The Use of Microorganisms as Ground-Water Tracers: A Review

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ABSTRACT

Microbial contamination of ground water results in numerous disease outbreaks each year. Tracing their movement in ground water is therefore essential. Bacteria, viruses, yeasts and spores have been used for this purpose and to trace underground movement of water in much the same manner as chemical tracers are used. Chemical tracers do not always reflect the movement of microorganisms in ground water. The use of certain bacteria and animal viruses is undesirable due to their pathogenic potential and difficulties in their differentiation from background, naturally-occurring organisms. Bacterial viruses appear to be the microorganisms most suited as a microbial tracer because of their size, ease of assay and lack of pathogenicity. Bacteriophages have been used to trace ground-water movement over distances of 1,600 meters and can be used under a variety of conditions.

The microbial contamination of ground water is a serious problem that has resulted in large outbreaks of waterborne disease. Almost half of the waterborne disease outbreaks reported every year are due to contaminated ground water. Overflow from septic tanks was responsible for 42% of the reported outbreaks (Craun, 1979). There are many sources of human pathogenic microorganisms which can cause ground-water contamination including leaky sewer lines, sanitary landfills, waste oxidation ponds, land application of waste water, etc. Recent studies at waste-water land application sites have indicated that viruses can travel long distances in the subsurface under the proper conditions (Keswick and Gerba, 1980).

The movement of bacteria and viruses into ground water is influenced by many environmental factors (Gerba *et al.*, 1975) which are difficult to define completely. Thus, the ability to trace microbial movement in ground water is essential in recognizing the potential for transmission of disease-causing microorganisms. Therefore, it is desirable to use tracer techniques to monitor the movement of microorganisms with percolating water through the soil systems and ground water when assessing new sites for land application of waste water, septic tank drainfields, investigation of sources of waterborne disease outbreaks, etc.

Most ground-water tracers used for hydrological and geological studies are chemicals, most often fluorescein dyes or halogen salts. They are used to determine directions and velocities of subsurface flow. Recently this subject has been reviewed by Davis *et al.* (1980). Chemical tracers, however, do not always reflect the movement of microorganisms in the ground water (Rahe *et al.*, 1979; Pyle, 1979; Buchtela *et al.*, 1968). Techniques are needed to predict the movement of suspected sources of ground-water pollution with highly distinctive tracer organisms which can be identified in the ground-water system. The criteria for selecting a suitable tracer organism for ground-water con-

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tamination is the time of survival as well as their retention in soil-water systems. Microbial tracers have the advantage of not being mutagenic (Nestmann *et al.*, 1980), not having potential toxic effects, and having a finite lifetime.

This paper reviews the use of microorganisms as tracers of ground-water movement and makes recommendation for the development and use of such tracers.

TYPES OF MICROBIAL TRACERS

Bacteria

Bacteria are the most commonly used microbial tracers because of their ease of growth and detection. Coliform bacteria and in particular *Escherichia coli* (a fecal coliform) are currently extensively used for monitoring ground-water quality. Coliform bacteria are excreted in large numbers in the fecal wastes of man and other warm-blooded animals and thus can be used to monitor the movement of septic and sewage wastes in ground water. The fecal coliform bacteria are not natural inhabitants of soil and ground water, and can be differentiated easily from common soil microflora by their ability to grow on selective media at elevated temperatures. Several studies (Caldwell, 1937a, 1937b, 1938; Caldwell and Parr, 1937; Reneau and Pettry, 1975; Viraraghavan, 1978; Martin and Noonan, 1977) have looked at the movement of these indicators of fecal pollution at pit latrines, septic fields and sewage disposal sites. Martin and Noonan (1977) traced fecal coliforms at a land disposal site in New Zealand over a distance of 900 m. The bacteria moved at a rate of approximately 150 m/day. Based on this rate of movement and the measured survival time for the bacteria, fecal coliforms were estimated to be traceable for at least 2.5 km from their source. The disadvantage of using fecal organisms as tracers is the difficulty in differentiating between those which derived from the suspected source and those from other sources (Ormerod, 1964). Therefore, some investigations have been conducted by utilizing fecal bacteria with specific characteristics such as a marker which allowed the tracer organisms to be distinguished from all background organisms in the system (Hagedorn *et al.*, 1978; Rahe *et al.*, 1978; Rahe *et al.*, 1979).

