



ASSESSMENT AND CONTROL
OF PATHOGENS
IN MUNICIPAL WASTEWATER
USED FOR IRRIGATION
TO PROTECT CROPS AND GROUNDWATER

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PROJECT SUMMARY

Population growth and water shortages will increase the need to use treated wastewater effluent for irrigation, particularly in areas where fresh water resources are limited. However, there are serious concerns about the transmission of pathogens and toxic chemicals from municipal and animal wastewater to agricultural land and crops and thus to human food and to groundwater. An increase in foodborne disease in the US has been attributed in part to the transmission of pathogens in the water used for irrigation of edible crops. Furthermore, there is limited knowledge on the long-term effects of irrigation with sewage effluent on soil and underlying groundwater. Thus, the aim of this research project is to assess the microbiological safety of wastewater irrigation of food crops and potential environmental hazards in order to protect the public health and our future groundwater resources. Molecular biology techniques will be used to evaluate pathogen survival, regrowth, and transport in vegetated and non-vegetated soil columns, water distribution systems, and field sites with a long history of wastewater application for crop irrigation. Studies will determine the movement of pathogens through the soil column as well as the factors affecting their survival and transport. This could lead to the development of management strategies that would minimize the introduction of pathogens into the environment and thus reduce the risk to human health.

OBJECTIVES

1. Determine the fate and transport of pathogens present in treated sewage using vegetated (grass and alfalfa) and non-vegetated soil columns irrigated at various efficiencies or flooded to simulate artificial groundwater recharge conditions with chlorinated secondary sewage effluent. The columns will also be used to determine the fate of organic compounds, such as pharmaceuticals and pharmaceutically active chemicals and disinfection by products under a companion project under National Program 201, Water Quality and Management (Wastewater Irrigation and Groundwater Recharge).
2. Determine if wastewater irrigation has an effect on groundwater quality by analyzing upper groundwater samples below agricultural fields, urban irrigated areas (golf courses, parks, landscaping), and/or groundwater recharge areas with a long history of municipal wastewater application for emerging microbial pathogens including but not limited to *Escherichia coli* O157:H7, *Salmonella*, and *Campylobacter*. The samples will also be analyzed for pharmaceuticals and other chemicals under a companion project.
3. Determine if bacterial pathogens present in treated sewage can regrow in conveyance systems used to transport wastewater to fields for irrigation of fresh fruits and vegetables and conduct laboratory studies using a model system to determine the physical and chemical factors that promote/inhibit pathogen regrowth so that cost effective prevention strategies can be developed.

NEED FOR RESEARCH

Description of the problem to be solved

Increasing populations, finite water resources and increasingly stringent treatment requirements for discharge of sewage effluent into surface water is increasing the need for water reuse practices in

the United States. However, due to recent foodborne outbreaks, public concern about the potential human health risks and environmental consequences of water reuse in agriculture is increasing. Thus, research is needed to increase our current knowledge on the long-term effects of wastewater irrigation on food, soil and underlying groundwater. In addition, the potential for pathogen regrowth in conveyance systems used to transport treated wastewater over long distances to the irrigated areas also deserves attention. Furthermore, proper assessment of water reuse practices will require microbial detection methods that are fast, sensitive and specific for pathogens of concern. Addressing these research needs will help assess the environmental and public health risks associated with wastewater irrigation so that future problems of food, soil and groundwater contamination can be anticipated or avoided.

Relevance to ARS National Program Action Plan

The research directly addresses national and global problems dealing with safety of food produced in fields that have been irrigated with treated sewage effluent or with effluent contaminated water. This project falls under National Program 108, Food Safety, Microbial Pathogens Component. The reduction of microbial pathogens in food products also relates to reducing environmental contamination from animal (and human) waste. This project is related to objective 1.6.1.1 "Identify sources and reservoirs of pathogens relative to on-farm and environmental situations" by determining the fate of pathogens in wastewater applied as irrigation to crops.

Potential benefits expected from attaining objectives

Benefits from attaining the objectives include safe use of sewage effluents for irrigation from the standpoint of food safety and groundwater protection. Water reuse will be more common and the practices will be safer for public health.

Anticipated products of the research

Anticipated products of the research include (1) improved techniques of sewage treatment and system management for safe and sustainable water reuse with minimum adverse health effects and in environmentally acceptable ways, and (2) new guidelines for irrigation with wastewater to protect groundwater and surface water quality and for control measures of pathogen regrowth in water distribution systems.

