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Pathogen survival in groundwater during artificial recharge

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Abstract Wastewater can be regarded as a valuable resource that can be re-used in various ways, creating more sustainable societies which can benefit both human communities and the environment. An issue when using reclaimed water is the potential presence of health hazards such as microbial pathogens. The presence of pathogens can often impact on the type of water reclamation project selected. Artificial recharge is one of the water re-use options available and has the advantage of storage of reclaimed water, as well as potential improvement of the quality of the stored water by processes within the aquifer. Microbial pathogens have been shown to be removed in aquifers due to the action of a variety of biological and abiotic processes. This paper discusses the activity of the processes on microbial pathogens and the implications for the use of artificial recharge and reclaimed waters.

Key words artificial recharge; groundwater; microbial pathogens; survival; water re-use

INTRODUCTION

Fresh, clean water is a primary requirement for both humans and the environment. Decreasing availability of adequate potable water supplies due to increasing human population centres, drought and global warming, are increasingly placing a strain on both human communities and surrounding environmental systems. Many small and large urban areas extract large amounts of freshwater from the environment and discharge most of this water back into the environment as wastewater. Often these discharged wastewaters, and excess stormwater, are inadequately treated prior to discharge, thus creating a potential hazard from chemical and biological contaminants. Wastewater is starting to be viewed as a valuable resource that can be re-used in various ways within communities, thus creating more sustainable societies with a complete water cycle that can benefit both the local population and the environment.

One method used for recycling water is artificial recharge. Artificial recharge has considerable potential for adding a water source to an aquifer for purposes such as short-term storage, conveyancing of water through an aquifer, achieving water quality improvements, re-pressurizing depleted aquifers, or preventing saline intrusion. Artificial recharge can be potentially used with a variety of water types ranging from potable water, river waters, stormwater, low level wastewaters such as industrial wastewaters, through to treated sewage. The ability to recharge any of these types of water to an aquifer can depend on the availability of that water, a prospective end use for the water, the proposed recharge method, pre-treatment requirements and the characteristics of the receiving aquifer.

ARTIFICIAL RECHARGE METHODS

Water can be actively recharged to an aquifer by a number of different methods. The method of choice depends on aquifer characteristics, depth to groundwater from the ground surface, the presence of confining layers, the availability of large amounts of cheap land for infiltration and the type of water to be recharged to the aquifer.

The simplest method of artificial recharge is spreading water over the ground surface and allowing the water to recharge to a shallow aquifer. This is one of the original recharge methods and is often used in arid regions to capture water released during periodic storm events through the use of a dam or bund, thus enhancing recharge of the water to an aquifer (El Sheikh & Hamdan, 2002; Salajegheh & Keshtkar, 2002). A logical step from simple surface spreading is to create a basin into which water can continuously flow and recharge to an aquifer. This reduces the area required to recharge the water and helps to contain the water. By cycling the water between two or more basins, thus allowing a wetting and drying cycle in each basin, the recharge process can be used to improve the quality of the recharged water using the natural treatment potential of the aquifer system (Fox, 2002). This is commonly called soil aquifer treatment (SAT).

Infiltrating water from the surface has the disadvantage that it requires a large area of land, the recharge rate and destination in the aquifer is difficult to control, and the presence of a confining layer above the target aquifer can prevent surface infiltrated water from reaching the aquifer. When one or more of these obstacles exist, the water can be recharged into a target aquifer using well injection. The water is injected into the aquifer using a dedicated injection well and then recovered either via the injection well (termed Aquifer Storage and Recovery or ASR) (Pyne, 1995) or using a dedicated recovery well downgradient from the injection well. While well injection can place water in a specific location within a target aquifer and beneath confining layers, they are more expensive to establish and operate, as well as being susceptible to problems such as clogging of well screens and aquifer material around the injection well.

PATHOGEN RISK IN WATER USED IN ARTIFICIAL RECHARGE

There are a range of water types that can be used to recharge target aquifers. These can include potable water, river water, captured storm water and treated wastewater. A major issue associated with the recharge of these waters is the chance of pollution, particularly faecal pollution. Faecal pollution of water increases the risk of pollutants harmful to human and livestock health being present in the water. Faecally contaminated water can contain a number of health risk factors, including disinfection-by-products, endocrine disruptors, organic chemicals, and microbial pathogens. It is the microbial pathogens that almost always are the immediate major health risk. Pathogenic microorganisms remain a health risk as long as they persist in aquatic environments. The longer they survive in an environment such as groundwater the greater the chance that they can become mobilized if the chemical, physical or hydraulic conditions are suitable. Increased persistence and survival also increases the chance of their dispersion during use of the recovered water.

