MICROBIOLOGICAL WATER QUALITY IN IRRIGATION WATER, TREATED WASTEWATER, AND UNTREATED WASTEWATER AND ITS IMPACT ON VEGETABLES IN SONORA, MEXICO

by

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As members of the Dissertation Committee, we certify that we have read the dissertation prepared by Pablo Gortáres-Moroyoqui entitled Microbiological Water Quality in Irrigation Water, Treated Wastewater, and Untreated Wastewater and Its Impact on Vegetables in Sonora, México and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy

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DEDICATION

This work is dedicated with much love to my family: Eva Luz (my wife), Juan Pablo (my son), and America (my daughter). Also, this work is dedicated to my parents. Margarita (my mother) and in the memory of Francisco (my father)

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ABSTRACT

In México, wastewater recycling is an important alternative source of water, particularly in arid regions like the state of Sonora, México. In El Valle del Yaqui, Sonora, México, where 500 million m^3 per year of wastewater is available for recycling in agriculture activities . The main objective of the three studies presented in this dissertation was to assess the microbial water quality of surface water, untreated wastewater, and treated wastewater for produce irrigation, as well as the impact of microbial water quality on vegetable contamination. The results suggest that the three sources of water meet Mexican and international guidelines for use in production of food crops.

Despite wide differences in the concentration of bacterial indicators (*Escherichia coli, Clostridium perfringens*) and enteric pathogens (*Cryptosporidium, Giardia*, and enteroviruses) in the various types of water studied there was little impact on contamination of the produce studied (carrots, lettuce, tomatoes, and peppers) at harvest. Apparently, the time between the last irrigation event and harvesting was sufficient to allow for die-off of enteric organisms that may have contaminated the produce.

INTRODUCTION

Problem Definition

El Valle del Yaqui has been a point of interest for many people either to invest, to work, or to live, particularly due to its agricultural development. El Valle del Yaqui is an integration of five counties --Bacum, Guaymas, Navojoa, Etchojoa, and Cajeme-- with a population around 700,000. El Valle del Yaqui is widely known as one of the most modern regions in Mexico in terms its irrigation system. El Valle del Yaqui (27^{o}N, 100^{o}W) is in the state of Sonora in north-western Mexico. The coastal plain, which is approximately 60 Km wide, is between the Gulf of California (west) and the foothills of the Sierra Madre Occidental (east), some of which can reach heights of 1,500 m. The major part, approximately 225, 000 ha, of El Valle del Yaqui is occupied by an irrigation district fed by the Alvaro Obregón Dam, which has a capacity of 3,000 x10^{6} m^{3}.

The irrigation water requirement for all agriculture (225,000 ha) is around 2.5 million cubic meter per year. However, during the last ten years severe problems were experienced in finding enough irrigation water due to the drought conditions. Reuse of treated wastewater could aid in meeting the demand for agriculture.

In México, wastewater recycling is an important alternative source of water, particularly in arid regions like the state of Sonora. In El Valle del Yaqui, Sonora, México, where 500 million m^{3} of wastewater per year are discharged including agricultural, industrial, and municipal. Recycling treated wastewater could be an excellent alternative to meet water demand for agriculture requirements. Since 1997, two wastewater treatment plants have been operating in Cd. Obregón, Sonora, which is the

main city in el Valle del Yaqui. The treatment capacity of these plants is around 1,500 liters per second (47.3 millions of m^3 per year).

Although, a number of studies have been carried out to evaluate water quality, treatment and reuse of irrigation wastewater and sewage (Gortáres, 1992; Gortáres, 1993; Gortáres, 1997), investigations have been focused on the detection of indicators and not pathogens. The purpose of this study was to better assess the quality of existing irrigation water and compare it to reclaimed wastewater. The potential of pathogens in the wastewater to contaminate crops was also assessed.

Literature Review

Water scarcity, irrigation water and water quality

Population growth has resulted in increased water demand and greater contamination of water from the disposal of waters in many parts of the world, including Mexico . In many areas the use of water is limited by its quality rather than by the quantity available (Maidment, 1993). According to Brooks *et al.* (1997) water quality involves a long list of individual components and chemical constituents. These authors claim that a water quality standard refers to the physical, chemical, or biological characteristics or properties of water in relation to a specified use related to physical, chemical and biological properties of waters. Changes in water quality due to watershed use can make water unusable for drinking but can be acceptable for fisheries, irrigation, or other uses. The pollution of water is a term generally used when the water quality is degraded or defiled in some way by human activities. However, water quality

degradation also results from natural events, such a large rainstorms, fires, or volcanic eruptions (Brooks *et al.*, 1997). This degradation either can be caused by point pollution or non-point pollution. Point pollution is a regulatory term meaning a source that discharges through a pipe at a known location, such as from industries and municipalities. While non-point pollution refer to pollution that occurs over a wide area and usually is associated with land use activities such as agricultural cultivation, grazing of livestock, and forest management practices (Brooks, *et al.,* 1997; Maidment, 1993; Metcalf and Eddy, 1994).

Water quality is important not only because of its linkage to the suitability of water for various uses and its impact on public health, but also because water quality has an intrinsic value. The quality of life is often judged on the availability of pristine waters. The availability of good and clear water is also a public health issue. Many human diseases, especially those causing cholera, diarrhea, and others are serious problems in the world, including United States where disease outbreaks have occurred (Maidment, 1993).