Customers of the research and their involvement

Customers of the research include the public, farmers and farm workers, water planners and managers, government regulators, consulting engineers, water districts and municipalities, wastewater treatment plant operators and water managers.

SCIENTIFIC BACKGROUND

Municipal sewage effluent is becoming an increasingly significant water supply for irrigated agriculture all over the world and could help reduce groundwater pumping as well as conserve water in arid areas. In the United States, increasing demands on the nation's water supplies have led to

reuse of wastewater following treatment (largely for nonpotable uses such as irrigation) particularly in areas with limited water resources. For example, in the Northern Monterey County portion of the Salinas Valley, over 11,850 acres are being irrigated with tertiary wastewater effluent for the production of various crops including artichokes, head lettuce, celery, broccoli, and cauliflower (Israel, 2000). Many of these crops are washed and bagged for raw consumption in many parts of the country. In Bakersfield, California, approximately 5,100 acres of corn, alfalfa, cotton, barley and sugar beets are being irrigated with more than 16.9 million gallons per day of primary and secondary wastewater effluents. In Tallahassee, Florida, approximately 18 million gallons per day of secondary effluent are being pumped through 8.5 miles of pipeline to irrigate 1,729 acres of food crops by spray irrigation. Although the use of wastewater for irrigation is increasing, the risks to public health and the long-term effects on groundwater quality are largely unknown and deserve attention.

New foodborne pathogens are emerging in the U.S. and irrigation water has been identified as a source of contamination (Mead, 1997). During 1999, thirty three percent of the foodborne outbreaks reported were caused by fruits and vegetables such as apple cider, cantaloupe, lettuce and alfalfa sprouts (Prier and Solnick, 2000) that had been irrigated with wastewater or water that had become contaminated. Recent outbreaks of foodborne illness have been associated with produce, including *E. coli* O157:H7 in lettuce (Hilborn et al., 1999) and *Cyclospora* in imported raspberries. These outbreaks together with the increase in wastewater irrigation practices have raised public concern regarding the safety of fresh fruits and vegetables (Meng and Doyle, 1997). In addition, there is growing concern about the long-term effects of wastewater irrigation on groundwater quality. Groundwater contamination with bacterial pathogens can occur by several routes including leaking sewer lines and land discharge by passage through soils and fissures. Groundwater supplies half of the nation's drinking water and accounts for 37% of agricultural irrigation supporting many billions of dollars worth of food production (USEPA, 1999). Thus, it is important to protect our groundwater resources from possible contamination through wastewater irrigation practices and artificial recharge of groundwater. This project will evaluate the safety of these practices to reduce human health risks and ensure groundwater quality.

Municipal wastewater contains a variety of viral and bacterial pathogens (Table 1) that have been excreted in the feces of infected individuals. Some of the most common pathogens found in raw wastewater include *Salmonella* (Asano, 1998), *Mycobacterium*, *Escherichia coli* (Tsai et al., 1993; Grant et al., 1996), and *Campylobacter jejuni* (Jones, 2001). Hence, the use of reclaimed water for crop irrigation requires pretreatment followed by disinfection in order to minimize the risk of disease transmission. However, wastewater treatment only reduces the amounts of pathogens in finished water and does not eradicate the disease agent (Asano, 1998). For example, it has been reported that even though *Campylobacter* can be reduced during treatment to 99.9%, there are 10^{10} *Campylobacters* left in the treated effluent (Arimi et al., 1988). This is of great concern since the infective dose of *Campylobacter* in humans is low (~500 cells). In fact, *Campylobacter* infections exceed those of previously most commonly reported enteric bacterial infections in the U.S. and it is the number one cause of all domestic foodborne illness (Okum, 2000). In addition, *Campylobacters* have been found in vegetables such as spinach, lettuce, and parsley (Kumar et al., 2001). Thus, the potential transmission of infectious disease by pathogenic agents is the most common concern associated with nonpotable reuse of treated municipal wastewater.