Hagedorn *et al.* (1978) studied the potential use of antibiotic-resistant strains of *Escherichia coli* and *Streptococcus faecalis* to monitor movement of subsurface-water flow in one soil series in western Oregon. These organisms are distinguished from naturally-occurring organisms by their ability to grow on media containing antibiotics which suppress the growth of the naturally-occurring organisms. They observed that these microorganisms could travel 500 cm in a septic drainfield under saturated conditions and could survive a 32-day sampling period. It was suggested that these organisms were suitable as indicators of microbial translocation and may be utilized satisfactorily as tracers. Similar experiments were conducted by Rahe *et al.* (1978) using strains of antibiotic-resistant *E. coli* to study the transport of fecal organisms through two western Oregon hillslope soils. The strains of *E. coli* were found to survive in significant numbers in the soils over periods of at least 96 hr and they appeared to be satisfactory as tracers of subsurface-water flow. Recently Rahe *et al.* (1979) reported a comparison study between antibiotic-resistant *E. coli* and a fluorescein dye as tracers of ground-water flow in a soil series in Oregon. The bacteria were recovered at the 45 cm depth from 15 m downslope 1 hr after inoculation. In addition, large numbers of the strains of *E. coli* survived during the 12 hr sampling periods. However, the failure to visually detect the fluorescein dye in ground-water samples raised serious concerns about the continued use of such dyes as ground-water tracers of microorganisms. The results indicated that consideration should be given to more frequent use of marked strains of bacteria as tracers since these organisms more closely represent subsurface movement of microorganisms in soil and ground water.

More recently, a series of tracer experiments were conducted at Canterbury, New Zealand by using a number of different species of bacteria (Sinton, 1980). The results indicated that *Bacillus stearothermophilus* and H_2S-producing strains of *E. coli* traveled over a distance of 920 m in the underlying ground-water system. Both species moved from the injection well at a rate of 200 m/day; however, *Bacillus stearothermophilus* was found to occur naturally in Canterbury soils and ground-water systems so the use of this species as a ground-water tracer was considered to be limited to periods of low rainfall when numbers were low. Furthermore, since both *E. coli* (H_2S+) and *Bacillus stearothermophilus* appeared to be present in raw and treated sewage effluent, they were found to be unsuitable as tracers in sewage polluted water. Tracer experiments were also conducted by injecting a mixture of antibiotic-resistant *E. coli* strains into a well site and monitoring their movements in downstream wells (Sinton, 1979). The rate of movement during this

experiment was found to be 350 m/day. However, this high velocity was considered to be due to the higher than normal ground-water table present during this study rather than the type of tracer. Pyle (1979) investigated aquifer contamination at Heretauga, New Zealand using H_2S-producing strains of *E. coli* together with rhodamine WT dye as ground-water tracers. It appeared that the movement of *E. coli* was faster than that of rhodamine dye. In addition, these bacteria were found to survive for at least 1 month in the ground water.

Other species of bacteria previously indicated to have potential as tracer organisms include *Bacillus chromobacterium, Streptococcus* and *Serratia sp;* the latter produces red pigmented colonies on nutrient agar when incubated at 25-30° C. Fournelle *et al.* (1957) studied the lateral movement of bacteria through shallow ground water near Anchorage, Alaska by introducing various types of bacteria in a well and then sampling for the presence of these bacteria in a series of test wells. The first study was conducted using *Serratia marcescens, Chromobacterium violaceum,* and *Bacillus globigii.* However, none of these bacteria were found to be satisfactory as tracer organisms due to an inability to distinguish these organisms from those naturally present in the soil. In separate studies, *Streptococcus zymogenes (S. liquefaciens)* was demonstrated successfully as a ground-water tracer in terms of its long-term survival and its capability to move in ground water; movement of as much as 15.2 m was observed in 70 days. Antibiotic-resistant strains of *Serratia marcescens* were used by Wimpenny *et al.* (1972) as water tracers in a heavily polluted river. Although the numbers of this tracer organism recovered on antibiotic-containing media were at least tenfold less than on normal media, it was considered to be a reasonable organism for use as a water tracer because it was easily distinguished on agar plates.

