted of running two times median of 4 then 2, then 5, then 3, followed by hanning. Smoother 3RSSH, twice comprises two runs of 3 simple smoothers: 3R, SS, and H. The 3R repeatedly used running medians of

length 3 until there were no changes. The SS, or splitting, used a special method to remove flat spots that often appeared after 3R. Hanning, H, was a running weighted average smoother against outliers.

The median-polish method was used to describe an additive model for a two-way table that involved three components--the row factor, column factor, and response. The row factor was the bacterial type, the column factor the location (effluent and wells), and the response the means of bacterial counts at Site 8. Using means as response made the analysis sensitive to outliers and afforded more conservative appraisals.

Information on additive behavior was swept out of the data table and into a common value term, a set of row effects, a set of column effects, and a table of residuals through several stages approaching best fit; all sum to the original data values. The common value served as a standard against which to measure patterns. The effect values help to quantify any substantial variation of bacterial type with location. The residuals from an additive fit serve to reveal patterns not readily apparent in the original data. A coded table of the residuals was used (Velleman and Hoaglin, 1981).

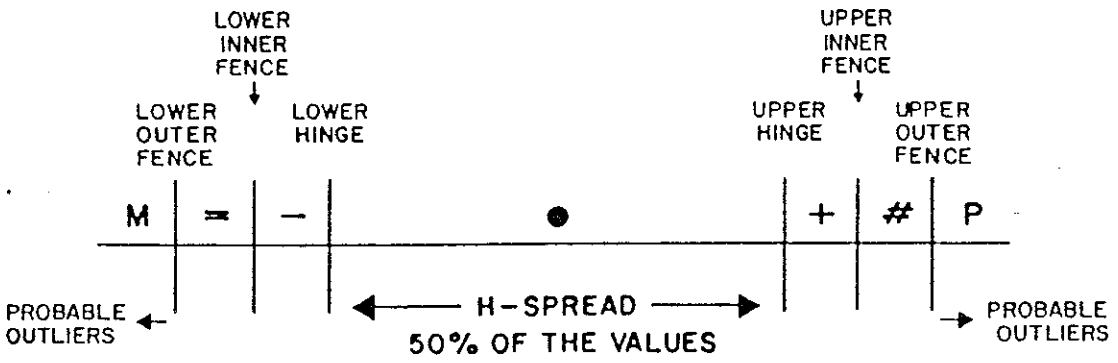
Since the table was not described well by an additive model, it was made more nearly additive by re-expressing the data values. The power of the re-expression was found by subtracting the slope relating the residuals to the comparison values from unity. The comparison value for each cell of the table was computed as a ratio of row and column effect product to the common value. Natural logarithm was found to be the best fit because the slope was unity and the power of re-expression was zero.

The two-way analysis of variance, i.e., ANOVA, uses the same additive model as the median-polish method, but it fits this model by finding row and column means using least-squares regression. On the other hand, the exploratory technique of median-polish fits medians using resistant line and iterative calculations. One major reason for use of medians in finding the additive fit, however, is to protect the results from being distorted by extraordinary values (outliers) and to show where the differences are.

Graphical Procedure

A two-way plot of the fit was performed using the graphical procedure described by Tukey (1977). The fit was taken as a constant sum of two parts such that one part depends only on the column and the other only on the row (e.g., common value + column PLUS row or 2/3 common value + column PLUS 1/3 common value + row) and was represented by dashed lines connecting all the constant intersections of horizontal and vertical ordinates on rectangular coordinates. The residual codes were plotted in the intersections of the ordinates over an invisible two-way plot of the fit.

The two-way plot of the fit was drawn by rotating the graph paper under the tracing paper 45° before tracing the fit dashed-lines and codes of residuals. The actual values of the individual residuals were shown on a vertical scale on the right side of the plot with the representing code. Key to these codes is shown in Figure 1.



INNER FENCES = HINGES ± 1.5 (H-SPREAD)
OUTER FENCES = HINGES ± 3 (H-SPREAD)

Fig. 1. Key to the residual codes (after Tukey, 1977; Velleman and Hoaglin, 1981).

Analysis of Contaminant Transport

Statistical Summaries

The TC, FC, FS, and PV were the contaminants whose transport from septic system to ground water is of concern. The Cl concentrations were used to

infer the amount of effluent present in any ground water sample collected. Summary statistics were conducted on Cl, TC, FC, and FS, in effluent and water from wells 1 to 5 at all sites, and in addition, for PV at Site 8.

The raw data exhibited means equal to or greater than medians, high coefficients of variation, and therefore positively skewed data populations. Test of normality was conducted on the raw data (Ryan *et al.*, 1982). The latter test, essentially equivalent to the Shapiro-Wilk test (NCASI, 1985; Shapiro and Wilk, 1965), is very powerful--especially for small numbers of samples ($n \leq 30$). In this test, the normalities were not confirmed at $p = 0.05$, and the raw data came from populations other than normal.

Since the data batches were not from normal distributions, it was erroneous to draw conclusions on contaminant transport from simple statistical parameters such as means or medians which represent only normal distributions. Therefore, a contaminant transport model was tested to simulate ground water contamination from septic system.

Probability Distributions and Curve Fitting Procedures

Since many statistical conclusions are based on normal distribution, it is often advantageous to transfer skewed distributions into natural log-normal forms. Natural log-normal distributions are distributions of varieties modified so that their logarithms obey the normal law of probability. Natural log-normal distribution arises from a theory of elementary errors combined by a multiplicative process, just as the normal distribution arises from a theory of elementary errors combined by addition. Because of the complexity of processes occurring in septic systems, it is reasonable to suggest that the processes causing differences in the data were multiplicative rather than additive.

The plume, background, and effluent concentrations of Cl, TC, FC, FS, and PV were best represented by a three-parameter natural log-normal distribution fitted by combining the maximum likelihood procedure for the two-parameter distribution described by Stedinger (1980) with a technique which provides a reasonable estimate of the third parameter:

$$\hat{a} = (1/n) \sum_{i=1}^{n} \ln(x_i - \hat{c}) \qquad (1)$$

$$\hat{b} = \{(1/n) \sum_{i=1}^{n} [\ln(x_i - \hat{c}) - \hat{a}]^2\}^{1/2} \qquad (2)$$

where $\hat{a}$, $\hat{b}$, and $\hat{c}$ were respectively the biased estimates of the population mean, population standard deviation and the third parameter. The third parameter (lower boundary or the threshold of the distribution) for plume and background concentrations was set at zero, and for effluent concentrations to one-half of the lowest observed concentration. This pre-setting of the third parameter was reasonable for the data accumulated and eliminated calculation of the third parameter by the full-quartile method (Stedinger, 1980). The maximum likelihood method was generally best for fitting log-normal distributions. However, Stedinger (1980) also found this method robust and that it performed well even when observations obeyed other than log-normal distribution.

In comparison to measured levels, the fitted distributions were very good--individual natural log-normal distributions could not be rejected at a significance level of $p = 0.05$ using the Kolmogorov-Smirnov goodness-of-fit test (Steel and Torrie, 1980). The statistic used with the latter test was the Lilliefors D-statistic (Lilliefors, 1967) which represented the largest absolute value of the differences between observed and predicted frequencies at both ends (top and bottom) of each interval for the individual distributions. The probabilities or frequencies were generated using the California formula of Viessman *et al.* (1972).

The Lilliefors D-statistic was used to determine whether the hypothesis that the natural logarithms of the data were normally distributed could be rejected. For the tracers in effluent and water from wells 1 to 5, 30 individual distributions were developed and compared to the

corresponding fitted ones with the Lilliefors D-statistic tables to verify the goodness-of-fit. The Lilliefors tables provided more power to identify non-normally distributed small data sets because they allowed confident rejection of the normal distribution hypothesis more frequently than the standard D-statistic tables. Therefore, the effects of possible errors in the probability distributions for the various constituents on the simulation results were small, and validated use of the simulated values with the transport model.