Table 1. Some pathogens of concern in municipal wastewater and sewage sludge (U.S. EPA, 1989)

Bacteria	Viruses	Protozoa
<i>Salmonella</i> spp.	Hepatitis A	<i>Cryptosporidium</i>
<i>Shigella</i> spp.	Rotavirus	<i>Giardia lamblia</i>
<i>Campylobacter jejuni</i>	Norwalk Agents	<i>Entamoeba histolytica</i>
<i>Escherichia coli</i>	Coxsackievirus	<i>Balantidium coli</i>
<i>Yersenia</i>	Echovirus	<i>Taxoplasma gondii</i>
<i>Vibrio cholerae</i>	Reovirus	

Wastewater containing pathogens can contaminate crops directly by contact during irrigation or indirectly as a result of soil contact. Therefore, it is essential that we understand the factors affecting the quality of irrigation water as it leaves the treatment plant and during its transport to the fields. Current guidelines for unrestricted irrigation with effluents apply only to the effluent as it is discharged from the sewage treatment plant and not as it leaves the distribution system. Studies have shown that although the water may leave the treatment plant with a uniform level of quality, it has the potential to exhibit a highly variable pattern of water age and quality throughout a distribution system (LeChevallier et al., 1996; Volk et al., 1999). Clogging problems due to microbial growth have been reported for sprinkler and drip irrigation systems using reclaimed wastewater (Asano, 1998). Long residence times of water in a distribution system can have the following negative effects; loss of disinfectant residual, formation of disinfection by-products, growth of biofilm colonies along pipe walls, and the protection and subsequent release of nuisance and pathogenic microorganisms from the biofilm over time (Volk et al., 1999). Thus, there is growing concern about the potential for pathogen regrowth in the water distribution systems particularly where the effluent is transported over long distances to the irrigated areas (mostly with pipelines). A better understanding of the physical, chemical, and biological activities that occur in distribution systems will help minimize the health risks associated with the reuse of wastewater in food production (Raucher, 1996). The USDA's food safety initiative calls for the development of cost effective prevention strategies to reduce the incidence of foodborne illness. Hence, part of this project will involve the study of operational factors affecting pathogen regrowth in conveyance systems to ensure that the quality of reclaimed water is not degraded prior to its use.

Proper assessment of water quality and reuse practices requires microbial detection methods that are fast, sensitive and specific for pathogens of concern since indicator microorganism are not adequate predictors of treatment resistant pathogens. Chlorination is the disinfectant most commonly used in wastewater treatment. It is now known that there are chlorine resistant microorganisms such as viruses (Metcalf et al., 1995) and protozoans (Steiner et al., 1997) that are not killed or inactivated during disinfection. In addition, chlorination can elicit a nutrient starvation and a reversible non-culturable (VBNC) state of coliform bacteria and other pathogens (Rockabrand et al., 1999). This aggravates the limitations posed by conventional cultivation techniques since VBNC pathogens will not grow in laboratory media. Therefore, culture-based methods underestimate the fecal coliform content and do not possess the sensitivity required to detect low numbers of pathogens that exist in the presence of high numbers of indigenous microorganisms in complex environmental samples such as water, manure or biofilms. Also, it is known that less than 1% of the bacterial population

from oligotrophic systems can be cultivated in the laboratory. Staley and Konopka (1985) have described this phenomenon as “the great plate count anomaly”. Bacterial pathogens that are not destroyed during treatment have the potential to multiply and become infective when the conditions become favorable and should not go undetected.