It can be assumed that any raw water or wastewater used in an artificial recharge scheme would be treated to some degree prior to being injected into an aquifer. However, microbial pathogens, in particular enteric viruses and bacteria, have been shown to be present in treated sewage and treated reclaimed waters (Aulicino *et al.*, 1996; Rose *et al.*, 1996; Abbaszadegan *et al.*, 1997; Gennaccaro *et al.*, 2003), to contaminate groundwater from sewage treatment and septic systems (Scandura & Sobsey, 1997) and stormwater infiltration (Pitt *et al.*, 1999). Reduction efficiencies of various treatment processes and methods also need to be viewed in perspective when considering the risks of re-using wastewater which has been treated to varying treatment levels (Rose *et al.*, 1996; Fane & Ashbolt, 2000; Gennaccaro *et al.*, 2003). In addition to the potential presence of microbial pathogens in treated effluent, their persistence in the environment once the reclaimed water is used can also be an issue that needs consideration.

It is known that pathogens do decay once released into the environment and that the decay can be influenced by a range of external factors and environmental pressures. Some of the factors influencing the survival of viruses and bacteria in environments such as soil and groundwater are listed in Table 1. Such factors include the environment into which they are added; physical and chemical conditions of the environment; Redox conditions; the type of pathogenic microorganism present; the

Table 1 Survival of viruses and bacteria in soil and groundwater.

Factor	Virus	Bacteria
Moisture content	Increases reduction in drying soils. Reduction rate varies between viral type.	Longer survival in moister soils
Moisture holding capacity	Viral dependant. Some viruses more susceptible to drying.	Survival tends to be less in sandy soils with lower water-holding capacity
Soil type	Adsorption to surfaces can increase survival times.	Clay coatings can inhibit predation and parasitism effects. Adsorption can increase survival times
pH	Indirect effects through adsorption capacity. Most enteric viruses stable between pH 3 and 9	Shorter survival times in acidic soils
Cations	Generally increased cations increases virus survival. The opposite has also been observed.	Increased cations increase adsorption that increases survival rates.
Soluble organics	May protect viral particles from inactivation. Some evidence to suggest may reversibly reduce infectivity.	Increased survival and possible regrowth when sufficient amounts of organic matter are present.
Temperature	Increased temperature decreases virus survival	Lower temperature increases survival rates
Sunlight	Minor influence at the soil surface. Not applicable in groundwater	Lowest survival at the soil surface where the light is most intense. Not applicable in groundwater
Microbial factors	The presence of indigenous microorganisms has been shown to decrease survival times. Survival varies between virus types.	Indigenous microbes tend to out compete introduced microorganisms.
Pathogen type or species	Different viruses vary in their ability to withstand environmental conditions. Survival can vary in different environments.	Varies depending on bacterial physiology, metabolism, spore formation, ability to form biofilms, etc.

Source: Modified from Roper & Marshall (1979), Yates & Yates (1988), Gerba & Bitton (1994).

presence and activity of the indigenous microbial population; the rate of adsorption; and the moisture content. The survival of protozoan and helminth pathogens is less well known. However, it is believed that many of the factors influencing bacterial and viral survival may have similar effects on protozoan cysts and helminth eggs.

Investigation by researchers has also shown that pathogenic microorganisms also have a wide range of survival times in soils and on crop surfaces depending on the environmental conditions (Feachem *et al.*, 1983). Quoted survival times should, however, be taken as "the usual case" only. Much longer survival times in soils and waters have been noted for some microorganisms. Thus, under optimal conditions, some pathogenic microorganisms could survive in soil or water for much longer periods of time than what is considered to be the norm. While it has been shown that a 99.9% reduction in viable pathogenic viruses and bacteria can occur in less than 20 days in soil (Abu-Ashour *et al.*, 1994), it needs to be considered, that with high loadings of microbial pathogens onto soils or sediments, even a 99.9% death/inactivation rate may mean that some pathogens could survive, thus creating a potential health risk. Thus, it must always be considered that microbial pathogens can be present in reclaimed water and therefore present a risk to human health during and after the use of the reclaimed water.