Microbiological water quality and waterborne diseases.

A waterborne disease is an illness caused by the ingestion of water contaminated by human or animal feces or urine containing pathogenic microorganisms. The elimination of these microorganisms has been realized by filtration and disinfection of the water supplies. These approaches have been practiced since the nineteenth century resulting in a dramatic decrease in the incidence of waterborne disease such as typhoid

fever and cholera. Until the late 1960s, it was thought that threats from waterborne disease had been controled in developing countries by proper water treatment. However, some waterborne diseases related to viruses and protozoa were detected, suggesting that these agents are more resistant to disinfection than enteric bacteria (Percival *et al.*, 2000)

Waterborne diseases are among the three major causes of illnesses and death in the world, such as cholera, hepatitis, giardiasis, and others. In developing countries, like many Latin American and Caribbean countries, acute diarrheas, not including typhoid fever, hepatitis and others, are among the ten major causes of illnesses resulting in thousands of deaths every year. Furthermore, a study carried out by the World Health Organization (WHO) established that in 1988 there was an average of 4.6 events of diarrhea per year in each child under five years in American countries (Cañez and del Puerto, 1992). It was also estimated that, in 1996, every eight seconds a child died from a water-related disease and each year more than 5 million people died from illness linked to unsafe drinking water or inadequate sanitation (Percival *et al.*, 2000). This increase in the number of cases of waterborne diseases during the last 30 years has been due to the apparition of both new and old agents, which are refereed to as emerging and re-emerging pathogens, respectively (Forrest and Gushulak, 1997; Miller *et al.* 1998)

The main disease-causing microorganisms in drinking water, water used in preparing food, or food that has been in contact with contaminated water include: bacteria, viruses, and parasites. Although these microorganisms can cause an amply variety of diseases, those diseases of special concern in developing countries are acute diarrhea, cholera, typhoid fever, and infectious hepatitis (Cañez and del Puerto, 1992;

Bitton, 1994). Sobsey *et al.* (1993) affirm that many waterborne outbreaks of gastroenteritis are documented ever year. However, no etiology has been found for approximately half of them, but it is suspected that many are of human viral origin. Many human viruses may infect the gastrointestinal tract and can be excreted into the environment. Once in the environment, these enteric viruses may reach water supplies, recreational waters, crops, and shellfish, through sewage, land runoff, solid waste, landfills, and septic tanks. The enteric viruses in the environment create a public health risk because they are transmitted via the fecal-oral route through contaminated water and low numbers are able to initiate infection in humans. Diseases caused by enteric viruses range from trivial to sever, or even fatal (Rose, 1986)

The enteric viruses include: rotaviruses, Norwalk and Norwalk-like viruses, adenoviruses, reoviruses, hepatitis A virus, and enteroviruses. The enteroviruses are the enteric viruses most commonly detected in polluted water. The enteroviruses such as polioviruses, coxsackie A and B viruses, and echoviruses can cause a variety of illness ranging from gastroenteritis to myocarditis and aseptic meningitis (Abbazadegan *et al.*, 1999). Abbazadegan *et al.* (1999) affirm that numerous studies have documented the presence of enteroviruses in raw and treated drinking water, wastewater, and sludge.

Rose (1986) states that the concern in regards to viruses is due five reasons: (1) scarce information concerning the occurrence and significance of viruses such as the Norwalk viruses because methods are not available to grow and study them; (2) currently available methods may recover less than 50 % of the viruses present; (3) studies suggest that enteric viruses can survive longer than bacteria in the environment and are not as efficiently removed by conventional treatment processes; (4) because of the low infectious dose, if even one virus particle is ingested by a susceptible individual, it could cause disease; and (5) the present bacterial indicator system used to evaluate fecal pollution is not always reflective of the presence of these viruses. Although some of these factors have been addressed in the United States, in México all of these are currently important.

Water regulations in the United States and Latin America

Since 1974 there have been national regulations for drinking water in the United States (U.S.). The Environmental Protection Agency is the federal authority in charge to develop and enforce environmental regulations in the U.S. In 1893, the Interstate Quarantine Act was developed to establish drinking water in the U.S. This Act resulted in the creation of the first water-related regulation in 1912. This regulation prohibited the use the common drinking cup on interstate carriers. But later it was understood that even when the cup was considered clean, the quality of the water to be pour into was more important. This gave rise to the first bacterial standard in 1914 and was the framework use by federal,

state, and municipal water facilities from 1914 to 1975 ensuring the safety of water for community water systems (Pepper *et al.*, 1996).

In recent years, the finding of enteric viruses and protozoan parasites in water gave rise to the Information Collection Rule that requires monitoring of these microorganisms in drinking water supplies from surface sources serving 100,000 people or more for a limited time to asses the adequacy of treatment in the U.S. On the other hand, water regulation in Latin American countries is in the early stages. Regulations requires just the detection of coliforms to assess the quality of the water use by the population (AWWA, 1999). Viruses and parasite testing is not required due to the higher cost of testing and the current lack of appropriate laboratories and trained personal, the latter may not be in effect for too long due to the increase effort of the Latin American countries regulatory agency to train personnel in these techniques.