Recent advances in recombinant DNA technology provide new tools to rapidly detect specific pathogens in the environment and thus, overcome the limitations of culture-based methods (Metcalf et al., 1995; Relman, 1998). Molecular techniques allow the direct identification of targeted foodborne pathogens such as *Escherichia coli* O157:H7 (Meng and Doyle, 1997) from environmental samples and can detect bacteria that are in a viable but non-culturable (VBNC) state. These methods have superior sensitivity to that of cell cultivation. PCR methods have the ability to detect up to one bacterial cell in a sample, they can detect the VBNC bacteria, and when amplifying mRNA (by an RNA PCR), they can distinguish live cells from dead cells. However, the ability to detect specific pathogens of concern from environmental samples presents a challenge to environmental microbiologists because of low DNA recovery rates and the presence of inhibitory substances such as humic acids in environmental matrices. The lead scientist has successfully studied environmental microbial communities using PCR and gene probes. In a study conducted in collaboration with New Mexico State University and the U.S. Environmental Protection Agency (ORD/NRMRL/SPRD), shifts in microbial communities were detected in samples taken from a contaminated aquifer. The data demonstrated that the aquifer contained a diverse microbial community, which had not been previously identified using conventional cultivation methods (Duran, submitted). Two of the organisms that were identified by molecular methods were later isolated in pure culture. However, the organism identified as archaeobacteria could not grow on laboratory media possibly because this is a novel organism and not much is known about its metabolism to allow synthesis of proper growth media. Another possible explanation is that this organism is a syntrophic archaeobacteria meaning that it cannot grow without the association of other bacteria and thereby cannot be isolated nor identified by culture-based techniques. The lead scientist also conducted a project in collaboration with the U.S. Environmental Protection Agency and the U.S. Department of Agriculture, Forest Service, to study the effectiveness of a restored riparian buffer zone to attenuate nitrogen inputs from agricultural practices using molecular methodology. Soil samples were collected from the restoration site and DNA was extracted and purified. Denitrifying bacteria were then identified through the detection of the *nirS* and *nirU* type nitrite reductase genes using the PCR technique (Duran, 2001). Thus, once the challenges of DNA isolation and recovery are overcome, PCR techniques and gene probe methodologies can provide a powerful and sensitive option for detection of microorganisms in environmental samples and will be the method of choice for this project.

Other related CRIS projects:

A CRIS search of active projects on animal manure and wastewater irrigation identified 22 projects, of which two are from this research unit. CRIS projects of relevance to this research include a project by the Western Regional Research Center in Albany, CA (#5325-42-000-023-00D) dealing with treatment of animal manure to prevent pathogen transmission and to gain a better understanding of pathogen ecology in agricultural settings. Another related project is being conducted by the U.S. Meat Animal Research Center in Clay Center, NE (#5438-42000-006-00D) dealing with the prevention of zoonotic pathogen transmission from animal manure to human food. Both of these

research projects are similar to my proposed research in that they use a molecular biology approach for the detection and identification of specific pathogens including *Campylobacter* from environmental samples; however, they are addressing potential contamination through animal waste rather than municipal waste used for agriculture. Another related project is being conducted by ARS in Athens, Georgia (#6612-13610-002-09R, “Subsurface transport of *Cryptosporidium* and *Giardia* from grazing lands to drinking water supplies”) to help understand the transport of pathogens in the subsurface to a stream, however it uses polystyrene microsphere in place of the pathogens. A project by the University of California, Riverside, (CRIS #5310-42000-001-02S) includes the fate and transport of pathogenic microorganisms in surface water, groundwater and the atmosphere from animal waste (beef or poultry) products. Also, a CRIS project (5344-42000-013-00D) is being conducted in my research unit as a companion study to my proposed project. This study addresses the fate and transport of organic chemical present in wastewater used for irrigation including endocrine disruptors. In addition, CRIS #4344-42000-13-01S by Arizona State University is being conducted within my laboratory to see if pharmaceutically-active compounds present in wastewater can pose a threat to groundwater quality.

Several CSREES projects were identified that are considered complimentary to the research proposed herein. However, some of these projects may no longer be active. CSREES # 96-35102-3839 “Role of subsurface drainage in transport of *Cryptosporidium parvum* oocysts” conducted by Cornell University, Ithaca, NY addresses the transport of *Cryptosporidium* in the subsurface through preferential flow paths in the soil. CSREES #ARZT-319650-G-21-512 “Role of irrigation water in contamination of imported and domestic fresh food” is a project using wastewater irrigation conducted by the University of Arizona, Tucson. The irrigation waters from canals used for crop irrigation were assessed for the presence of pathogens, however the impact of irrigation on groundwater quality was not addressed. CSREES #PEN03571 “Wastewater irrigated forests for timber and wildlife” conducted by Pennsylvania State University uses municipal wastewater as irrigation to study abundance and distribution of plant communities.