Contaminant Transport Model and Stochastic Analysis

The transport of contaminant M (here TC, FC, FS, and PV) from septic tank to ground water was evaluated in the following computational steps:

1. The volumetric fraction of effluent (E) undiluted by ambient ground water in each ground water plume sample (samples from wells 1 to 3 and 5) was estimated by Eq. (3) using Cl as tracer

$$E_{i,j} = \frac{Cl_{i,j} - Cl_{b,j}}{Cl_{e,j} - Cl_{b,j}} \qquad (3)$$

where, for every plume sample i and iteration j in a Monte Carlo stochastic simulation (Wonnacott and Wonnacott, 1977), Cl_i, Cl_b, and Cl_e were respectively the plume, background (well 4), and effluent Cl concentrations. The Monte Carlo simulation showed the probability of having effluent present in the ground water at wells located downgradient from the drainfields.

2. For all iterations in which $E_{i,j}$ was > 0, the contaminant transport from the septic tank to ground water was described as the percentage ratio of the actual increase in contaminant above background to the increase in contaminant above background if there were no retention by soil. Mathematically,

$$\% \text{ MT} = \frac{M_{i,j} - M_{b,j}}{E_{i,j}(M_{e,j} - M_{b,j})} \cdot 100 \qquad (4)$$

where % MT was the percentage of contaminant transported and $M_{i,j}$, $M_{b,j}$, and $M_{e,j}$ were the plume, background, and effluent concentrations of contaminant M and interaction j in a Monte Carlo simulation. The contaminant concentration, assuming no retention by soil, was computed from the estimated amount of effluent in the sample, with no reduction in M concentration except by dilution, accounted for by use of Cl as a reference. The E factor in Eq. (4), the amount of effluent intercepted in the sample, mitigated the uncertainty regarding the actual sampling location in the plume versus the true plume center since the well point did not necessarily intercept the center of the plume.

The values of % MT, the percentage of contaminant transported in Eq. (4), were calculated 150 times for each sample in the Monte Carlo analysis and constrained within limits of actual possible values from 0 to 100%.

This number of iterations produced a fairly smooth empirical probability distribution. However, further smoothing was needed and conducted with the 4253H, twice smoother (Velleman and Hoaglin, 1981). Values of % MT of < 0 were set equal to 0, and values > 100 were set equal to 100.

The Monte Carlo simulation evaluated the effect of uncertainties (included in the wide range found for plume, background, and effluent concentrations of Cl and contaminants) on estimates of contaminant transport. The stochastic analysis method represented the selected variables of plume, background, and effluent concentrations of Cl and contaminant as probability distributions rather than discrete values, and thus the apparent movement of contaminants from septic system to ground water were best described in probabilistic terms. Such a description is superior to one based on discrete values because the uncertainty in the values of variables is reflected directly in transport estimates and not open to the same degree of subjective interpretation.

Assumptions of the Contaminant Transport Model

The main assumptions used in the contaminant transport model and the likelihood of their validity were:

1. Chloride is a conservative tracer, i.e., it moves freely through the soil with no loss by sorption or addition by desorption, so any change in Cl concentration is due to dilution and dispersion. That this assumption is valid was confirmed by Johnson *et al.* (1979), who found no retention of Cl by soils similar to those in southcentral Wisconsin.

2. Septic tank effluent is the only non-background source of Cl and contaminant. No serious violations of this assumption were found.

3. Unassimilated or unsorbed contaminant M and Cl released from a septic tank and transported to any sampling well move at equal rates through the soil. This assumption is necessary to estimate M dilution in individual samples based on Cl concentrations, but is difficult to assess in natural systems. Aftergrowth and die-off of bacterial contaminants after their release to the soil may violate this assumption of the contaminant transport model. The soil-associated virus may be desorbed and redistributed within the soil matrix under seepage beds following changes in certain characteristics of the soil solution, e.g., pH and ionic properties (Gerba and Bitton, 1984). This assumption was recognized as an unknown source of uncertainty in the analysis. However, its effects were mitigated by the curve fittings and the simulation analysis. This is why curve fittings and simulations were essential components of the transport model.

RESULTS AND DISCUSSION

Water Table Fluctuation

The monthly depths of the water table from the land surface and the monthly rainfall and atmospheric temperature at individual sites were plotted to find the relative influences of effluent recharge and climatic

factors on ground water table fluctuation near the individual septic systems. Figure 2 is representative of the situation at all sites except Site 8; the other plots, not shown here, exhibited similar patterns. Figure 3 represents Site 8.

The water table at the sites (except Site 8) was within 3.0 m (10 ft), of the land surface. Ground water levels in the monitoring wells at the sites generally fluctuated with a maximum of ± 102 cm/yr (40 inches/yr), with a winter low period (Dec.-Jan./Feb.), a spring peak (Mar.-Apr./May), a slow summer decline (Jun.-Jul./Aug.), and small rise in fall (Sep.-Oct./Nov.). There was no pattern to the monthly water use in the households. Therefore, the fluctuation in the levels of the water table in the vicinity of the sites seems to be mainly due to climatic factors. Seasonally, water levels generally rose sharply during the spring in response to recharge from snowmelt and rainfall. Water levels generally declined slowly throughout the summer because precipitation was mostly evaporated or transpired by plants and did not percolate to the ground water reservoir. A small rise occurred following recharge from fall rains, followed by a decline during the winter when precipitation was stored on the frozen land surface as snow. The ground water levels fluctuated more during the fall of 1980 than that of 1981 due to the unusual, near-record wet period for August and September 1980. The clogging layers at the soil-gravel interfaces restricted effluent percolation to a slow unsaturated flow which recharges the ground water slowly, thus having little influence on the water table fluctuations compared to the seasonal climatic factors.

Ground water levels at Site 8 (Figure 3) showed no perceptible fluctuation in response to precipitation and atmospheric temperature changes, in contrast to the situation at the rest of the sites. That behavior was due to the unique location of Site 8 on Lake Wisconsin; surface water from the lake was presumably interfering with the aquifer close to the lake by charging that part of it under Site 8 in the summer and winter when the water table dropped but the water level of Lake Wisconsin was kept constant by the dams on the Wisconsin River (Holmstrom *et al.*, 1982; USGS, 1980, 1981).

Statistical Summaries

Ground water samples were monitored closely for 7 months and occasionally for a continuing period of 2 years at 17 septic systems but showed no evidence of indicator bacteria; only samples from Site 8 showed counts of TC, FC, and FS >1/100 mL. The analyses for indicator bacteria following 5 years of system operation showed similar results, i.e., only ground water samples from Site 8 showed counts >1/100 mL; all other samples showed counts <1. Summary statistics for EC, Cl, TC, FC, FS, and PV levels in effluent and wells 1 to 5 at Site 8 are presented in Table 1.