Occurrence of waterborne pathogens in waters of Latin America

According to World Health Organization (WHO), "Infectious diseases caused by pathogenic bacteria, viruses or by parasites are the most common and widespread health risk associated with drinking-water". These organisms are transmitted through human and animal excreta. Fecal contamination can occur if there are cases or carriers in the community, then increasing the chances of finding the excreted organisms in the water. Use of contaminated water for drinking, food preparation, contact during washing or bathing, and even inhaling water aerosols may result in infection (WHO, 1999). WHO recommend that water that is going to be used for drinking and household purposes do not contain water-borne pathogens. In addition, the most used indicator for water

microbiological quality, *Escherichia coli*, must not to be present in 100-ml samples of any water intended for human consumption. However, scientists have found the presence of *E. coli* in pristine places, particularly in tropic areas.

In Latin America, most of the water regulations for drinking water are based on the use of fecal coliform as an indicator of water quality. However, coliform-free water is not an indication that the water is completely safe to drink. Pathogens such as rotavirus, enterotoxigenic *Escherichia coli* (ETEC), *Shigella, Campylobacter jejuni* and *Cryptosporidium parvum* are important etiological agents of diarrheal disease in Latin America. The main reason that most of the studies on water quality are done for bacterial testing is because of the cost of performing test for viruses and parasites are more expensive. But the increase use of novel techniques such as Polymerase Chain Reaction (PCR) should help to look for pathogens other than bacteria (Naranjo *et al.*, 1990).

Microorganism survival and transport at environmental conditions

The survival of pathogenic organisms in wastewater, soil, and crops depends on many factors such as (Rowe and Abdel-Magids, 1995): 1) Indoor/outdoor environment, 2) Soil moisture content, 3) Methods of wastewater disposal, 4) Crop type, 5) Distance of crop parts from soil surface, 6) Seasons and temperature, 7) Wastewater and soil pH, 8) Time pathogen remained in wastewater before it use on soil and crops 9) Treatment level of wastewater, 10) Methods of soil cultivation or soil disturbance, 11) Depth of soil, 12) Sunlight, 13) Organic matter in soil, and 14) Antagonistic soil microorganisms.

According to Yates and Yates (1991) there are two major factors controlling microbial fate in the subsurface: survival and movement. In general, both survival and movement are controlled by the specific type of microorganism, the physical and chemical properties of the soil, and the climate of the environment. The survival characteristic of microorganisms is usually studied as a net decay rate. Decay or inactivation is the irreversible destruction of contaminants (microorganisms in this case) by chemical, physical, or biological process.

Wastewater reuse and its implications

In n many arid environments an alternative source of water is required to meet the growing demands of the community, agriculture, and industry, thus wastewater has become a viable option (Rose, 1986). The utilization of wastewater in arid regions in the world has been a common practice, especially in countries such as Australia, Israel, México, Saudi Arabia, South Africa, and the United Arab Units. Also, countries with semiarid and humid regions are involved in wastewater reclamation and reuse. The most numerous wastewater reuse projects in the United States are in Arizona, California, Colorado, Florida, Georgia, Kansas, South Carolina, and Texas (Rowe and Abdel-Magid, 1995). Although the use of wastewater for agricultural irrigation has been practiced for centuries, recently a conservative approach in fully utilizing this source of water has been taken. The uncertainty of the health risks to an exposed population through wastewater irrigation practices because of the possible presence of enteric pathogenic organism is one of the major disadvantages of wastewater reuse (Rose, 1986). Pathogenic bacteria, parasites, and viruses are all found in sewage and may survive treatment processes.

Furthermore in the environment, many are able to exist for prolonged periods of time and outbreaks associated with wastewater irrigation have been documented.

Sadowski *et al.* (1978a) and Rose (1986) have demonstrated that crops directly irrigated with wastewater become contaminated with enteric microorganisms. Although enteric viruses do not grow on contaminated vegetables, they can survive long enough to cause disease in humans. Studies developed by Tierney *et al.* (1977) and Ward and Irving (1987), in which enteric viruses have added to sewage effluent used for crop irrigation have shown that viruses can remain viable from 3 to 5 weeks. Furthermore, Badawy *et al.* (1985) affirm that enteroviruses and rotaviruses can survive 1-4 months on vegetables during commercial and household storage.

Emerging pathogens

Morse (1995) defines emerging infectious diseases as infections that have newly appeared in a population or have existed but are rapidly increasing in incidence or geographic range. The author claims that factors precipitating diseases emergence include ecological changes, human demographic behavior, international travel and commerce, technology and industry, microbial adaptation and changes, and breakdown in public measures as well as deficiencies in public health infrastructure. Correspondingly, Forrest and Gushulak (1997) establish that emerging pathogens have been defined as clinically distinct infectious diseases whose incidence in humans has increased. Most of the emerging pathogens are not actually "new" pathogen-causing diseases, but may be "reemerging". Many of these simply have not been identified before, while others have

existed but have been sequestered from a population such that they have not caused significant widespread disease. In the same way, the Centers for Disease Control and Prevention (CDC, 1994) defined emerging pathogen as an infectious agent whose incidence in humans has increased dramatically within the last 20 years, or one that has probability of increasing in the future.

According to Morse (1995) "most emerging infectious appear to be caused by pathogens already present in the environment, brought out of obscurity or given a selective advantage by changing conditions and afforded an opportunity to infect new host populations"

Forrest and Gushulak (1995), mention that the major factors which can facilitate the development of new diseases include environments, reservoir/vector components (animal factors), microbial characteristics, and human. These authors suggest that the human factors are the most important and can include agricultural and economic development, changes in human demographic and behavior, international travel and commerce, technology and industry changes, and breakdown or deficiencies in public health systems.