APPROACH AND PROCEDURES

Experimental Design

Objective 1 - Soil Column Studies

The main purpose of this study is to assess the safety of water reuse practices, mostly for urban and agricultural irrigation, so that the risk to human health and groundwater contamination are minimized. Ten soil columns in 8 ft long x 1 ft wide stainless steel pipes were set up in a greenhouse at the U.S. Water Conservation Laboratory to study the movement of pathogens in systems involving irrigation with sewage effluent, artificial recharge with sewage effluent, and recharge and irrigation with Colorado River water. The columns were filled with a sandy loam from the McMicken Flood Control reservoir northwest of the City of Surprise. This is a desert soil in the Mohall-Laveen Association that has had no agricultural use. The hydraulic conductivity of the soil was determined with a laboratory permeameter test as 280 mm/day, using a disturbed sample. To avoid particle segregation, the soil was placed in the columns in air-dry condition followed by compaction with a rod. The effects of different infiltration rates and recharge conditions on the survival and transport of pathogens and effects on groundwater quality will be determined in the soil

columns. Because of space and other physical limitations, only ten columns could be set up. Thus, the schedule in Table 2 was developed so as to include as many different treatments as possible, including different crop and soil conditions (legume, non-legume, and bare soil), different modes of water application (irrigation and recharge), different irrigation efficiencies, and different sources of water (effluent and Colorado River water). Initially, there will be no replications since variability issues theoretically do not exist in these engineered columns. In addition, this orientation-type study will permit us to maximize the use of the columns and obtain the maximum information that will allow the observation of trends, particularly with regard to the different irrigation efficiencies, which have not been previously published. However, depending on the results, replicate treatments may be used in the future to firm up some of the conclusions. The irrigation efficiencies in Table 2 will be determined as ET divided by amount of water applied and expressed as a percentage. ET will be calculated from the weight loss of the column as measured with load cells on which the columns are resting. Estimates of irrigation efficiency will also be obtained from EC values of irrigation water and leachate.

Table 2. Schedule of irrigation and recharge studies for soil columns in the greenhouse

Column #	Cover	Irrigation Efficiency	Water Source
1	grass	50%	Effluent
2	grass	70%	Effluent
3	grass	90%	Effluent
4	alfalfa	50%	Effluent
5	alfalfa	70%	Effluent
6	alfalfa	90%	Effluent
7	bare soil	70%	Effluent
8	grass	70%	Colorado River Water
9	bare soil	recharge mode	Effluent
10	bare soil	recharge mode	Colorado River Water

The sewage effluent to be used in the column studies will be representative of typical wastewater treatment for non-potable uses such as irrigation of crops, parks, playgrounds, golf courses and residential yards. The effluent should have, as a minimum, primary and secondary treatment followed by chlorination. Also the effluent should primarily be of residential origin with not much industrial input. The Goodyear and Tolleson wastewater treatment plants in Arizona meet these requirements. However, since the soil columns will also be used in a companion project to study the transport of organic chemicals including pharmaceuticals, sewage effluents from both wastewater treatment plants were collected and sent to Dr. David L. Sedlak's laboratory of the Civil and Environmental Engineering Department at the University of California at Berkeley for analysis of pharmaceuticals. The results demonstrated that very low concentrations of pharmaceuticals were present in the Goodyear effluent while the Tolleson effluent contained concentrations that were more in line with typical values (Table 3). Hence, sewage effluent from the Tolleson wastewater treatment plant will be used in the column studies. Colorado River water will also be used as a comparison to wastewater irrigation.

Table 3. Concentrations of selected pharmaceuticals in the Goodyear and Tolleson effluent

Pharmaceuticals	Concentrations	
	Goodyear	Tolleson
Ibuprofen	17 ng/L	247 ng/L
Naproxen	22 ng/L	699 ng/L
Indomethacine	<3 ng/L	55 ng/L
Metoprolol	20 ng/L	133 ng/L

The sewage effluent and drainage water from the columns will be analyzed for selected pathogens including but not limited to *E. coli* O157:H7, *Campylobacter*, and *Salmonella*. Once we determine which pathogens present the greatest risk to groundwater, the columns will be sampled at different depths including the root zone of vegetated columns to obtain detailed pathogen transport data. All microbiological analysis will be carried out by polymerase chain reaction (PCR) technology and/or gene probe methodology using published detection sequences specific for the pathogens of concern.