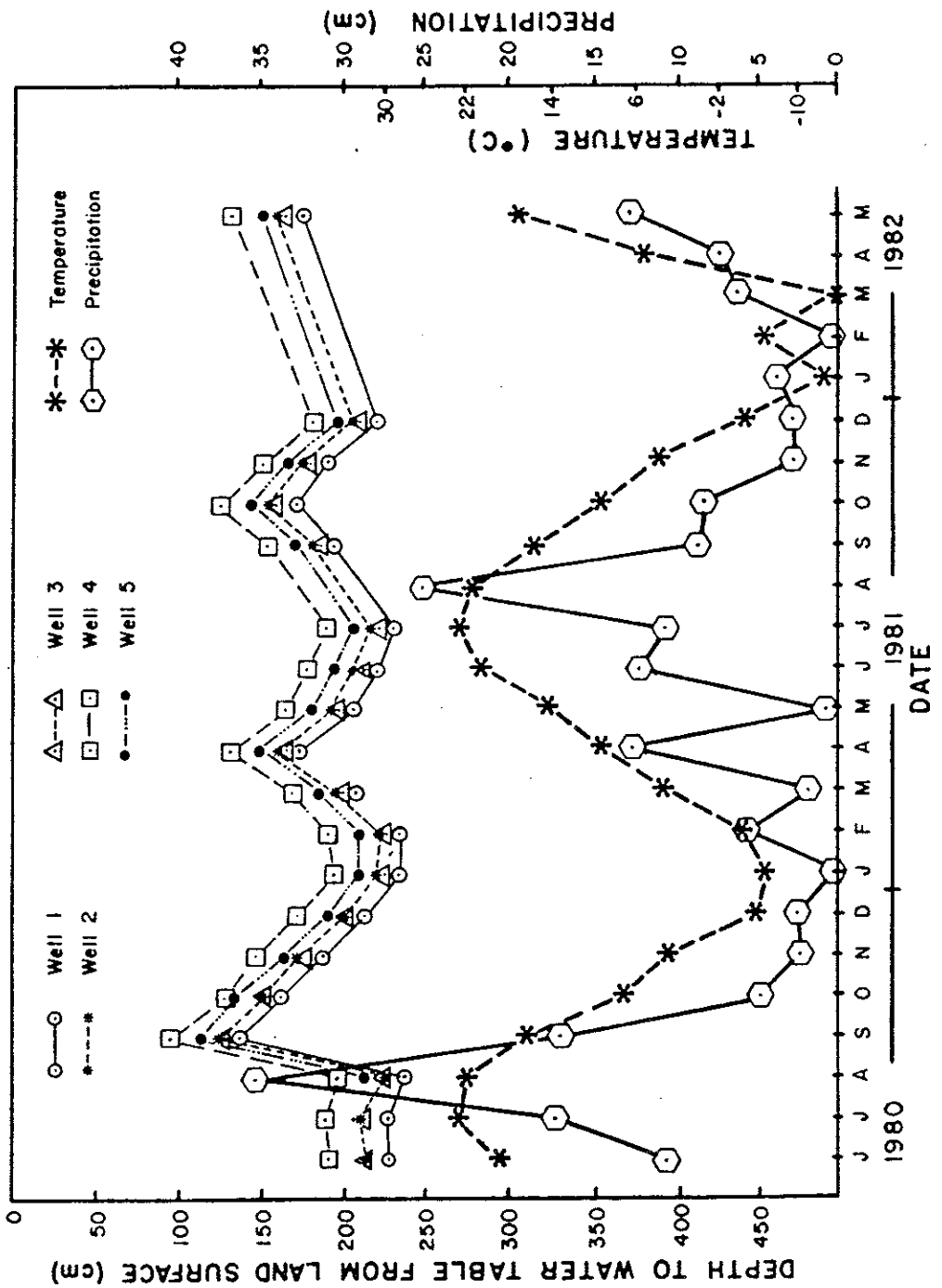


Fig. 2. Monthly ground water levels in five wells, atmospheric temperature, and total precipitation at Site 10.

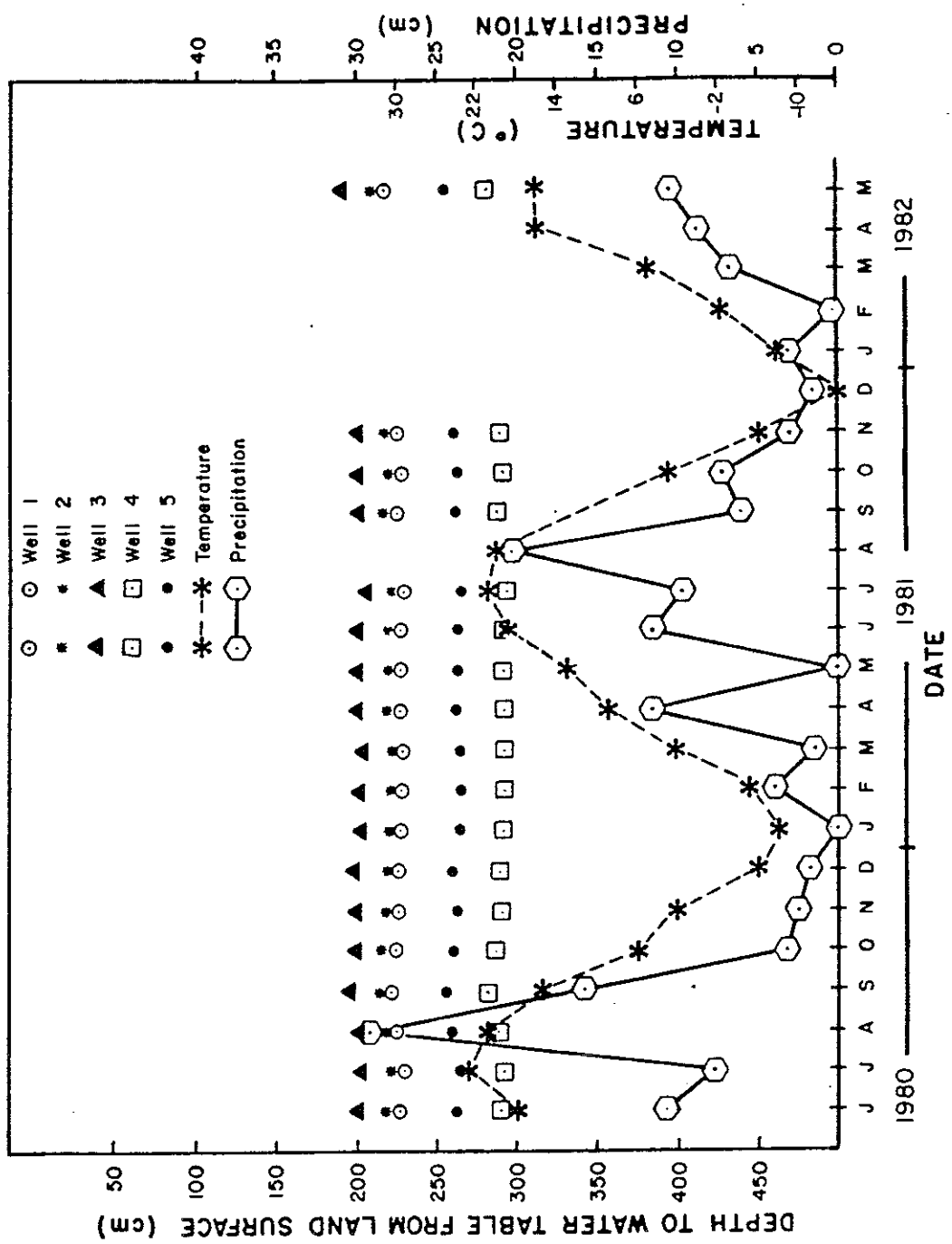


Fig. 3. Monthly ground water levels in five wells, atmospheric temperature, and total precipitation at Site 8.