Serratia indica has been employed to trace sewage dispersion around a sea outfall (Robson, 1956; Putman *et al.,* 1956) and in a bay of the Oslofjord in Norway (Ormerod, 1964). A faster-growing, antibiotic-resistant strain of *Serratia indica* developed by Robson (1956) was used by Rippon (1963) for tracing water movements in the River Blackwater estuary. Methods for obtaining suitable liquid cultures of *Serratia indica* for transporting mature cultures in the field were described by Ormerod (1964). The field investigation indicated that most of the tracer bacteria were found in a region 6-10 m below the surface of the sea water. Pike *et al.* (1969) have used *Serratia indica* and spores of *Bacillus subtillis var nigar* to trace offshore dispersion of sewage released from submarine outfalls in England. *Bacillus subtillis var nigar* produces black pigmented colonies on media containing tyrosine. The results suggested that the continuous release of *S. indica* into the sea was suitable for assessing the pollution field around a discharge, while adding single doses of either tracer provided information on dispersion processes and minimum transit-times to the shore from a point offshore. It should be noted, however, that caution and concern over dangers inherent in the further use of *Serratia sp.* (Davis *et al.,* 1970; Dobson, 1968; Sattar *et al.,* 1972) for water tracing has been expressed and is well justified (i.e., this organism is potentially pathogenic to man).

Marti *et al.* (1979) studied several bacterial tracers and found significant differences in their travel through the aquifer under study. *Bacillus pomilus* was removed after a travel distance of 45 m. The speed at which *Streptococcus fecalis, Serratia marcescens* as well as a fluorescent dye over a distance of 90 m was found to be 220-240 meters per day. *Bacillus subtilis,* on the other hand, moved at a speed to 150 m per day.

Bacteria have the following advantages as ground-water tracers. They are easy to grow in large numbers and to assay. Further, through the use of various markers they usually are distinguishable from other flora likely to be present in the ground water and the soil. However, care should be taken in the choice of organisms since they could be potential pathogens; even *E. coli* has enterotoxigenic strains which can cause disease in man. Some are capable of growth in the environment, thus producing erroneous results; they are large enough to be filtered out in certain soils; they can adsorb to a variety of surfaces removing them from circulation; and their movement probably does not reflect the movement of other types of microorganisms like viruses. In addition, even with markers like antibiotic resistance it is often difficult to distinguish test organisms from those naturally present because markers can be lost during interaction with natural populations. It also would seem to be imprudent to inject antibiotic resistant microorganisms into ground water, because of the possible transfer of resistance to potential human pathogens especially when the water might be consumed later. The odds of this can be reduced greatly by the use of bacteria which do not carry

the genetic information for antibiotic sensitivity on plasmids. Thus prudence should be used in the selection of bacterial species to be used as tracers and should be used only when experimental conditions dictate or where the movement of ground water is traced back to a source of pollution where organisms are already present.

Yeasts

Limited research has been done on the use of yeasts as ground-water tracers. Wimpenny *et al.* (1972) used two pigmented yeasts to test their ability as water markers in the river Taff, Great Britain. Both yeasts were detected in the middle of the river 2926 m below the dosing point in 60-80 minutes. The selective medium for yeasts described in this study eventually eliminated growth of the natural population of bacteria. However, the authors indicated that yeasts could be used satisfactorily as river markers only if the wild type yeasts in the river were low or absent.