Sampling of sewage effluent and DNA extraction will be carried out using a protocol by Smalla (1995) with minor modifications. All water samples will be concentrated in Sterivex filters (Millipore) and DNA extraction will take place inside the filters followed by ethanol precipitation. Preliminary results for wastewater sampling and DNA recovery have resulted in good yield of high quality DNA as determined by spectral analysis (Table 4). DNA extraction from soil and root samples will be carried out using a modified bead-mill procedure (More et al., 1994; Van Elsas and Smalla, 1995). All community DNA will be purified using a glass milk purification kit (Bio 101) and will be subjected to PCR tests to assess the presence of specific pathogens using published primer sequences (Table 5). Real-time PCR will be performed using a GeneAmp 5700 (Perkin Elmer) and should allow for the enumeration of pathogens originally present in the sample. This information together with the infective dose (Kowal, 1985) of the organisms can be valuable for determining if the pathogens are present in significant numbers to cause a human health risk. All procedures will be optimized in the laboratory using DNA from stock organisms that will be purchased from the American Type Culture Collection (ATCC). Positive and negative controls will be included in each sample analysis. The positive control will contain DNA from the ATCC stock organisms and the negative control will contain distilled water. General PCR practices will be followed to avoid contamination of the samples and non-specific amplification (Griffin and Griffin, 1994). Separate areas in the laboratory will be devoted to sample processing, nucleic acid purification, PCR mixture preparation, and examination of PCR products. All laboratory surfaces and equipment will be cleaned with 90% ethanol and/or 10% bleach. In addition, aerosol filter pipette tips will be used. If non-specific amplifications (false positives) are observed through the dissociation profiles or upon agarose gel electrophoresis, the AmpErase uracil-N-glycosylase (UNG) (Applied Biosystems) will be used to remove previously amplified copies and prevent reamplification of carryover PCR products. When gene probes are used, the Southern blot procedure will be followed as detailed in Ausubel et al. (1999). Southern blotting is a technique for identifying a DNA sequence by providing a chemiluminescent complimentary probe that binds to it. Thus, hybridization and colorimetric detection of bound probe will be performed using the DIG Nucleic Acid Detection Kit (Roche).

Table 4. DNA yield from wastewater effluent samples and soil column drainage

Sample	DNA concentration
Tolleson effluent	58.5 µg/ml
Sewage effluent from top of the column	24.9 µg/ml
Sewage effluent from bottom of the column	34.9 µg/ml

Table 5. List of pathogens and their PCR-target sites

Pathogens	PCR-Target sites	References
<i>E. coli</i> O157:H7	Shiga-like toxin gene (<i>stxI-stxII</i>) <i>eaeA</i> gene	Gannon et al., 1997 Oberst et al., 1998
<i>Campylobacter jejuni</i> 16S rRNA gene	Flagellin gene (<i>flaA-flaB</i>) Waage et al., 1999	Kirk and Rowe, 1994
<i>Salmonella</i>	phoP, H-li and Hin genes	Way et al., 1993 Wang, et al., 1997

Contingencies

This research relies on cooperation with the City of Tolleson to obtain wastewater. If this falls through, another source of wastewater can easily be found. Arrangements were made with the Central Arizona Water Conservation District to obtain Colorado River water from the Central Arizona Project (CAP) Aqueduct at a point where the canal has 100% Colorado River water through a companion project, if this falls through, we can collect water from the Colorado River or change to a different water source.

Collaborations

Necessary (within ARS); collaboration with research hydraulic engineer, Herman Bouwer, at the U.S. Water Conservation Laboratory is required for the set up and operation of the columns, as described in his Project Plan under National Program 201.

Objective 2 - Field Studies

Studies to investigate the potential detrimental effects of animal wastes released into the environment on groundwater quality have recently been conducted. However, the effects of municipal wastewater irrigation on underlying groundwater quality have not been addressed and deserve attention. For example, Cho and Kim (2000) have studied the effects of livestock wastewater on underlying groundwater quality and found that the increase in bacterial-community diversity in the contaminated aquifer was probably due to the infiltration of livestock wastewater.