Each change occurring in the food chain, even realized for encompassing human, technological, environmental factors, creates a new selection pressure that drives microbial adaptation and emergence potential (Miller *et al.*, 1998).

Meng and Doyle (1997) affirm that many microorganisms previously unrecognized as food-borne or harmful are emerging pathogens transmitted by food. They mention that pathogens recognized as significant causes of human illness include

Escherichia coli, Listeria monocytogenes, Aerobacter butzleri, Helicobacter pylori, Cryptosporidium parvum, and *Cyclospora*.

According to Miller *et al.* (1998) the food-borne pathogens that have emerged within the past 20 to 25 years include: *Campylobacter jejuni, Clostridium botulinum* (infant botulism), *Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella enteriditis, Vibrio cholerae* (Latin America), *Vibrio vulnificus, Yersinia enterocolitica,* Norwalk and Norwalk-like viruses, Rotavirus, *Cryptosporidium parvum, Giardia lamblia, Toxoplasma gondii,* and bovine spongiform encephalopathy

Food safety

According to Nguyen and Carlin (1994) minimally processed fresh (MPF) fruit and vegetables are fresh, raw fruits or vegetables processed in order to supply a ready-to-eat or ready-to-use product. The main features of MPF fruit and vegetables include: 1) the presence of cut surfaces or damaged plant tissues, 2) minimal processing that cannot ensure sterility or microbial stability of the product, 3) active metabolism of the plant tissue, and 4) confinement of the product. These features are adequate for microorganism proliferation.

MPR fruits and vegetables are an important and rapidly developing class of foods so it is essential to know if these foods can serve as vehicles for many different food-borne pathogenic microorganisms. In most cases, these MPR foods are consumed without cooking, making the presence of pathogens a concern. Pathogens can reach MPR food from several sources including, but no limited, irrigation or wash water, infected

operator, fertilizers of animal waste and municipal biosolids, and operation facilities with poor sanitation (Han *et al.*, 2000).

There are two critical points related to the level of pathogens on vegetables when they are growing in the field, the first one is how pathogens can reach vegetables and the second one how pathogens can attach vegetable surfaces. The former one is related to transport processes and different mechanisms can occur depending on if the vegetables are root growth such as carrots or onions, surface growth such as leaf vegetables (lettuce or cabbage), or aerial growth such as tomatoes or peppers. The last point, attachment, is related to sorption mechanisms. No matter how pathogens reach vegetable surfaces, it is possible to assume that sorption mechanisms are likely to be the same in the three different types of growth vegetables mentioned above.

Food safety is one of the greatest health public concerns in the United States. In the US each year food-borne illness affects 6 to 80 million persons, cause 9 000 deaths, and cost an estimated five billion US dollars. Many of the food-borne diseases are caused by emerging pathogens.

According to Majkowski (1997) the food-borne paradigm has shifted, in the past an outbreak affected a small local population, had a high attack rate, and involved locally prepared food products with limited distribution. However, now outbreaks involve large populations and maybe multi-state and even international. In many cases, the pathogenic organism has a low infective dose and sometimes is never isolated from the food product. Delay in identifying the causative agent can allow the outbreak to spread, increasing the number of cases. Tauxe (1997) mention that a series of outbreaks were investigated for

the Centers for Disease Control and Prevention (CDC) and linked with a variety of pathogens found on fresh fruits and vegetables harvested in the USA and elsewhere. In this study, various possible points of contamination were identified, including contamination during production and harvest, initial processing and picking, distribution, and final processing.

Shuval *et al.* (1986) claimed that the microbiological contamination of vegetables is most important because of the survival time can be from several days until months. This is particularly important when microorganisms are present on the most moist and protected vegetables areas. Monge *et al.* (1996) mention that in some studies carried out using untreated wastewater for irrigation, the sanitary quality of vegetables was reduced. However, according to Castro and Flórez (1990) only 48 percent of *E. coli* present on vegetables originated from irrigation water.

Vegetables and food-borne

Food-borne diseases are a persistent challenge and concern to health worldwide. New and emerging pathogens appear, and new food vehicles continue to be implicated as a result of the changing industrial ecology of food production and consumption (Tauxe *et al.*, 1997). The presence of numerous genera of spoilage bacteria, yeasts, molds, and occasionally pathogens on fresh produce has been recognized for many years. Numerous microorganism capable of causing human illness have been isolated and outbreaks of human gastroenteritis have been linked to the consumption of contaminated vegetables (Beuchat, 1996; Beuchat *et al.,* 1998).

Consumption of fresh vegetables has greatly increased at the expense of processed products. Consumers perceive fresh vegetables to be more nutritious than their processed counterpart (Garg *et al.*, 1990). Hotchkiss and Banco (1992) mention Americans consumed 37% more fresh vegetables in 1988 than in 1971. In addition, they affirm that annual per capita fresh fruit consumption increased by 10.3 Kg over the same period. In the same way, Tauxe *et al.* (1997) affirmed that consumption of fresh fruits and vegetables has increased in the United States in the past two decades. Furthermore the geographic sources and distribution of fresh produce have expanded greatly

The Centers for Disease Control and Prevention (CDC) reports that the number of food-borne disease outbreaks doubled between 1973 and 1987, and 1988 and 1991 (mentioned by Tauxe *et al.*, 1997). During 1995, major outbreaks were associated with *Salmonella* serotype Stanley on alfalfa sprouts, *Salmonella* Hartford in unpasteurized orange juice, *Shigella* spp. on lettuce and onions, *Escherichia coli* O157:H7 on lettuce, and hepatitis A virus on tomatoes (Tauxe *et al.*, 1997).