Wood and Ehrlich (1978) performed both laboratory and field tests using a suspension of baker's yeast (*Saccharomyces cerevisiae*) to trace movement of microorganisms in artificially recharged ground water. Laboratory studies showed that yeast cells were able to travel through fine sand at least 60 cm in 30 min, indicating their potential use for tracing microbial movement in aquifer systems. In the field experiments where 35 lbs. of yeast cells were injected into wells, yeast cells were found to penetrate more than 7 meters into a sand and gravel aquifer in less than 48 hours. Results of the field tests also indicated that yeast cells moved through the aquifer faster than bromide and iodide, suggesting the chemical tracers did not represent the movement of yeast cells adequately. In evaluating the use of yeasts it should be noted that the distance over which the yeast cells were followed in these experiments was short and their survival is dependent on nutrients present in the environment. They are also of a much larger size than bacteria and viruses.

Lycopodium Spores

The spores of the club moss *Lycopodium clavatum* have been used primarily by speleologists as tracers to determine subterranean drainage characteristics in limestone karst regions (Gardner and Gray, 1976; Atkinson *et al.*, 1973; Smith and Atkinson, 1974; Aley and Fletcher, 1976). The spores are small cellulose bodies 30 μm in diameter. This technique involves coloring *Lycopodium* spores with biological strains, injecting the dyed spores into the flow system at sinkholes and trapping the spores with plankton nets at potential resurgencies. The spores are then examined under a microscope. A detailed description of the technique has been given by Gardner and Gray (1976). They cited the following advantages of using these spores in karst regions. Their size and density reportedly allow them to move readily with ground water. They are resistant to detrimental factors in the environment and appear to be "harmless" nonpathogens. In addition, they can be stained with five different colors of biological stains permitting their use as multiple tracers.

Atkinson *et al.* (1973) evaluated the performance of *Lycopodium* spores as ground-water tracers in comparison to other tracers in open karst regions and observed that the rate of travel of *Lycopodium* spores were comparable to that of fluorescein dyes. However, results of the tracer experiments conducted by Buchtela *et al.* (1968) showed that *Lycopodium* spores traveled faster than Rhodamine B dye and suggested that a combined use of spores and dyes was necessary to more accurately estimate the movement of underground water.

Although the dyed-spore tracing technique may be suitable for investigating water flow in areas of open ground-water systems, it is considered to be of little value in finer-textured aquifers because of the size of the spores (Aley and Fletcher, 1976). Furthermore, these spores do not share surface properties with those found on pathogenic microorganisms and may be poor indicators of pollution travel even if they are good tracers of water movement. Another major disadvantage is that the dust of the spores is explosive and care must be taken in their handling.

Animal Viruses and Bacteriophages

Animal viruses such as hepatitis and the Norwalk agent are known to be transmitted by contaminated ground water. Other animal viruses pathogenic to man have been isolated from ground water. Because all human enteric viruses are capable of causing disease they cannot be used safely as tracers. To overcome this problem, vaccine strains of poliovirus type 1 LSc has been used as a tracer (Table 2). But the vaccine strain of poliovirus can be responsible for serious disease in man, although the odds are extremely low. Thus, caution must be observed carefully even if the vaccine strain is used. Most animal enteric viruses are not known to infect man. Thus, bovine enterovirus type 1 has been used to trace the movement of viruses from