One benefit of artificial recharge over other forms of water re-use is the increased potential for the removal of contaminants such as microbial pathogens present in the injectant through processes present in the aquifer (Dillon *et al.* 1999; Toze *et al.* 2001). While water is treated before being recharged to an aquifer there is always the potential for a small number of microbial pathogens, particularly viruses and protozoa to pass through the treatment system and be present in the treated water. In addition, failure of the treatment plant (e.g. equipment breakdown) is known to occur which has an even greater chance of enabling microbial pathogens to be present in the recharged water. Thus, the storage capacity and treatment ability of aquifers can be used as a critical treatment barrier in water re-use schemes.

FACTORS INFLUENCING MICROBIAL PATHOGEN DECAY IN GROUNDWATER

A compilation of published decay rates, along with the factors implicated in the decay of pathogens in the environment is listed in Table 2. The most common influences on the survival of the microorganisms tested cited were temperature and the type of pathogenic microorganism studied. Other influences mentioned included dissolved oxygen, water chemistry and the source or type of water tested. It was not clear why factors such as dissolved oxygen, water chemistry and water source would have a significant influence on the decay rate of the pathogens. It is possible that they could have had a secondary effect through their influence on the indigenous microorganisms, particularly in relation to viruses that are more resistant to factors such as pH and EC than bacteria. It is unclear how the susceptibility of different pathogens to these factors varies, as the different studies were conducted under a range of concentrations/magnitude. More information is also needed to determine the actual statistical significance of these different controlling factors under both sterile and non-sterile conditions.

Table 2 Published decay rates of microbial pathogens and indicator organisms in groundwater and sediment and the major factors influencing the decay rate.

Author	Organism	Decay rate (day^{-1})	Influencing conditions						Sterile vs non-sterile	Field /Lab.
			Temp	MT	WC	WS	DO	MO		
Yates *et al.* (1985)	Poliovirus 1 (12°C)	0.026–0.125	✓	✓	×	(✓)			NS	Lab.
	Poliovirus 1 (23°C)	0.357 - 0.676	✓	✓	×	(✓)			NS	Lab.
	Echovirus 11 (12°C)	0.054–0.151	✓	✓	×	(✓)			NS	Lab.
	Echovirus 1 (23°C)	0.188–0.628	✓	✓	×	(✓)			NS	Lab.
	Coliphage MS2 (4°C)	0.012–0.064	✓	✓	✓	(✓)			NS	Lab.
	Coliphage MS2 (12°C)	0.030–0.162	✓	✓	✓	(✓)			NS	Lab.
	Coliphage MS2 (23°C)	0.187–0.578	✓	✓	✓	(✓)			NS	Lab.
Yates *et al.* (1990) (different sites)	Poliovirus 1 (nonfiltered)	0.035–0.667	✓	✓	×	✓		×	NS	Lab.
	Poliovirus 1 (filtered)	0.026–0.625	✓	✓	×			×	S	Lab.
	Coliphage MS2 (nonfiltered)	0.030–0.323	✓	✓	×	✓		×	NS	Lab.
	Coliphage MS2 (filtered)	0.028–0.385	✓	✓	×	✓		×	S	Lab.
Yates *et al.* (1990) (single site)	Poliovirus 1 (nonfiltered)	0.161–2.000	✓	✓	×				NS	Lab.
	Poliovirus 1 (filtered)	0.179–0.667	✓	✓	×				S	Lab.
	Coliphage MS2 (nonfiltered)	0.208–1.111	✓	✓	×				NS	Lab.
	Coliphage MS2 (filtered)	0.130–1.429	✓	✓	×				S	Lab.
Jansons *et al.* (1989)	Poliovirus 1	0.030–0.090	✓	✓			✓	(✓)	NS	Field
	Coxsackievirus B5	0.050	✓	✓			✓	(✓)	NS	Field
	Echovirus 6	0.110	✓	✓			✓	(✓)	NS	Field
	Echovirus 11	0.100	✓	✓			✓	(✓)	NS	Field
	Echovirus 24	0.050	✓	✓			✓	(✓)	NS	Field
Nasser & Oman (1999)	Poliovirus	0.003–0.054	✓	✓				✓	NS	Lab.
	Hepatitis A virus	0.009–0.041	✓	✓				✓	NS	Lab.
	Coliphage MS2	0.011–0.021	✓	✓				✓	NS	Lab.
	E. coli	0.017–>0.08	✓	✓				✓	NS	Lab.
Bitton *et al.* (1983)	Poliovirus 1	0.046		✓				✓	NS	Lab.
	Coliphage f2	1.420		✓				✓	NS	Lab.
	E. coli	0.160		✓				✓	NS	Lab.
	Streptococcus	0.030		✓				✓	NS	Lab.