Tauxe *et al.* (1997) mention that the distribution pattern for fresh produce in the United States generally disperse production lots widely, and contamination of produce frequently is intermittent and low level. These authors suggest that trace-back of produce to its origins is particularly difficult due to the complex network of growers, packers, shippers, re-packers, distributors, brokers, retailers, and consumers, which often involves several states as well as countries.

Although consumer education about basic principles of food safety is an important component of prevention, by itself it is insufficient. Food-borne diseases reach

the consumer through long chains of industrial production, in which many opportunities for contamination exists. The general strategy of prevention is to understand the mechanisms by which contamination and disease transmission can occur well enough to interrupt them (Tauxe, 1997).

Jackson (1990) suggests that scientific advances in methodology and epidemiology have resulted in a renewed awareness of food-borne disease, and increase contact among nations of the world has stimulated rapid global distribution of food as well as food-borne pathogens. In addition, this author affirms that new food vehicles are being identified for old, familiar, and new pathogens (emergent). Some organisms of recent interest such as *Bacillus, Yersenia, Campylobacter, Listeria, Sporothrix, Giardia, Cryptoporidium*, and *Anisakis* are the foci of new investigations, as are the more familiar food-borne pathogens which include *Salmonella, Shigella, Clostridium, Staphylococcus, Entamoeba* and *Ascaris.*

Jackson (1990) affirms that if human illnesses are grouped by organ system, gastrointestinal infections rank second in incidence, respiratory disease are first, circulatory disease third, and skeletomuscular injuries fourth. In the U.S. this means, on the average, one digestive tract episode per person per year; death from gastrointestinal infections range from 35,000 to 40,000 annually. With the portion of gastrointestinal infections attributed, conservatively, to food as the vehicle being one third of the total, the cost of food-borne illness is about $40 billion for acute cases.

Disease outbreaks caused by consumption of contaminated fruits and vegetables occur less frequently than those caused by consumption of contaminated meat and

poultry. However, food-borne illness outbreaks have been reported from vegetables and fruits contaminated with pathogenic microorganisms (Albrecht *et al.*, 1995).

Beuchat *et al.* (1998) suggest that an increased per capita consumption of fresh and lightly processed produce in the United States and other countries, coupled with an increase in importation of produce from regions where standards for growing and handling produce may be compromised, has resulted in heightened interest in outbreaks of human gastroenteritis that may be attributed to consumption of contaminated fresh produce, particularly salad vegetables.

The contamination of fresh produce can be not only due to water quality insufficiency and scarceness sanitary conditions during growing of vegetables but also to several factors, which are described by Beuchat (1996), who mentions the mechanisms by which fresh produce can become contaminated with pathogenic microorganisms and serve as vehicles of human disease. The main factors, grouped in two categories, involved in these mechanisms pre-harvest and post-harvest factors. Pre harvest factor include: animal and human feces, soil, irrigation water, green or inadequate composted manure, air (dust), wild and domestic animals, and human handling. Post harvest factors include: animal and human feces, human handling (workers and consumers), harvesting equipment (field to packing shed), wild and domestic animal, air (dust), wash and rinse water, packing processes (sorting, cutting, packing, and other further processing equipment), ice, transport vehicles, improper storage (temperature and physical environment), improper packing, cross-contamination, improper display temperature, and improper handling after wholesale or retail purchase.

Brackett (1992) mention that some pathogenic organisms of concern in minimally processed produce include: *Listeria monocytogenes, Clostridium botulinum, Shigella* sp., *Salmonella* sp., parasites and viruses. Therefore, every step from production through consumption will influence the microbiology of fresh produce. For a better understanding of the production/processing/distribution of fresh produce system, this author, grouped it into the following four broad areas: 1) production, 2) processing, 3) storage, and 4) marketing.

Some studies related to vegetables contaminated

Several years ago, Maxcy (1978), evaluated the magnitude of contamination and the nature of representative microbial contaminants on fresh lettuce. Also specific microbes of public health interest were added to test portions to determine their fate during storage of lettuce as salad at room temperature. The microbial plate counts, on fresh lettuce commonly were over 10^5 CFU/g and the diversity of the microflora indicated favorable microenvironments for many types of bacteria. Inoculated *Salmonella typhimurium, Escherichia coli*, and *Staphylococcus aueres* fared well on lettuce salad and were able to grow at room temperature.

Food preparation for consumption outside the home is a rapidly growing segment of the food industry at the United States of America and in many countries. The common way for vegetable production has inherent opportunities for contamination from such sources as manure for fertilizer, contaminated irrigation water, wild animals, and personal contact in the harvesting process (Maxcy, 1978). However, Dunlop and Wang (1961) found few pathogens on lettuce irrigated with sewage.

Several studies (Ercolani, 1976; Fowler and Foster, 1976; Gould, 1973; Hall *et al.*, 1967)) have demonstrated that lettuce as prepared for salads by traditional methods and evaluated immediately may carry a total microbial load of millions per gram. According to Priepke *et al.* (1976) and Maxcy (1978) some of these microorganisms may grow during storage. They argue that more rapid growth would be expected at warmer conditions than room temperature.