Table 1. Levels of EC, Cl, TC, FC, FS, and PV in Plume Wells, Background (Well 4), and Septic Tank Effluent at Site 8

	EC	Cl	TC	FC	FS	PV
			WELL NO. 5			
n	29	27	15	15	15	19
M	2.0	305	54	<1	<1	<1
$\bar{x}$/M	0.80	1.0	8611	19	9.0	17
R	0.35-2.7	6.0-912	<1-7.0×10^{6}	<1-226	<1-99	<1-300
COV	2.1	0.82	3.8	3.0	2.7	4.1
			WELL NO. 1			
n	29	25	15	15	15	19
M	0.50	11	30	<1	<1	<1
$\bar{x}$/M	0.98	1.7	14	7.0	16	17
R	<0.20-0.76	3.1-88	<1-3.2×10^{3}	<1-99	<1-99	<1-200
COV	3.5	1.2	2.1	3.6	2.0	3.0
			WELL NO. 2			
n	26	26	15	15	15	19
M	0.39	6.4	101	<1	<1	<1
$\bar{x}$/M	0.97	1.4	26	100	11	27
R	0.22-0.50	ND-30	<1-3.0×10^{4}	<1-1.3×10^{3}	<1-99	<1-300
COV	4.7	0.90	3.0	3.3	2.3	2.9
			WELL NO. 3			
n	27	26	15	15	15	19
M	0.36	8.0	81	<1	<1	<1
$\bar{x}$/M	1.0	1.0	12	12	9.0	6.0
R	0.19-0.50	0.26-26	<1-9.0×10^{3}	<1-110	<1-99	<1-100
COV	4.0	0.75	2.5	2.7	2.9	3.7
			WELL NO. 4			
n	27	26	15	15	15	19
M	0.41	1.0	101	<1	<1	<1
$\bar{x}$/M	1.0	3.1	8.1	293	8.0	1.0
R	0.20-0.58	0.09-16	<1-5.0×10^{3}	<1-4.0×10^{3}	<1-99	<1-<1
COV	4.6	1.5	1.9	3.5	3.1	-
			EFFLUENT			
n	34	24	29	29	29	13
M	2.1	349	4.0×10^{5}	6.3×10^{4}	9.0×10^{3}	1
$\bar{x}$/M	0.95	1.0	144	502	48	70
R	1.0-3.1	44-776	3.5×10^{3}-6.4×10^{8}	2.0×10^{3}-5.5×10^{8}	450-8.3×10^{5}	1-503
COV	3.0	0.47	2.8	3.5	3.5	2.0

EC = mmhos/cm of electrical conductivity; Cl = mg/L of chloride; TC, FC, FS, PV = count/100 mL of total coliforms, fecal coliforms, fecal streptoccoci, and poliovirus, respectively.

n = number of samples, M = medians, $\bar{x}$/M = mean to median ratios, R = ranges, and COV = coefficients of variation.

Time-series Smoothing Analysis

Total Coliforms

Time-series smoothing analyses on TC counts recovered from Site 8 are shown in Figure 4. TC counts in ground water wells at Site 8 increased with time. The counts of TC in well 5 increased from <1/100 mL in June to 7.0 x 10^6/100 mL in October. TC also accumulated in well 1. Elevated numbers of TC in well 1 occurred in October and December (1.6 x 10^3 and 3.2 x 10^3/100 mL). However, higher numbers of TC were detected in wells 2 and 3 than in well 1. Maximum counts of TC were observed in wells 2 and 3 in November (1.6 x 10^4 and 4.4 x 10^3/100 mL). High counts (2.6 x 10^3/100 mL in September and 2.8 x 10^3/100 mL in November) were recovered from the control well (well 4), located upgradient from the septic system.

The TC in ground water at Site 8 may have many sources. Besides occurring in the intestinal tract and feces of warm-blooded animals, TC occur in the feces of cold-blooded animals, as well as on plant surfaces and in soil (Geldreich, 1978; Gerba *et al.*, 1975). TC also travel long distances in ground water. Gerba and McNabb (1981) cited experiments where coliform bacteria spread horizontally over 0.9 km. Furthermore, many coliforms are capable of replication outside the intestine, especially in nutrient-rich waters such as percolating septic tank effluents; this may exaggerate the apparent risk of pathogen occurrence. The problem of differentiating between TC from the septic system at Site 8 and soil-derived strains necessitates the use of supplementary bacterial indicator groups to detect unequivocal ground water contamination from the septic system.

Fecal Coliforms

Figure 5 depicts time-series smoothing analylses of FC counts. The FC counts increased in ground water over time, however, such increases were not as pronounced as those of TC. From June to October, no FC were recovered from well 1, where the lowest TC counts generally occurred. In all other wells, few or no FC were detected over the same period. The highest numbers of FC were in samples collected from the control well during November and December (2.0 x 10^3 and 2.2 x 10^2/100 mL).

A shortcoming of FC as an indicator of enteric pathogens is that FC have shorter survival times in soil and ground water than some enteric pathogens (Kaufman and Orlob, 1956). FC may die off prior to the disappearance of a given enteric pathogen, and ground water that appears to be free of fecal contamination may in fact still contain pathogens.

The major advantage of this test, however, is that FC are less likely than TC to exhibit aftergrowth in nutrient-enriched ground water under septic systems, and do survive long enough to be useful indicators (Van Donsel *et al.*, 1967). The FC counts therefore complement the TC test to differentiate between fecal and non-fecal contamination.