Mazzeo & Ragusa (1989)	Coliphage T2	0.170	✓					NS	Lab.
	E. coli	0.360	✓					NS	Lab.
	Streptococcus faecalis	0.31	✓					NS	Lab.
McFeters (1974)	*E. coli*	0.360		✓				NS	Field
	Faecal Streptococci	0.240		✓				NS	Field
	Salmonella typhimurium	0.220		✓				NS	Field
Keswick *et al.* (1982)	Poliovirus 1	0.210		✓	✓		×	S	Field
	Coxsackievirus	0.190		✓	✓		×	S	Field
	Rotavirus	0.360		✓	✓		×	S	Field
	Coliphage f2	0.390		✓	✓		×	S	Field
	E. coli	0.320		✓	✓		×	S	Field
	Salmonella typhimurium	0.130		✓	✓		×	S	Field
	Faecal Streptococci	0.230		✓	✓		×	S	Field
Nieme (1976)	Coliphage T7	<0.048	✓	✓	✓		✓	NS	Lab.
Davies *et al.* (1995)	*E. coli*	0.107		✓				NS	Field
	Faecal Streptococci	0.012		✓				NS	Field
	Clostridium perfringens	<0.012		✓				NS	Field
Toze & Hanna (2002)	*E. coli*	1.000		✓	✓		✓	NS	Lab.
	Salmonella typhimurium	0.909		✓	✓		✓	NS	Lab.
	Aeromonas hydrophila	0.588		✓	✓		✓	NS	Lab.
	MS2	0.294		✓	✓		✓	NS	Lab.
	Poliovirus	0.370		✓	✓		✓	NS	Lab.
	Coxsackievirus	0.141		✓	✓		✓	NS	Lab.
Gordon & Toze (2003)	*E. coli*	0.909	✓	✓		✓	✓	NS	Lab.
	MS2	0.370	✓	✓		✓	✓	NS	Lab.
	Poliovirus	0.034	✓	✓		✓	✓	NS	Lab.
	Coxsackievirus	0.098	✓	✓		✓	✓	NS	Lab.

Temp, Temperature; MT, Type of microbial pathogen; WC, Water chemistry; WS, Water source; DO, Dissolved oxygen; MO, Indigenous microorganisms.
NS, Non-sterile; S, Sterile.
✓ = Factor linked by author as having an influence on the decay rate; × = factor shown by author to have no influence on the decay rate; ✓ = Factor identified in this article to be potentially a significant influence on the decay rate.

Temperature is clearly the factor that has been identified in the majority of the studies reviewed in this article as having the biggest influence on the decay rate of the microorganisms. It was also inferred by several of these authors that increases in the decay rate associated with increased temperature could be due to an increase in the activity of indigenous groundwater microorganisms (Jansons *et al.*, 1989; Nasser & Oman, 1999; Gordon & Toze, 2003). The majority of the studies listed in Table 2 were carried out under non-sterile conditions. This suggests that the temperature effects on the decay rates noted in the different studies could be due to the influence that the temperature had on the indigenous groundwater microbial population. Similarly, dissolved oxygen and the chemistry of the groundwater could be influencing the activity of the indigenous microorganisms. Gordon & Toze (2003) examined a range of factors that could influence enterovirus decay in groundwater. They determined that the activity of the indigenous groundwater microorganisms was the most significant factor. They also determined that other factors such as temperature, the presence of oxygen and nutrients had a secondary influence on the decay of the viruses by influencing the activity of the groundwater microorganisms. However, in an earlier study that examined the significance of various factors, Yates *et al.* (1990) found that temperature was the only factor that had any significance on the decay of microbial pathogens and indicator organisms in groundwater. They also showed that in the samples they tested, the presence of indigenous groundwater microorganisms did not have any statistically significant influence, in comparison to the decay of the pathogens in sterilized groundwater. Medema & Stuyfzand (2002) studied the removal of various indicator microorganisms during the recharge of potable water through sand dunes and determined that the principal form of removal was not decay of the microorganisms but instead adsorption to the aquifer material.