Rosas *et al.* (1984) reported a study in Xochimilco county, part of Mexico City, related to the microbiological quality of vegetables irrigated with wastewater. Xochimilco is one of the most fertile agricultural areas in the Valley of Mexico and where a large portion of fresh vegetables are produced for the consumption of Mexico City. The crops included in this study were radish, spinach, lettuce, parsley, and celery. The highest bacterial counts were found in leafy vegetables such as spinach with 8,700 MPN/100 g for total coliforms and 2,400 MPN/100 g for fecal coliforms and lettuce with 37,000 MPN/100 g for total coliforms and 3,600 MPN/100 g for fecal coliforms. The total coliforms values found in irrigation water ranged from $4X10^4$ to 29 $X10^4$ CFU/100 ml and for fecal coliforms the values ranged from $5X10^2$ to $30X10^2$ CFU/100 ml.

Monge and Chinchilla (1996) carried out a study in Costa Rica of eight different vegetables. *Cryptosporidium* spp oocysts were found in 5.0% (4 samples) of cilantro leaves, 8.7% (7 samples) of cilantro roots and 2.5% (2 samples) of lettuce sampled. They reported that for cilantro roots and lettuce a positive linear correlation ($P < 0.05$) was established between the presence of *Cryptosporidium* spp oocysts and fecal coliforms and *E. coli.*

Odumeru *et al.* (1997) studied the microbiological quality of ready-to-use vegetables including chopped lettuce, salad mix, cauliflower florets, sliced celery, coleslaw mix, broccoli florets, and sliced green peppers before and after processing. All vegetables, with the exception of green peppers, showed up to 1-log decrease in aerobic colony counts after processing. They reported that microbial population increased to processing levels after four days of storage at both 4 and 10^{o}C. Green peppers had the highest bacterial counts while cauliflower and chopped lettuce had the lowest counts at both storage temperatures. *Listeria monocytogenes* was detected in 13 of 120 (10.8%) of vegetables samples stored at 10 ^{o}C but not in 175 samples stored at 4 ^{o}C after 7 days. *E. coli* was detected in 2 of 120 (1.7%) processed vegetable samples after 7 days of storage at 10^{o}C and one of 65 (1.5%) unprocessed vegetables. *E. coli* not was detected in vegetables samples stored at 4 ^{o}C.

In addition, the above mentioned authors affirmed that *E. coli* levels on unprocessed and processed green peppers was not significantly different. Similar results were reported for chopped lettuce (9.0 MPN/g). *Listeria monocytogenes* was detected in one of 65 (1.5%) unprocessed samples (celery). *L. monocytogenes* was not detected on unprocessed iceberg lettuce, cauliflower, green and red cabbage, carrots, broccoli, and green peppers. Pathogenic bacteria including *Salmonella* spp., *Campylobacter* spp., *Yersinia enterocolitica* (serotype O:3) and verocytotoxigenic *E. coli* (VTEC, including O157:H7) were not detected in any vegetables tested.

Ortega *et al.* (1997) carried out a study in Peru. Samples of vegetables including cabbage, celery, cilantro, green onion, ground green chili, leeks, lettuce, parsley, yerba

Buena, basil, and huacatay were collected at several small markets. They reported that of the total of vegetables examined, 14.5% contained *C. parvum* oocysts and only 1.5 had *Cyclospora* oocysts. They also detected *Cryptosporidium parvum* and *Cyclospora cayetanensis* oocysts using scanning electron microscopy on the surface of vegetables after washing with distilled water.

Seo and Frank (1999), based on their study about lettuce leaves inoculated with *E. coli* O157:H7, found *E coli* attached to the surface, trichomes, stomata, and cut edges. In addition, they demonstrated that these bacteria were entrapped 20 to 200 μm below the surface in stomata and cut edges. Furthermore, these authors claim that many live *E. coli* were found in stomata and cut edges after of chlorine treatment (20 mg/l for 5 minutes). Also, they found that *E. coli* did not preferentially adhere to biofilm produced by *Pseudomonas fluorescens* on the leaf surface. Romberger *et al.* (1993 establish that leaf surfaces are usually considered to be hydrophobic due to the presence of a waxy cuticle.

Hirotani *et al.* (2002) demonstrated the presence of indicator microorganisms of fecal pollution on surfaces of vegetables obtained in retail local market in the U.S.A. and Mexico. They found at least one of the indicators, among coliphages, fecal streptococci, total coliforms, and fecal coliforms, on the vegetables tested which included: tomato, lettuce, cabbage, leek, bell pepper, long pepper, carrots, radish, celery, spinach, Chinese cabbage, and parsley

Odumeru *et al.* (1997) suggested a zero tolerance for *Salmonella* spp., VTEC, *Campylobacter* spp., and pathogenic *Y. enterocolitica* for ready-toe-use vegetables. For *L. monocytogenes* they mentioned a limit of ≤ 100 CFU/g.

Dissertation Format

This dissertation consists of three manuscripts prepared for publication and presented as appendices. The three manuscripts are the results of investigations carried out in El Valle del Yaqui Sonora, México. Appendix A is a study of the microbiological water quality of a large irrigation system. This manuscript has been submitted to Journal of Food Protection. Appendix B is an assessment of aerated lagoons for production of irrigation water. Finally, Appendix C is related to the impact of irrigation water quality on microbial contamination of produce.