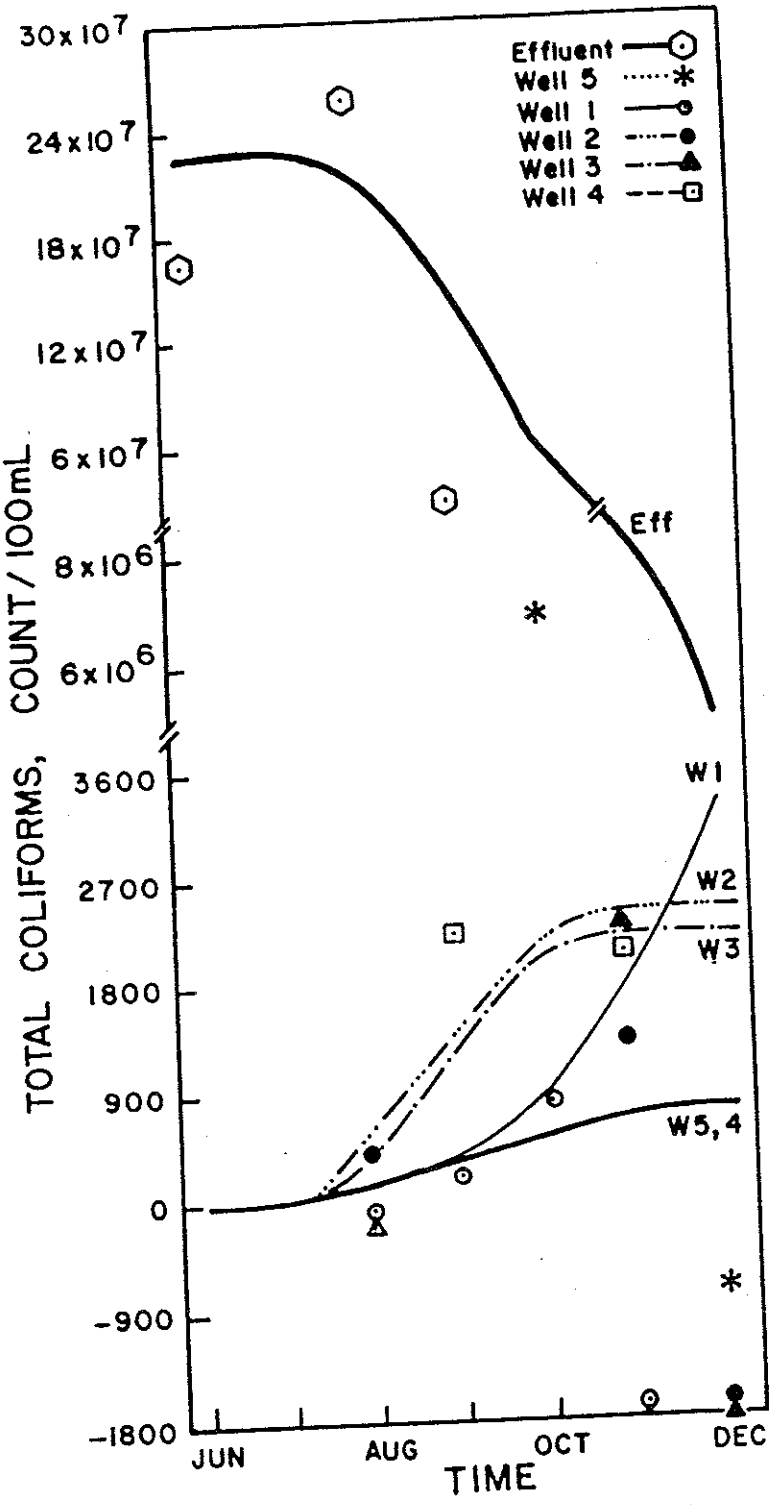


Fig. 4. Total coliforms in six locations at Site 8, smoothed by 3RRSH, twice (Velleman and Hoaglin, 1981). The vertical distance from any smoothed (fitted) line to its corresponding symbol is the rough (error); smooth plus rough equal data.

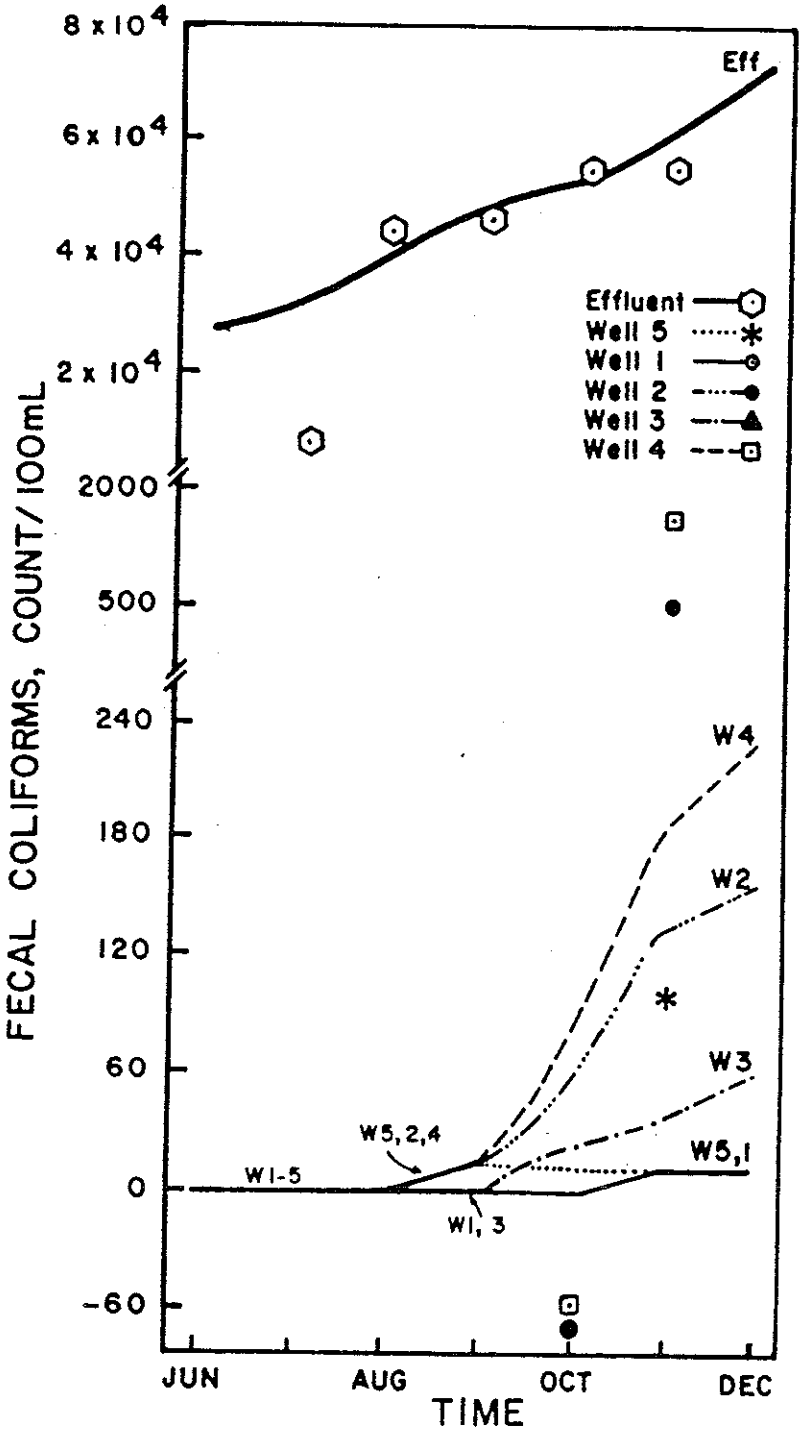


Fig. 5. Fecal coliforms in six locations at Site 8, smoothed by 4253H, twice (Velleman and Hoaglin, 1981). The vertical distance from any smoothed (fitted) line to its corresponding symbol is the rough (error); smooth (fit) plus rough equal data.

Fecal Streptococci

Time-series smoothing analyses on FS counts at Site 8 are shown in Figure 6. The highest FS counts were detected in December (99/100 mL in all wells including the control), coinciding with high counts of TC and FC. Water from well 1, however, showed high FS counts in June and July (42 and 48/100 mL), but the lowest FC counts for the same months; low TC counts occurred in June but not in July.

FS, like TC, originate in the intestine of warm-blooded animals and are discharged with feces. Unlike TC, however, they also include organisms of plant origin such as *Streptococcus faecium* var. *casseliflavus* (Mundt and Graham, 1968) and atypical *S. faecalis* (Kibbey *et al.*, 1978), and of soil and insect origin such as *S. faecalis* var. *liquefaciens* (Geldreich, 1970). FS persist longer than FC in ground water maintained naturally at cool temperatures (Geldreich, 1970). This might explain the more frequent detection of FS than FC in ground water at Site 8. The FS test was essential for determining possible fecal contamination because FS survive longer than FC forms, and because FC (in contrast to FS) do not occur naturally in significant numbers in ground water; thus, use of these two indicator groups together negates the shortcoming of either used alone.

Because of the natural occurrence of FS in ground water, FS counts below 100/100 mL, in the absence of FC are not deemed significant from the standpoint of ground water fecal contamination (Geldreich, 1970). FS levels in ground water at Site 8 did not exceed this limit (Figure 6). Therefore, the septic system at Site 8 does not appear to contribute fecal contamination to local ground water; the FC recovered must be due to a source other than the septic system, as demonstrated by the high recoveries of FC in the control well especially in cooler months (Figure 5).

Median-polish Analysis

Two-way median-polish analysis of bacterial type (TC, FC, and FS) by location (effluent and wells 1 to 5) at Site 8 is graphed in Figure 7. Figure 7 reveals that the effects of TC were approximately 38 times greater than those of FC, which were in turn approximately 11 times greater than the effects of FS. These effects were significant at a level of 0.05. The effects of location were in the increasing order: effluent > well 2 > well 5 > well 3 = well 4 > well 1. Such order clearly indicated that the effects of background well were no less than the effects of other wells. The row effects of bacteria at individual ground water wells were much smaller than the common value; only row effect of effluent was larger. Consequently, the septic system at Site 8 cannot be blamed for the presence of indicator organisms in the ground water; there must be another source.