The combination of different water types during artificial recharge also has the consequence of effectively creating different zones within the aquifer. Often the water being injected is aerobic, thus an aerobic zone is created in the near vicinity of the injection well, with decreasing redox potential out into the aquifer as the oxygen is consumed by abiotic and biological reactions. This would create differing pressures on microbial pathogens introduced into these environments, little of which is well understood. The survival of the microbial pathogens would probably be influenced by temperature variations, oxygen concentrations, and variations in the metabolic activity of the indigenous microbial population, and the presence of biofilm. Other factors such as changes in water chemistry, transport rates and movement through the aquifer material, could also be important in determining the survival potential of different microbial pathogens.

In addition, many artificial recharge schemes have large amounts of microbiological biofilm produced in the aquifer, particularly around the injection well. Microbial pathogens have the potential to interact with biofilm matrix. For example, Quignon *et al.* (1997) found that viruses have a tendency to accumulate in biofilms. The accumulation in biofilms could have a significant impact on the survival potential of the pathogens. Banning *et al.* (2003) found that while *E. coli* could enter biofilms formed in a model aquifer system, their persistence in these biofilms was limited, particularly in comparison with *Pseudomonas aeruginosa*. However, very little other information is available on the interaction of different pathogens with biofilm in

aquifers and the corresponding effect on their survival under different conditions. As large amounts of biofilm can be present in the initial recovered water of artificial recharge methods such as aquifer storage and recovery, it is important that more research is undertaken on biofilm formation and pathogen–biofilm interactions in aquifers.

INFLUENCE OF PHYSICAL AND CHEMICAL CONDITIONS OF AN ENVIRONMENT

The physical conditions of an environment have also been shown to influence the survival of different microbial pathogens. Light at the soil surface has a measurable bactericidal and virucidal effect. The resistance of hepatitis A virus in seawater to UV irradiation was tested by Lévêque *et al.* (1995). They were able to demonstrate that infectious virus particles were no longer detectable after 15 min of irradiation. Sinton *et al.* (1999) found that sunlight had a significant effect on the survival of enteric bacteriophage and bacteria. They found that the enteric bacteriophage had a significantly greater survival than the faecal coliforms. Of course, sunlight has no effect on pathogens once they enter an aquifer. The only time sunlight could have an influence on the survival of pathogens present in water recharged to an aquifer would be either before the water is recharged, for example while stored in a temporary holding basin or in a recharge basin, or after the water has been recovered.

Temperature has been observed to have an affect on the survival of enteric viruses. Blanc & Nasser (1996) observed negligible die-off of viruses (<1 $\log_{10}$ decrease) at 10°C over 20 days. Much greater reduction in viral numbers was observed at 23°C (as high as a 5 $\log_{10}$ decrease) over the same time period. They found that poliovirus and the bacteriophage MS-2 had a much greater reduction in numbers than hepatitis A virus and the bacteriophage PRD-1. Abad *et al.* (1997) found that astrovirus decreased 2 log units more over 90 days when held in dechlorinated drinking water at 20°C than in the same water incubated at 4°C. Gordon (2001) demonstrated that enteroviruses held in non-sterile groundwater at 4°C had negligible decay while these viruses rapidly decayed in the same groundwater held at 28°C. Increasing temperature was also shown to have a significant effect on viral, bacterial and helminth pathogens in dewatered biosolids (Abad *et al.*, 1997).

Oxygen concentrations have also been observed to have an influence on pathogen survival. Both Jansons *et al.* (1989) and Gordon & Toze (2003) found that increased oxygen concentrations reduced the number of infective enteroviruses in groundwater. Gordon & Toze (2003) suggested that the influence of oxygen was due to the effect it had on the activity and population structure of the indigenous groundwater micro-organisms. Walter *et al.* (1995) attempted to isolate cytopathogenic viruses from the water column and sediments of two Austrian rivers. They found that 54% of the water column samples were positive for viruses but viruses could only be recovered from 3% of the sediment samples. They concluded that efficient virus inactivation may be occurring in rivers which are not heavily polluted and carry high oxygen content, probably due to a healthy, active indigenous microbial population in the river sediments.