Table 1. Use of Bacteria, Yeasts and Spores as Tracers

Tracer Microorganism	*Type*	*Maximum Distance of Movement (m)* *Horizontal*	*Vertical*	*Reference*
Fecal coliforms	B	900	15-22	Martin and Noonan, 1977
Bacillus stearothermophilus	B	920	18	Sinton, 1979
Escherichia coli (H_2S+)	B	920	18	Sinton, 1979
E. coli (antibiotic resistant)	B	920	18	Sinton, 1979
E. coli	B	125	5	Pyle, 1979
Total coliforms	B	15	0.6	Viraraghavan, 1978
Fecal coliforms	B	15	0.6	Viraraghavan, 1978
Fecal streptococcus	B	15	0.6	Viraraghavan, 1978
Pseudomonas sp.	B	15	0.6	Viraraghavan, 1978
E. coli (antibiotic resistant)	B	15	1.1	Rahe *et al.*, 1978
E. coli (antibiotic resistant)	B	5	0.75	McCoy and Hagedorn, 1980
E. coli	B	NR	1.3	Glantz and Jacks, 1967
Streptococcus zymogenes	B	15	NR	Fournelle *et al.*, 1957
Bacillus sp.	B	River	River	Wimpenny *et al.*, 1972
Serratia marcescens	B	River	River	Wimpenny *et al.*, 1972
E. coli	B	Sea water	Sea water	Pike *et al.*, 1969
E. coli	B	1	5	Kudryavsteva, 1972
Fecal coliforms	B	5	NR	Johnson and Urie, 1976
Bacillus subtillis	B	900	NR	Marti *et al.*, 1979
Bacillus pumilus	B	<40	NR	Marti *et al.*, 1979
Serratia marcescens	B	90	NR	Marti *et al.*, 1979
Streptococcus faecalis	B	90	NR	Marti *et al.*, 1979
Lycopodium spores	S	NR	NR	Smith and Atkinson, 1974
Saccharomyces cerevisiae	Y	8	8	Wood and Ehrlich, 1978

B = bacteria; S = spores; Y = yeasts; NR = not reported.

septic tank drainfields (Scandura and Sobsey, 1981).

Bacteriophages were first evaluated as water tracers in a river by Wimpenny *et al.* (1972). A lambda-like bacteriophage of *E. coli* K 12 was used and found to be an excellent tracer of water movement especially in polluted rivers. The advantages of using bacteriophages as water tracers were fully discussed by the authors; among these are the following: (1) The phage is nonpathogenic to man and domestic animals; (2) it is specific for its host bacteria; (3) assay is simple and rapid; (4) it has good survival characteristics.

Type T4 bacteriophage, a tailed phage, was used by Fletcher and Meyers (1974) to trace ground-water movement in the carbonate rock terrain in the Ozark region of southern Missouri. The phage was injected on the surface, and was traced in the cave stream approximately 1600 meters from the point of injection site, demonstrating a long potential migration distance for viruses in ground water. The first arrival of the phages was found approximately 16 hours after injection, and the peak of phages passed the analysis site within 24 hours.

Martin and Thomas (1974) employed type 2

Table 2. Viruses Used as Tracers

Virus	*Maximum Distance of Movement (m)* *Horizontal*	*Vertical*	*Reference*
Coliphage T4	1,600	NR	Fletcher and Meyers, 1974
Coliphage ϕ174, T4	900	18	Noonan and McNabb, 1979
Poliovirus, type 1, Coliphage f2	180	18.3	Schaub and Sorber, 1977
Type 2 phage of *Aerobacter aerogenenes*	680	NR	Martin and Thomas, 1974
Bovine Enterovirus, type 1	35	NR	Scandura and Sobsey, 1981
Poliovirus, type 1, LSc	NR	NR	Stramer and Cliver, 1981
Poliovirus (vaccine strain)	<40	NR	Marti *et al.*, 1979

NR = not reported.

phage of *Aerobacter aerogenenes* 243 to follow the movement of shallow ground water in South Wales over a distance of up to 680 m. Ten liters of phage suspension were introduced through piezometers into sandstone strata. Phage was detected in their monitoring wells for 9 days at a distance of at least 680 m yielding a velocity of 1.5-7.5 m/hr (36-180 m/day). They concluded that the use of phage was advantageous where non-polluting substances were required. In light of recent evidence concerning mutagenicity of some tracer dyes (Nestmann *et al.*, 1980) this may be especially significant.

Noonan and McNabb (1979) used T_4 and ϕX 174 phages to trace ground-water movement at a land disposal area in New Zealand. They injected the phages to a depth of 14 m below ground level and followed their movement over a distance of 920 m in 7 days. The coliphage T_4 was observed to travel the 920 m to a monitoring well within approximately 96 hours. The rate of movement was about 300 m/day which was similar to that found by Sinton (1979) using a bacterial tracer.