The dissertation author was responsible for all the research presented in the manuscripts, with the following exceptions: Appendix B: Chemical water quality parameters were done by the laboratory staff of the treatment wastewater plant facility. Appendix B and C: Vegetables were planted with the help of the experimental field staff.

PRESENT STUDY

The methods, results, and conclusions of this study are presented in the manuscripts appended to this dissertation. The following is a summary of the most important findings in this document.

The manuscript "Microbiological Water Quality in a Large Irrigation Systems: El Valle del Yaqui, Sonora Mexico" is determined the microbial water quality of a large system and how quality varies with respect to canal system (upper and lower), canal size, impact to near-by communities and the travel distance of irrigation water. Ninety percent of the samples contained fecal coliforms levels lower than those recommended by WHO (Gerba and Rose, 2003) and were within Mexican Guidelines (Secretaria del Medio Ambiente, Recursos Naturales y Pesca, 1997). No significant difference was found in the water quality due to canal system, canal size (main vs. lateral), and the vicinity of sampling sites to communities or towns. However, there was a significant difference with travel distance from the origin of the water. This variation was more significant for *E. coli* ($p < 0.011$) and total coliforms ($p< 0.022$).

The manuscript in Appendix B, "Assessment of Aerated Lagoons for Production of Irrigation Water" is a study whose primary objective was to determine if aerated lagoon used to treat municipal water, in Ciudad Obregón, could meet the World Health Organization and Mexican Guidelines for reclaimed wastewater to be used for food crop irrigation. In addition, the impact of microbiological quality on vegetables (carrots, lettuce, and tomatoes) was assessed. Data for treated wastewater appear to indicate that the treated wastewater effluent can be used in agricultural to irrigate restricted crops. The

concentrations of all organisms studied were higher in RWW than in TWW, however, no significant difference was observed on the microbiological quality of vegetables regardless of the type of irrigation water used.

The manuscript "The Impact of Irrigation Water Quality on Microbial Contamination of Produce" is presented in Appendix C. The main objective of this study was to assess the potential for contamination of vegetables during irrigation with untreated wastewater and surface water. Untreated municipal wastewater from a small town (El Tobarito) in El Valle del Yaqui, Sonora, México and surface irrigation water from storage dam were used to irrigate three different vegetables (carrots, lettuce, and green peppers). All of the studied microorganisms (total coliforms, *Escherichia coli*, *Clostridium perfringens, Cryptosporidium* oocysts, *Giardia* cysts, coliphages, and enteroviruses) were present in untreated wastewater, but only total coliforms, *E coli*, and *Clostridium perfringens* were present in the surface irrigation water. Total coliforms, *E. coli,* and *C. perfringens* were detected on all vegetables regardless of irrigation source water. A greater concentration of total coliforms, *E. coli*, and *C. perfringens* was found on carrots followed by lettuce and green peppers. *E. coli* levels were 10 to 100 times greater on produce irrigated with untreated water than those irrigated with surface water.

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APPENDIX A:

MICROBIOLOGICAL WATER QUALITY IN A LARGE IRRIGATION SYSTEM: EL VALLE DEL YAQUI, SONORA MÉXICO

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Abstract

The primary objective in this study was to determine the microbial water quality of a large irrigation system and how quality varies with respect to canal size, impact of near-by communities and the travel distance from the source in the El Valle del Yaqui, Sonora, Mexico. In this arid region, 220,000 hectares are irrigated with eighty percent of irrigation water being supplied from an extensive water supply system including three dams on the Yaqui River watershed. The stored water flows to the irrigated fields through two main canal systems (severing the upper and lower Yaqui valley) and then through smaller lateral canals that deliver the water to the fields. A total of 146 irrigation water samples were collected from 52 sample sites during three sampling events. Not all sites could be accessed on each occasion. All of the samples contained coliform bacteria ranging from 1,140 to 68,670 MPN/100 ml with an arithmetic mean of 11,416 MPN/100 ml. Ninety-eight percent of the samples contained less than 1,000 MPN/100 ml *Escherichia coli*, with an arithmetic mean of 291 MPN/100 ml. Coliphage were detected in less than 30% of the samples with an arithmetic average equal to 141 PFU/100 ml. No significant difference was found in water quality between to the two major canal systems (upper and lower), canal-size (main vs. lateral), and the proximiy of sampling sites to human habitation (presence of various villages and towns along the length of the canals). There was a significant decrease in coliforms ($p<0.011$) and *E. coli* (<0.022) concentrations as travel distances increase from the City of Obregon.

Keywords

Irrigation water, *Escherichia coli*, viruses, coliphage, enteric viruses, *Cryptosporidium*, *Giardia*

Introduction

Irrigated land comprises around 15% of the arable world's land and produces 36% of the overall food supply (Ayers and Wescot, 1989). Irrigation water may be contaminated by agricultural runoff from storm events, waterfowl, and sewage. The microbial water quality of irrigation water is important when used to grow vegetables that are eaten fresh or minimally processed. The growing demand for fresh produce has been satisfied by an increase in importation of fresh produce from countries where hygiene standards may be compromised. Enteric protozoan pathogens have been detected in irrigation waters which can contaminate produce during irrigation (Stine *et al.*, 2005; Thurston-Enriquez *et al.*, 2002). Few studies have looked at the microbial quality of irrigation systems. In existing studies the irrigation water was obtained from rivers or other sources which had sewage discharges (Brenner *et al.*, 2000; Ceballos *et al.*, 2003; Okafo *et al.,* 2003; Pianetti *et al.*, 2004).