Microbial pathogens can be quite susceptible to moisture content of soils and the subsurface. Increased virus reduction has also been observed as the surrounding moisture decreases (Gerba & Bitton, 1984). Another study indicated that poliovirus 1

was sensitive to moisture contents between 5% and 25% with the greatest sensitivity at 15% (Hurst *et al.*, 1980). It was surmised that the soil moisture saturation point was the cause of the greatest inactivation of the virus. The influence of the air–water interface has been examined as well. Thompson & Yates (1999) studied the inactivation of several bacteriophages due to interactions at the air-water interface. They demonstrated that the different bacteriophages varied in their inactivation at this interface. They also showed that the ionic strength of the solution and the concentration of surface active compounds in the solution were important in bacteriophage inactivation.

Air–water interface interactions and soil moisture content are of particular importance to pathogen survival in artificial recharge schemes such as soil aquifer treatment and surface infiltration, but are of less significance in an artificial recharge scheme where the water is directly injected into an aquifer. Transportation and adsorption effects of microbial pathogens are much more important in artificial recharge, and a good understanding of the degree of adsorption would be needed to determine what influence, if any, adsorption had on the survival of microbial pathogens in an aquifer.

ADSORPTION AND TRANSPORT IN SOILS AND AQUIFERS

Other non-biological influences on the survival of microorganisms in soils, sediments and the subsurface include adsorption to solid surfaces. An increased ability to adhere to surfaces generally reduces the die-off rates in soils and groundwater for both bacteria and viruses (Gerba & Bitton, 1984; Matthess *et al.*, 1988). Medema & Stuyfzand (2002) determined that removal of indicator microorganisms during infiltration of potable water through a coastal sand dune system was principally due to adsorption of these organisms to the surface of aquifer materials, particularly those coated with iron hydroxides.

For bacteria, adhesion to surfaces increases the ability to obtain nutrients that flow past them. For both bacteria and viruses, attachment allows integration into biofilms that decreases predation effects and other influences such as treatment processes and changes in the surrounding environment. The sorption of bacterial cells to clay has also been demonstrated to be advantageous to their survival. Roper & Marshall (1979) investigated the effect of microbial predators and parasites on *E. coli* cells adsorbed to montmorillonitic clay. They found that the interaction between the bacterial parasite *Bdellovibrio* and the *E. coli* cells was reduced by the presence of the montmorillonitic clay. They also demonstrated that colloidal clay had little effect on the predation of the *E. coli* cells by microbial predators, but that predation was significantly reduced by crude clay. The inference of this study was that clays protect microbial cells by creating a barrier between them and microbial predators and parasites. This would have implications regarding the influence of predation/parasitism on bacterial pathogens in aquifers containing clay materials.

SURVIVAL OF DIFFERENT MICROBIAL PATHOGENS IN THE ENVIRONMENT

Research has shown that the type of microbial pathogen in an environment can have a major influence on the potential microbial pathogen risk. Different microbial

pathogens have different survival potentials in different environments as demonstrated in numerous studies (Table 2). Sobsey *et al.* (1995) compared the survival rates of hepatitis A virus, poliovirus, echovirus and the bacteriophage MS-2 in laboratory columns packed with course sand, loamy sand, clay loam or organic muck. They found, overall, that poliovirus had the largest reduction rate and echovirus the least. However, there was some variation in these results between soil types, with echovirus numbers being reduced more in organic muck than poliovirus. They also determined that increased organic matter and clay content in the soil, as well as increases in the organic content of the pore water, decreased the viral numbers eluted out of the columns. Enriquez *et al.* (1995) compared the survival of adenovirus, poliovirus and hepatitis A virus in different water types. Adenovirus was found to be slightly more resistant than poliovirus in wastewater and significantly greater than both hepatitis A virus and poliovirus in seawater and tap water. In a similar study, Nasser & Oman (1999) studied the inactivation of hepatitis A virus, poliovirus, F^+ bacteriophage and *E. coli* in groundwater and wastewater effluents at various temperatures. They found that the bacteriophage had the longest survival times of the microorganisms tested and *E. coli* had the shortest survival times. Poliovirus and hepatitis A virus had similar inactivation patterns with survival times between those of the bacteriophage and *E. coli*. Jansons *et al.* (1989) found that poliovirus was the most stable of a group of enteroviruses in groundwater under increasing oxygen concentrations, the most sensitive being type 6 strain of echovirus.