Effluent Transport

The movement of effluent from a septic system at Site 8 was inferred from Cl concentrations in plume ground water wells 5, 1, 2, and 3 located at increasing distance downgradient from the drainfield by solving Equation 3. The simulation results were expressed as probability distributions of

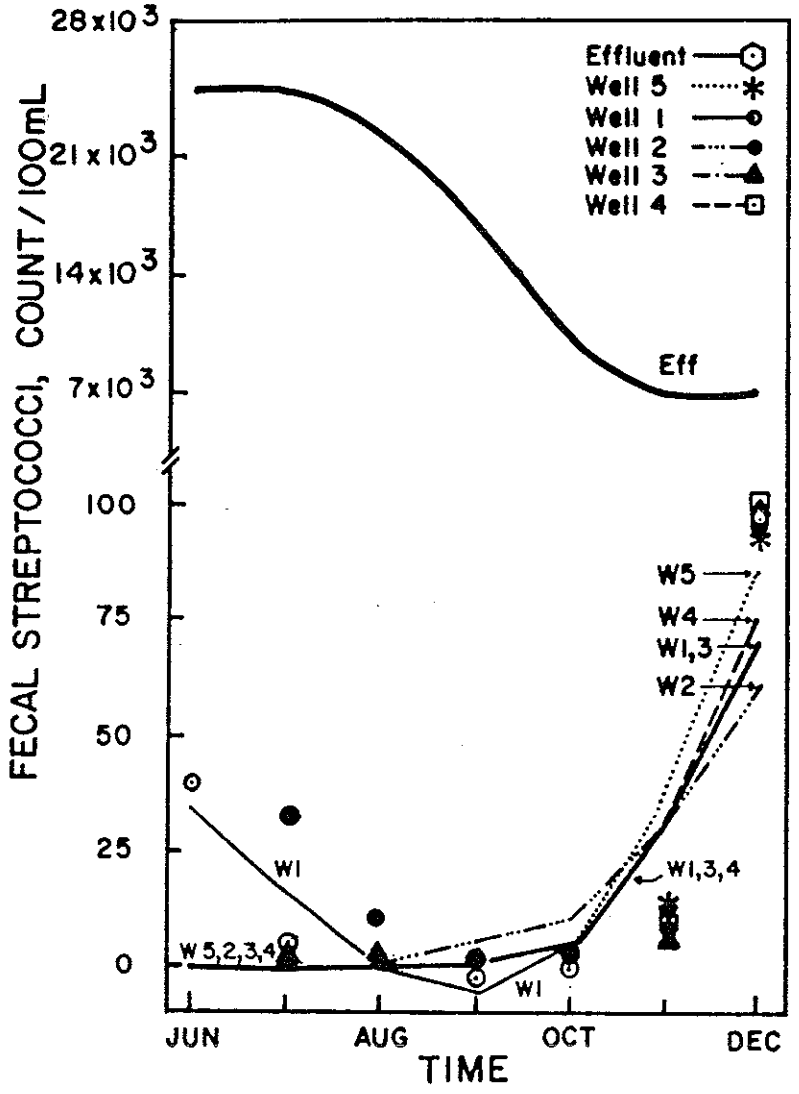


Fig. 6. Fecal streptococci in six locations at Site 8, smoothed by 4253H, twice (Velleman and Hoaglin, 1981). The vertical distance from any smoothed (fitted) line to its corresponding symbol is the rough (error); smooth plus rough equal data.

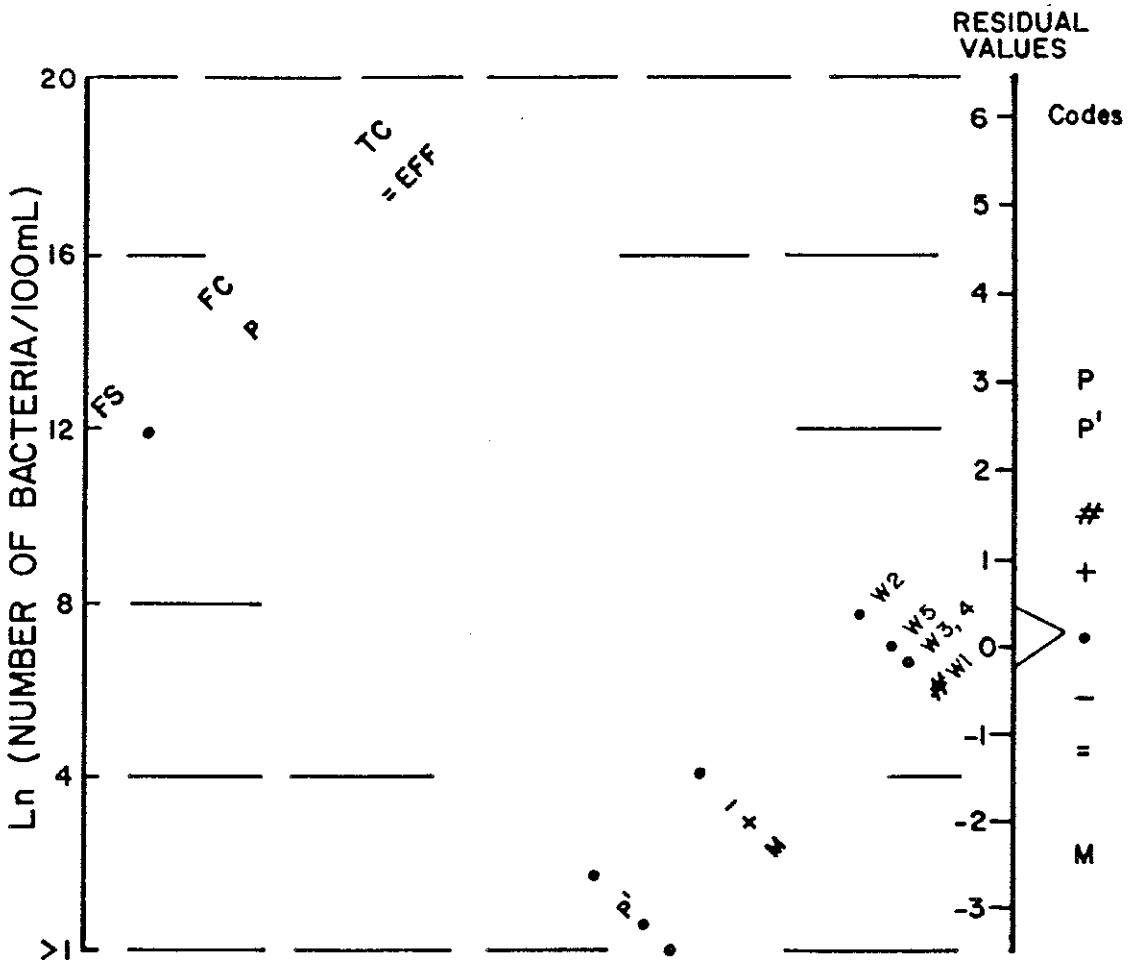


Fig. 7. Two-way analysis plot for total coliforms (TC), fecal coliforms (FC), and fecal streptococci (FS) by sampling locations from septic tank effluent (EFF) and wells 1 to 5 (W1 to 5).

the percentages of effluent in ground water wells 5, 1, 2, 3, and 5 plus 1 to 3 combined at Site 8. A summary of the simulated values of the amount of effluent in ground water at Site 8 is reported in Table 2. The percent of effluent in ground water decreased as distance from the drainfield increased in the direction of ground water flow at Site 8. The plume of pollution was sampled in wells 5 and 1 to 3 at Site 8 with a frequency of 100%; all $E_{i,j}$ values were >0, i.e., the wells were definitely intercepting the contaminant plume.