Coliphage f2 has been used as a tracer virus to examine its accumulation and movement in the soil and ground water beneath a rapid infiltration land application site (Schaub and Sorber, 1977). This study was conducted by continuous addition of stock f2 phage into the waste water over a 7-day application period. Sewage effluent, soil, and ground-water samples were obtained prior to, during, and after dosing of the wastewater for f2 bacteriophage. The tracer virus was found to penetrate into ground water at the same rate as the percolating primary effluent and was observed in an 18.3 m deep observation well beneath the land application site within 48 hours after application. Both pathogenic animal viruses indigenous to sewage and tracer f2 were detected in the ground water at a horizontal distance of 183 m from the application site, indicating the potential use of f2 phage for tracing movement of pathogenic animal viruses.

DISCUSSION AND RECOMMENDATIONS

Properties desirable of a microbial tracer are listed in Table 3. Of all the groups of micro-organisms described, bacteriophages fit these criteria most closely. In addition, because of host specificity, phages can be mixed, injected together, then distinguished on different hosts thereby permitting simultaneous multiple tracers. One drawback is that bacteriophages might be removed by adsorption to the soil. However, it may be possible to develop a strain phage in the laboratory which has poor adsorption properties by genetic selection (Burge and Enkiri, 1978). Research along these lines should include phages which have been shown to be similar to animal viruses in their behavior in soils (Gerba *et al.*, 1981). Methods are available for the concentration of phages from large volumes of water. With the use of these techniques it is possible to detect as little as 1-2 phages in a 20 liter volume of water (Goyal *et al.*, 1980). Phage assay requires 12-24 hours which may be a drawback in some applications, but for most studies would pose no difficulties when samples are collected over long periods of time; furthermore, samples can be frozen for long-term storage and assayed later. Phages are also available which have no known host common in

Table 3. Properties Desirable of a Microbial Tracer

1. The tracer should be nonpathogenic (i.e., not capable of causing disease in man, animals or plants).
2. The tracer should not be present normally in ground water or at least be distinguishable readily from micro-organisms found in water and have stable markers for such identification.
3. The tracer should be stable in the environment for suitable lengths of time.
4. The tracer should not affect the flow of ground water.
5. A tracer should move with the flow of ground water and not be filtered, absorbed or otherwise removed by the travel in ground water.
6. A tracer should be readily assayable in low concentrations by inexpensive methods.
7. A tracer should not interact with other microorganisms in ground water to produce changes in its or the ground-water organisms properties.
8. When it is desirable to use several tracers at one time, they should be readily distinguishable.

Table 4. Characteristics of Microbial Tracers

Organism	*Size (μm)*	*Average Time Required for Assay (days)*	*Essential Equipment Required*
Bacteria	1-10	1-2	incubator[a]
Spores	25-33	½	microscope plankton nets
Yeast	2-3	1-2	incubator[a]
Viruses:			
Animal (enteric)	0.2-0.8	3-5	incubator tissue culture laboratory
Bacterial	0.2-1.0	½-1	incubator[a]

[a] Many may be assayed at room temperature.

the subsurface environment which can support their replication. It may be possible with phages that infect *E. coli* to develop a strain that requires a specific nutrient which is only supplied in the laboratory. This is similar to the situation where vaccine strains of polio virus or other strains which require certain substances for their replication (i.e., guanidine) (Vaughn *et al.*, 1981). For the most part, however, human and animal viruses are not suitable for tracer work because of potential public health problems.

Besides the development of a tracer phage work should continue on low-level virus detection and concentration methods to make the present system (Goyal *et al.*, 1980) more widely applicable. An immunochemical (the use of labeled antibodies) assay has the potential of reducing virus detection to 1-3 hours but requires further development for application to their detection in water. By producing a "standard" phage with defined properties, it could be possible to compare different soil/aquifer systems and to evaluate possible land treatment sites for their suitability.

If other microorganisms can be demonstrated to be free of all potential health hazards like the phages they too may be used as tracers but for now it appears that, although not perfect, bacteriophages can be used to trace the movement of microbial pathogens in ground water.

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