Also, Chee-Sanford et al. (2001) examined two swine farm lagoons and found the presence of tetracycline resistance determinants in the underlying groundwater, which could be detected as far as 250 meters downstream from the lagoons. Thus, the purpose of this objective will be to evaluate laboratory-derived results under natural field conditions in order to better understand microbial transport behavior in the subsurface and evaluate the effects of wastewater irrigation on groundwater quality. It is imperative that we combine both laboratory and field studies because although column studies provide a greater degree of control, they cannot account for the many factors that control microbial mobility in situ such as hydrological characteristics of the aquifer, preferential flow, predation, motility, lysis, changes in cell size in response to alterations in nutrient conditions, spore formation, and attachment to soil surfaces. Sites will be selected on the basis of depth to groundwater (preferably shallow), availability of wells that pump primarily upper groundwater, and cooperation with farmers, irrigation districts, and municipalities. Two potential field sites in Phoenix, Arizona, are currently being evaluated based on the criteria described above. One of these sites is the Buckeye Irrigation District, which uses approximately 30,000 acre-feet per year of treated sewage effluent produced by the 91st Avenue Wastewater Treatment Plant for the irrigation of crops. The other site is the Roosevelt Irrigation District, which uses 33,000 acre-feet per year of treated sewage effluent produced by the 23rd Avenue Wastewater Treatment Plant for the irrigation of edible food crops such as vegetables and melons. Experiments will be designed to determine if there are significant differences in the microbiological quality of groundwater at existing groundwater recharge sites and below agricultural fields where wastewater irrigation has been conducted over long periods of time compared to selected control sites. Monitoring wells previously installed by the USGS to sample for pesticides and industrial chemicals will be evaluated for the use of microbiological sampling. At least two to three well volumes will be flushed prior to collecting groundwater samples (in duplicate) for microbiological and geochemical analysis. Care will be taken to conserve the integrity of the samples collected and to prevent microbial contamination during sampling. Autoclaved glass bottles will be used and filled all the way to the top to avoid any headspace. The groundwater samples will be chilled immediately after collection and will be transported to the laboratory on ice where they will undergo analysis for microbial pathogens using procedures described for sewage effluent in objective 1. Geochemical measurements will also be conducted. In the event that we require concentration of bacteria prior to nucleic acid analyses, we will use a hollow-fiber, tangential-flow filtration system described by Kuwabara and Harvey (1990).

Contingencies

Arrangements will be made with irrigation districts, municipalities, and landowners to permit sampling water from existing wells. There is enough interest in the effect of sewage irrigation on groundwater quality that adequate cooperation should not be a problem.

Collaboration

- Necessary (within ARS); collaboration with research hydraulic engineer, Herman Bouwer, at the U.S. Water Conservation Laboratory is required for site characterization and selection of field sites.

- Necessary (external to ARS); collaboration with the U.S. Geological Survey (USGS) is being developed to use their existing monitoring wells at various field sites through a companion project.

Objective 3 - Pathogen Regrowth

Research projects on pathogen regrowth have been conducted primarily for drinking water distribution systems (Le Chavallier et al, 1996). However, the potential for pathogen regrowth in conveyance systems used to transport sewage effluent from wastewater treatment plants to agricultural fields has not been addressed, possibly because this is a growing practice and it has not been given much thought. Thus, our objective will be to conduct field and laboratory experiments to study pathogen regrowth in wastewater distribution systems and to evaluate the factors that lead to deterioration of water quality during its transport. In addition, recommendations for the control of wastewater quality in the distribution systems will be made in order to avoid possible direct contamination of crops. Initially, we will examine only one field site but we are hopeful that this project will generate interest among the research community and regulatory agencies to expand this project in the future to evaluate other sites. Several factors will be considered in the selection of our field site, including the length of the conveyance system (>5 miles), types of crops produced (preferably fresh vegetables), and accessibility of monitoring data and sampling sites. Field data will be collected and recorded at the time of each sampling (once a week) and will include the plant's effluent production, water temperature, turbidity, pH, and disinfectant residual. Water samples (in triplicate) will also be collected from three different locations, at the plant effluent, at midpoint in the distribution system, and at the end of the distribution system. The water samples will be transported on ice to the laboratory where they will be subjected to molecular characterization of bacterial pathogens following DNA extraction and PCR procedures described in objective 1. In addition, total coliform and *E. coli* enumerations will be performed using the membrane filter method with m-Endo media following standard procedures (AWWA, 1999). Data from this study will be used to evaluate whether the number of bacteria (pathogens) in the effluent increases as it is transported from the treatment plant to the irrigated fields and to examine if there is a relationship between pathogen regrowth and any chemical, physical or biological parameters.