Indicator Bacteria Transport

Possible ground water contamination by pathogens from septic systems was investigated by simulating transport of indicator bacteria to ground water wells located downgradient from the drainfield at Site 8. The transport summary counts of TC, FC, and FS into individual plume wells from the simulation are shown in Table 3. Table 3 shows that the indicator bacteria were not transported to the ground water, being completely removed by the soil under the seepage bed; the overall means of effluent TC, FC, and FS transported were <1 count/100 mL ground water sample--the Public Health drinking water standard (USEPA, 1986; WHO, 1984).

The results of indicator bacteria transport from the Monte Carlo procedure further substantiate the conclusion from time-series analysis results that the septic system at Site 8 did not pollute ground water by bacterial pathogens as judged by the absence of effluent indicator bacteria from the ground water wells compared to the background.

Table 2. Percentage of Septic Tank Effluent Intercepted in Plume Wells at Site 8, as Calculated by a Monte Carlo Simulation and based on Chloride Data

	Number of Samples	Mean	90% Confidence Interval	Median
		---------- % $E_{i,j}$ ----------		
Well 5	150	68	59-78	50
Well 1	150	3.1	3.0-3.1	3.2
Well 2	150	2.0	1.6-2.4	1.0
Well 3	150	1.5	1.4-1.5	1.5
Overall	600	19	16-21	14

% $E_{i,j} = [(Cl_{i,j} - Cl_{b,j})/(Cl_{e,j} - Cl_{b,j})] \cdot 100$, where, for every plume sample i and iteration j in a Monte Carlo stochastic simulation (Wonnacott and Wonnacott, 1977), E is the volumetric fraction of effluent in each ground water plume sample; Cl_i, Cl_b, and Cl_e are respectively the plume, background (well 4), and effluent chloride concentrations.

Table 3. Total Coliforms, Fecal Coliforms, and Fecal Streptococci Transport to Plume Wells from Septic System; from the Monte Carlo Simulation

	Well 5	Well 1	Well 2	Well 3	Overall
n	150	150	150	150	600
		TOTAL COLIFORMS TRANSPORT (% MT)			
$\bar{x}$	0.02	0.04	4.4	0.41	1.2
M	0.02	0.00	1.0	0.02	0.02
C.I.	0.01-0.02	0.01-0.07	2.7-6.1	0.20-0.62	0.78-1.7
		FECAL COLIFORMS TRANSPORT (% MT)			
$\bar{x}$	0.02	0.00	0.43	0.24	0.17
M	0.00	0.00	0.02	0.00	0.00
C.I.	0.01-0.03	0.00-0.00	0.25-0.61	0.13-0.35	0.12-0.23
		FECAL STREPTOCOCCI TRANSPORT (% MT)			
$\bar{x}$	0.00	0.22	0.35	0.20	0.19
M	0.00	0.27	0.28	0.11	0.09
C.I.	0.00-0.00	0.19-0.24	0.31-0.39	0.15-0.24	0.17-0.21

n, $\bar{x}$, M, and C.I. are respectively number of samples, means, medians, and 90% confidence intervals.
% MT = $\{[(M_{ij} - M_{bj})(Cl_{ej} - Cl_{bj})]/[(M_{ej} - M_{bj})(Cl_{ij} - Cl_{bj})]\} \cdot 100$, where, for every plume sample i and iteration j in a Monte Carlo stochastic simulation (Wonnacott and Wonnacott, 1977), % MT is the percentage of contaminant M (total coliforms, fecal coliforms, or fecal streptococci) transported; Cl_i, Cl_b, and Cl_e are respectively the plume, background (well 4), and effluent chloride concentrations; M_i, M_b, and M_e are respectively the plume, background and effluent concentrations of contaminant.

The most probable source of the indicator bacteria observed appears to be intruding surface water from Lake Wisconsin. Evidence of such recharge under Site 8 was revealed in Figures 2 and 3 and is reported to be common on lakeshore sites by Dudley and Stephenson (1973). Because the water table dropped slowly in summer compared to winter, surface water recharged the near-shore aquifer faster in winter; thus, bacterial filtration was probably less efficient in winter recharge compared to summer recharge. This might explain the increase of indicator bacteria with time from summer to winter in Figures 4 to 6. Comparing these figures for the summer (June through August) showed that the frequency of appearance of FS is more than

that of TC and the latter more than that of FC. This might be related to their survival times in ground water, which followed the same trend, i.e., FC die off faster than TC which in turn die off faster than FS in ground water.

Another possible explanation is that increases in indicator bacteria (Figures 4 to 6) coincide with high rainfall in August and September 1980 (Figures 2 and 3) which might have flushed bacteria from the surface soil to the ground water. Under favorable field conditions, particularly when temperature of ground water decreases (from September to December), survival of the flushed bacteria may extend 3 to 4 months, as reported by Gerba *et al.* (1975).

Poliovirus Transport

The septic tank retained most of the inoculum dose of the poliovirus, likely by sorption to suspended particles which settle to the bottom of the tank with the sludge. However, polioviruses managed to escape in the effluent that flowed to the soil under the seepage beds. Of the 3.3×10^8 counts of poliovirus inoculated into the septic tank, a mean of 70 counts/100 mL escaped the tank with the effluent over a period of 109 days (see effluent column, $\bar{x}/M = 70$, $M = 1$, Table 1). Such a level of virus escape cannot be ignored because it may take only one virulent virus to infect a susceptible human.

The simulation analysis on poliovirus transport to plume wells 5, 1, 2, 3, and all wells combined at Site 8 is demonstrated by the probability distributions in Figure 8. Vaccine poliovirus leaving the tank in effluent was transported from the septic system to ground water well 5 at a maximum level of 44% with a probability of at least 50%; increasing the probability decreases the poliovirus transport exponentially.

Extremely important was the greater likelihood that viruses were encountered in the wells at a greater distance from the septic tank drainfield than those close to it (Figure 8). This phenomenon can be explained. Laboratory studies with soil columns and batch experiments with suspensions of soils or clays have shown that the ionic composition of the water containing the added viruses was a very important factor affecting virus sorption (Carlson *et al.*, 1968). In a likely mechanism of virus sorption, an increase in ionic strength of a virus-containing the solution reduces the thickness of the double layer of charges around the viruses and soil particles, allowing them to move close enough together to be bonded by London-van der Waals forces (Lance, 1978). Similarly, viruses desorb when the ionic strength of the suspending medium decreases (Landry *et al.*, 1979). Far greater fractions of septic tank effluent were recovered from well 5 than from other wells, as shown by the Cl concentrations in Table 1 and the simulation results in Table 2. The average EC--an indicator of ionic strength--of the ground water samples from well 5 was approximately four times higher than values obtained for samples from other wells (Table 1). Thus, virus retention by sorption in the unconfined aquifer sediments would be greater in the presence of more septic tank effluent around well 5 because effluent increased the ionic strength of ground water and allowed sorption to take place.