Studies of the attenuation of selected microbial pathogens during recharge of treated effluent have confirmed that pathogen type is also a significant factor influencing pathogen attenuation during artificial recharge. Toze & Hanna (2002) found that viruses persisted longer than bacteria in an aquifer receiving tertiary treated effluent using aquifer storage and recovery. It was also determined in this study that differences in attenuation varied between different groups of bacteria and viruses, even between closely related viral species such as poliovirus and coxsackievirus. A similar trend in attenuation rates between different pathogen types was observed in a shallow aquifer receiving secondary treated effluent (Toze *et al.*, 2003).

The apparent differences in resistance to environmental pressures of different microbial pathogens stresses the inherent importance of knowing what type of pathogen could potentially be present in the water used as the injectant. Without this knowledge, it would be virtually impossible to undertake an adequate risk analysis on the potential microbial pathogen contamination of that water source.

INFLUENCE OF NON-PATHOGENIC MICROORGANISMS

It has been determined that the presence of a native population of microorganisms can have a profound negative influence on the survival of introduced microorganisms. Kim & Unno (1996) observed an increase in the rate reduction of recoverable poliovirus numbers in a biological wastewater treatment system as the number of bacteria increased. The number of infectious viral particles removed from solution by the bacterial cells was observed to initially occur rapidly, but then the number of recoverable poliovirus became stable. In contrast, it was observed in the same study that a mixed culture of bacteria and metazoa was able to reduce poliovirus numbers to

below detection limits. The authors determined that the first rapid reduction of viral numbers was due to adsorption of the virus particles to the bacterial cells, while the second reduction was due to the predation of the bacterial cells by the metazoa.

Direct active antiviral activity has also been demonstrated in some bacteria. Hirotani *et al.* (1990) showed that *Rhodopseudomonas capsulata* could produce a compound with antiviral activity that could inactivate over 80% of coliphages in a wastewater sample within 24 h. Cliver & Hermann (1972) found that some strains of coxsackievirus were particularly susceptible to enzymatic degradation. Their results also suggested that the inactivation of the virus was not merely due to the action of proteolytic enzymes, but that a smaller molecular weight compound may also be involved. A similar study by Walker & Toth (2000) examined the effect of bacterial proteases on simian rotavirus. They found that alkaline protease had the most effect and could significantly reduce the titre of infective rotavirus under conditions favouring the activity of the enzyme.

Deng & Cliver (1985) determined that microbial activity in human and animal wastes were at least partially responsible for the inactivation of hepatitis A virus. Jansons *et al.* (1989) also suggested that the presence of microorganisms may have at least some influence on the survival of enterovirus strains in groundwater. Kelly *et al.* (1961) showed that the use of a specific respiratory enzyme inhibitor could reduce the virus-removing potential of activated sludge by 20%. Aeration of the sludge was found to increase the removal of the viruses. These results suggest that the removal of the viral particles was linked to the metabolic activity of the microorganisms present in the sludge. Bogosian *et al.* (1996) demonstrated that the survival of an *E. coli* strain added to water or soil was greatly dependent on the sterility of the soil or water. The seeded *E. coli* cells declined in number much quicker in the non-sterile soils than in the sterile soils. They were able to demonstrate that this decline was due to inactivation of the *E. coli* cells rather than through the induction of a viable—but nonculturable state. The addition of nutrients into sediments and aquifers has been shown to increase the metabolic activity of the indigenous bacterial population (Capuano *et al.*, 1995; Metge *et al.*, 1995). Thus the addition of partially treated wastewater into soil and the subsurface will most likely increase the metabolic activity of the native population. This may well have an increased reductive effect on introduced microorganisms in these environments.

In a series of studies Toze & Hanna (2002), Gordon *et al.* (2002) and Gordon & Toze (2003) demonstrated that the principal factor controlling pathogen decay during artificial recharge at a series of Australian sites was the activity of the indigenous groundwater microorganisms. These studies determined that the absence of the indigenous groundwater microorganisms significantly reduced the decay of bacterial and viral enteric pathogens. Other factors such as temperature, oxygen presence or nutrient concentrations individually had little influence on the decay of the studied pathogens, but appeared to have a secondary influence on pathogen decay during artificial recharge by influencing the activity and population dynamics of the groundwater microorganisms. In an associated study, Gordon (2001) examined the decay of poliovirus and coxsackievirus in groundwater obtained from five very different locations around Australia. He found that location had no significant influence on the decay rate of these two viruses. As it can be expected that the

groundwater microbial populations are different in the various groundwater samples tested, this suggests that either the action undertaken by the groundwater microorganisms on the viruses is similar among a range of bacteria, or that there are enough similar bacteria in the different groundwater samples (e.g. pseudomonads) to cause the decay.