The purpose of the laboratory studies will be to investigate the chemical, physical and operational factors that influence the regrowth of pathogens in a distribution system through use of a bench scale model (annular reactor, BioSurface Technologies Corp.). We have chosen to use of an annular reactor as a model because it is a simple and economic simulated distribution system that has become the system of choice for monitoring biological fouling, regrowth, and biocorrosion in drinking water systems at bench scale (Camper et al., 1998; Characklis et al, 1998). In addition, its operational controls will allow us to model parameters observed in our real-time distribution system while carrying out experiments under controlled conditions that are difficult to control in the field. The annular reactor contains an inner cylinder whose rotational speed can be adjusted to provide liquid/surface shear and transport conditions similar to that of real-time water distribution systems. It also permits the control of temperature and residence time of the water and is suitable for work with pathogenic microorganisms. In this study, we will monitor water quality and bacterial regrowth by sampling the annular reactor's influent and effluent using sterile tubes. Sampling will take place weekly for approximately 8 weeks to allow for biofilm formation. The samples collected will undergo the same type of analysis as for the field samples. In addition, the annular reactor contains

detachable coupons (slides), which will permit the monitoring of coliforms and selected pathogens that may be present in the biofilms throughout the experiment. Two slides will be removed from the reactor and replaced with sterile slides to determine reproducibility of results. A series of experiments will be carried out to test the effects of temperature, nutrient levels (carbon and phosphorus availability), and/or disinfection residual on water quality. Information from this study will be used to make recommendations for sewage treatment and conveyance strategies that use parameters observed in the field so that they can be applicable to the real-time distribution system under study. Overall, this research will help ensure the absence of pathogens in irrigation water and hence, safety of the food produced.

Contingencies

The research relies on cooperation with local municipalities to obtain wastewater. Arrangements will be made with wastewater irrigation projects to permit sampling water from conveyance systems and provide treatment plant monitoring data.

Collaborations

Necessary (within ARS): collaboration with research hydraulic engineer, Herman Bouwer, at the U.S. Water Conservation Laboratory is required for site characterization and selection of field sites.

Necessary (external to ARS): none

Physical and Human Resources:

The wastewater irrigation group addressing the microbial aspects of the study consists of a microbiologist (100%) and one technician. Physical resources include 300 sq feet of lab space for the microbiologist and a dedicated greenhouse for the soil columns. The microbiology laboratory is equipped with a 5700 GeneAmp (PE Appliedbiosystems) for real-time PCR and an imaging system (Biorad) for chemiluminescent detection of gene probe hybridizations. There is also general laboratory support including a water quality chemistry lab, a soils lab, and a machine shop. Support for the column studies is also provided by a research hydraulic engineer in a related project and additional labor through a cooperative agreement with Arizona State University. Field facilities include municipal sewage treatment plants and sewage irrigated fields in Arizona and California in addition to shallow wells for sampling the upper groundwater in sewage irrigated areas west of Phoenix, Arizona.

MILESTONES AND OUTCOMES

By the end of FY2002, the initial screening of pathogens in sewage and column effluents will be completed and should determine the presence of specific pathogens of highest concern to groundwater contamination. By the end of FY2003, we expect results regarding the fate and transport of pathogens from field studies as well as the completion of pathogen regrowth assessment in distribution systems. Studies on the effects of irrigation and groundwater recharge with sewage effluent will continue until dynamic equilibrium or end conditions are reached. If adverse effects are observed, procedures for mitigating these effects will be developed and tested on the columns by FY2004 (Table 5).

Table 5. Milestones and outcomes

Research Study-Component	Months of Study			
	11	22	33	44
Pathogen Transport/ Column Studies	Operation and management for irrigation and groundwater recharge procedures, development of sampling and DNA extraction protocols completed	Operation continued, establish PCR procedures for selected pathogens, initial screening of pathogens going into and out of the columns completed	Operation continued, evaluation of fate and transport of pathogens completed	Final reports and manuscript prepared, develop recommendations and future studies
Pathogen Transport/ Field Studies	Site characterization and sample collection completed	Optimization of DNA extraction and analysis protocols completed	Detail sampling to evaluate fate of selected pathogen(s), analysis by PCR completed	Interpretation of results, final reports and manuscript prepared, develop recommendations for future studies
Pathogen Regrowth/ Laboratory and Field Studies	Field site characterization, operation and management of annular reactor completed	Operation and sampling of annular reactor continued, sampling at different points in the water distribution completed	Molecular analysis of samples completed	Interpretation of results, final reports and manuscript prepared, develop recommendations for the control of pathogen regrowth

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