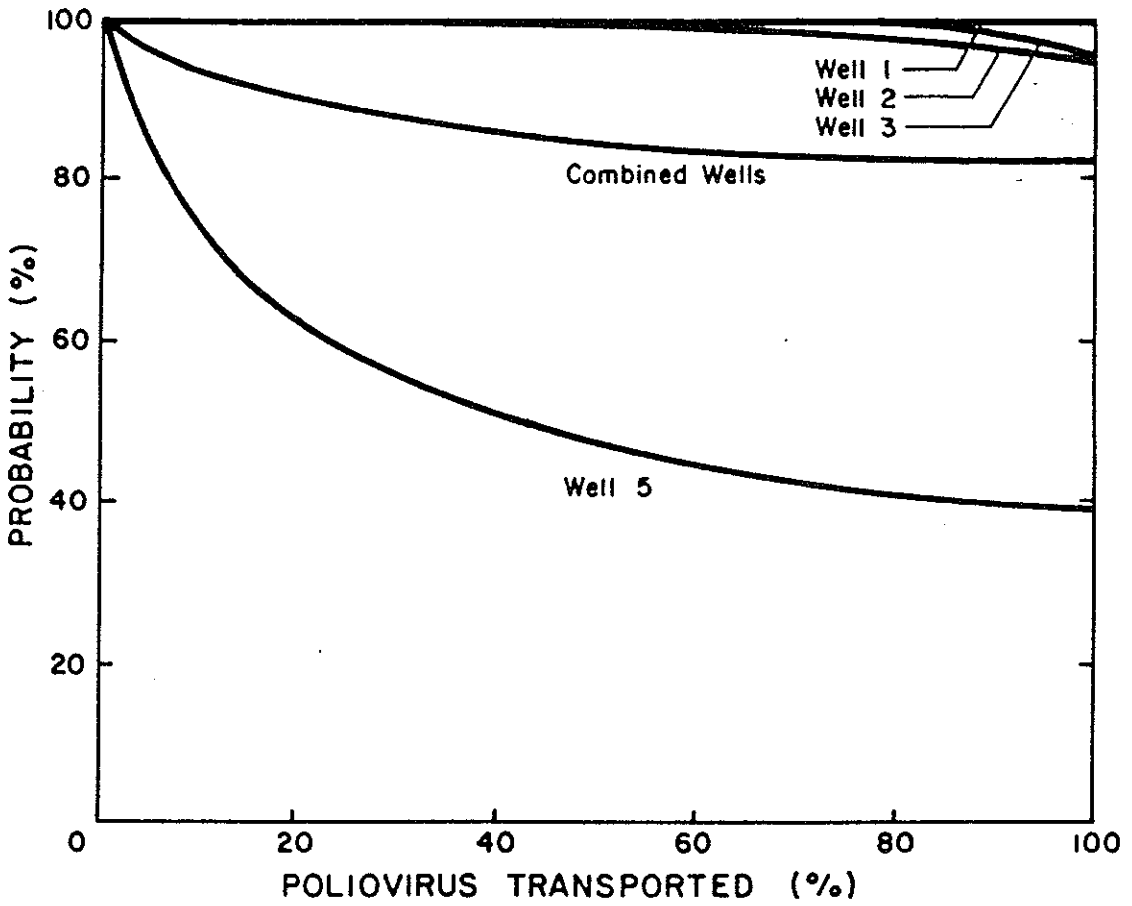


Figure 8. Probability of poliovirus transport to plume wells at Site 8.

Laboratory and field studies showed virus survival times of up to several months under anaerobic conditions in ground water (Duboise *et al.*, 1976; Hurst *et al.*, 1980a, 1980b; Lefler and Kott, 1974). Aerobic conditions are needed for high virus inactivation rates. Polioviruses might have remained infectious while sorbed to the aquifer sediments at high ionic strength near well 5. The poliovirus may have gradually desorbed from the sediments under anaerobic conditions and traveled freely in ground water to wells 1, 2, and 3 at distances of 0.3, 3, and 6 m from the drainfield; sorption is not an irreversible reaction and desorption may occur as such variables as pH, flow rate, ionic strength, and soluble organics change.

The capsid, or surface of a virus particle is composed of numerous ionizable proteins which are subject to protonation and deprotonation, depending upon the pH and ionic strength of the soil solution. The pH at which a particle has a net zero charge is termed the isoelectric point (IEP). Since each virus type has different surface characteristics, its

IEP is unique. The IEP's of several enterovirus types listed by Murray and Parks (1980) range from 3.8 to 8.5, indicating great variability for different virus types.

The significant breakthrough of vaccine poliovirus to the ground water from the septic system at Site 8 might be linked to the characteristics of the virus itself, principally its IEP and size. The virus used at Site 8 had two IEP's, one near pH 6.5, the other near pH 4.5 (Murray and Parks, 1980). Each IEP defines a specific conformation of the virus. Between these values, the overall charge is weak and heterogeneously distributed around the surface. Below pH 4.5, the particle behaves as a positively-charged species, above pH 6.5 the net charge is negative. The pH range of effuent at Site 8 was 6.5 to 8.1, with a mean of 7.2; the soil beneath the drainfield was calcareous drift overlying loess with a pH > 7. Under these conditions, vaccine poliovirus predominantly possessed negative charges, so that sorption to the negatively-charged soil particles was limited. On the basis of these considerations, vaccine poliovirus transport to and in ground water from the septic system at Site 8 was reasonable.

Poliovirus is 27 nm in diameter, or 35 times smaller than the average bacterium. An average bacterium is still 25 times larger than the encased cluster of polioviruses normally encountered in the soil (Bitton, 1980; Floyd *et al.*, 1976). The filtering capability of soil would, therefore, be much lower for poliovirus than bacteria.

The clogging mat or "crust" formed naturally at the gravel-soil interface under the drainfield was efficient in filtering bacteria but failed to filter poliovirus. Poliovirus appeared able to penetrate through the pores of the newly-formed clogging mat at Site 8; the septic system at Site 8 was a conventional system only 2 months old when the viral study was initiated.

The formation of a clogging mat is gradual and with time a dynamic equilibrium is reached between the rate of bacterial degradation and organic matter accumulation forming a "mature" mat. A mature mat might be able to retard poliovirus. However, a crust of approximately 7 months of age could not stop poliovirus from reaching the ground water at Site 8. Thus, poliovirus contamination of ground water through septic tank leachate resulted from a well-functioning conventinal system at Site 8.

Ideally, to ensure public health, virus should be removed in a properly-designed and maintained septic tank before effluent reaches the drainfield. Also, the indicator bacteria (TC, FC, and FS) were not tracers for the presence of viruses; the soil retained the indicator bacteria but not virus. Viral contamination of ground water from septic systems is a more serious problem than bacterial contamination, and attention should be focused in this direction.

The vaccine poliovirus transport is obviously significant. The soil cannot entirely retain the poliovirus, and it appears to move freely and with little or no retardation in ground water. It is essential to bear in mind, however, that the behavior of vaccine poliovirus is not representative of all virus types since these differ in their surface characteristics and IEPS.

Studies relating the movement through soils of indicator virus types that cover the IEP range of important pathogenic viruses are needed.

CONCLUSIONS

1. Indicator bacteria are not transported to local ground water but are completely removed by the soil under the seepage bed. The means of numbers of TC, FC, and FS transported were <1 count/100 mL of ground water, in keeping with the U.S. Public Health Service drinking water standard. On the other hand, the inoculum of PV at one system escaped in the effluent entering the soil under the seepage bed. Of the 3.3×10^8 counts of poliovirus inoculated into the septic tank, a mean of 70 counts/100 mL escaped from the tank with the effluent and an overall mean of 62 counts/100 mL was transported to ground water (88% transport).
2. Greater likelihood that viruses are encountered in water from wells at a greater distance from the septic tank system than those close to it corresponding with the higher ionic strength closer to the septic system. Viruses appear to be sorbed to the sediment of the unconfined aquifer closer to the septic system due to high ionic strength, and to desorb farther away from the drainfield as a result of reduction in ionic strength.
3. Indicator bacteria (TC, FC, and FS) are not indicators for the presence of viruses; the soil retains the indicator bacteria but not the much smaller virus. Virus contamination of ground water through septic tank leachate results from a well-functioning conventional system.

ACKNOWLEDGEMENT

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