However, the potential for an indigenous microbial population to inactivate introduced microorganisms is a poorly understood process. Some of the reasons for this can be attributed to the difficulties associated with detection, enumeration and identification of different microbial types. This has made it very difficult to directly study the interaction between different microorganisms at a cellular level. It has been suggested that indigenous microorganisms could produce virucidal compounds that can cause viral decay. Ward *et al.* (1986) believed that virucidal action was either closely associated with microbes or a very short-lived compound. Noble & Fuhrman (1997) isolated heat-labile, high molecular weight molecules or colloids up to 0.2 μm in size that were responsible for 20–25% of viral degradation in coastal waters. O'Brien & Newman (1997) also suggested from studies *in situ* that heat labile or possibly volatile inactivating compounds could be responsible for viral decay.

The ability for a virucidal effect to cross the limiting membrane that affected poliovirus but not coxsackievirus suggests that a compound is responsible for the decay. One possibility is that indigenous microorganisms may be influencing decay of the viruses through the production of extracellular enzymes. Cliver & Hermann (1972) investigated the influence of various enzymes on the decay of poliovirus and coxsackievirus and found several protein digesting enzymes resulted in viral decay. Coxsackievirus was found to be more susceptible to protease than poliovirus. They also found that the decay of poliovirus type 1 and coxsackievirus A9, A7 and B1 could occur in cell free extracts from *Pseudomonas aeruginosa*. These cell free extracts were also fractionated using ultrafiltration, and viral decay was observed in fractions containing molecules smaller than 500 molecular weight, suggesting that decay was not merely enzymatic. In another study investigating microbial activity on virus reduction in saturated soils Nasser *et al.* (2002) determined that protease-pronase had a significant effect on Coxsackievirus A9 but no effect on poliovirus, hepatitis A virus or MS2 bacteriophage. They also extracted extracellular enzymes from *P. aeruginosa* and found that this enzyme extract caused a decay of the coxsackievirus and hepatitis A virus but not poliovirus or MS2. Ward *et al.* (1986) found that a considerable amount of viral protein was being broken down and some cleavage of RNA was observed in the presence of indigenous microbes. They also determined that microbes were required for viral decay and any treatment that removed them, i.e. filtering, resulted in a loss of decay. They also demonstrated that the observed viral decay was not the result of predation.

A greater understanding of the processes influencing pathogen decay in environments such as groundwater can be expected to improve due to recent developments in detection methods, and an associated developing understanding of microbial interactions in the environment. This knowledge of pathogen decay is crucial for the determination of risk levels associated with microbial pathogens in the environment as well as in artificial recharge schemes such as ASR and for the efficient and sustainable operation of artificial recharge schemes.

CONCLUSIONS

The multitude of conditions described above influencing the movement and survival of introduced microorganisms in soil and groundwater point to the fact that each site is potentially different. There have been attempts to mathematically model microbial movement and survival in soil and the subsurface (examples are given by Sim & Chrysikopoulos (1999) and Yates & Yates (1988). An extensive review by Schijven & Hassanizadeh (2000) provides much detail on some of the equations and the processes involved in the formation of models for virus removal during passage through soils. While these models can provide some input into the prediction of microbial pathogen risk, much of the data still needs to be determined on a site-by-site basis.

As indicated by this paper, there are a wide range of conditions potentially influencing microbial movement and survival. This is evident from the range of decay rates listed for a number of different enteric organisms in Table 2. The results displayed indicate that there can be a wide variation in the decay rates between the different organisms as well as between different studies studying the same organism, suggesting some influence of local groundwater conditions or some other factor specific to that area or recharge scheme. Thus, the degree of movement and survival of microbial pathogens will always be highly site specific. This means that an assessment of the soil or groundwater conditions and their effect on introduced microbial pathogens will need to be done each time an artificial recharge scheme is being investigated. Artificial recharge schemes, like most water re-use projects, can involve a range of processes that may, or may not relate to the information provided above. It is important that a firm understanding of pathogen fate and behaviour is achieved, particularly where a water source of less than potable quality is used as the injectant. Improved understanding of these processes should lead to reduced costs for analysis of pathogens in water types; a potential reduction in treatment costs; and a wider potential for the use of water types of lesser quality.

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