This study focused on the occurrence of microbial indicators and enteric pathogens, and the potential impacts of human habitation, canal type, and water travel distance in a developing country. The source of water was a reservoir, and the canal system had no known sewage discharges into the system.

Methods

The study was conducted in El Valle del Yaqui (27^{o} N, 100^{o} W), which is located in the state of Sonora in the northwestern region of Mexico. The coastal plain, which is approximately 60 km wide, is between the Gulf of California (west) and the foothills of the Sierra Madre Occidental (east). Approximately 220,000 ha of El Valle del Yaqui is supplied with irrigation water by the Alvaro Obregon (*Oviachic*— a Yaqui name) dam, which has a capacity of 3,000 x 10^6 m^3. This dam is part of the Yaqui River watershed.

The irrigation system in El Valle del Yaqui is one of the largest and most modern agricultural areas in Mexico and comprises a canal network of 2,774 km (Gortáres and Castro, 1993). It is divided into the upper zone (UZ) and lower zone (LZ). Each zone has one main canal (upper main canal and lower main canal). The main (15 m in width) canals branch into smaller primary canals, and then into smaller secondary (one meter in width) canals running perpendicular to the primary canals. The secondary canals further branch into tertiary canals (less than one meter in width) which eventually deliver water to the irrigated fields. The upper main canal has a length of 120 km, a flow rate 110 m^3/s, and serves an irrigated area of 100,000 ha. The lower main canal has a length of 100 km, a flow rate of 120 m^3/s, and is used to irrigate 120,000 ha. Both main canals have earthen banks approximately one meter above the surrounding farmland. The combined length of all the primary and secondary canals is 2,554 km. Approximately 2,500 million cubic meters of water pass through this system every year.

Sampling

Fifty-two sampling sites were selected at random locations using a map of the system to represent different distances from the reservoir sources. The sample sites were both in the upper and lower irrigation zones. Samples were collected from the main, primary, and secondary canals (Fig. 1). Selected sites close to Ciudad Obregón were considered as close to the dam source (1 to 20 km) while sampling locations between 50 and 60 Km from Ciudad Obregon) were considered as distant. There are several small towns in the El Valle del Yaqui in the irrigated areas. Sample locations were also categorized as close to towns or distant (greater than 2 km). The number and distribution of sampling locations is shown in Table 1. Sampling was conducted three times during the irrigation season (February, March, and November 2000). It required three consecutive days to collect all of the samples. Water samples were always collected in the morning using sterile one-liter polycarbonate bottles. All samples were placed in a cooler with ice (approximately 4 ^{o}C), transported back to the laboratory, and analyzed within 6 hours of collection.

Water samples for the protozoan parasites and enteroviruses were collected according to the ICR Microbial Laboratory Manual (USEPA, 1996). Positively charged 1MDS filter cartridges (CUNO Inc., Meriden, CT) were used to process 400 liter virus samples and Filterite polypropylene cartridges (Filterite Corporation, Timorium, MD) were used to process 100 liter parasite samples.

Chemical and microbial analysis

Water samples were analyzed for temperature (T), pH, dissolved oxygen (DO), turbidity (TUR), total coliforms (TC), fecal coliforms (FC), and coliphages (CPH). In addition, five sample locations were chosen to test for enteroviruses (EV), *Cryptosporidium* oocysts (CRY), and *Giardia* cysts (G) during the November sampling. These locations were chosen based on the higher levels of total coliforms and *E. coli* found during the February and March sampling.

The Colilert Quanti-tray system was used for coliform and *E. coli* quantification (IDEXX Laboratories Inc., Westbrook, ME).

The double-layer agar was used to detect coliphages using *E. coli*, strain ATCC 15597 (American Type Culture Collection, Rockville, MD) (Pepper *et al.,* 1995) . Four replicates of five and one milliliter aliquots of water were assayed for each sample. The strain of *E. coli* used detects both somatic and male specific coliphages.

Cryptosporidium oocysts and *Giardia* cysts were detected using an indirect fluorescent antibody procedure (USEPA, 1996). The Ensys Hydrofluor-Combo kit (Strategic Diagnostics, Inc., Newark DE) was used for staining.

Samples for enteroviruses were processed according to protocols 9510C and 9510D from the Standard Methods for the Examination of Water and Wastewater (APHA, 1998). Samples were assayed on the BGM cell line as described in protocol 9510G using cytopathic effects (CPE). The cell cultures were observed for CPE for 14 days. If no CPE was observed they were passed a second time onto fresh monolayers.

Virus concentration was calculated by the Most Probable Number Method (MPN), also from APHA (1998).

Temperature, pH, and dissolved oxygen were measured in the field according to procedures described in Standard Methods for the Examination of Water and Wastewater (APHA, 1998). Turbidity was measured using a turbidimeter (Hach Chemical Co., Loveland, CO).

Data Analysis

Total coliforms, *E. coli,* and coliphage results were analyzed to determine the microbiological quality variation as a function of factors including, water travel distance, type of canal (main, primary, and secondary), irrigation zone (upper or lower) as well as whether they were within 2 km of a town or village. Statistical analysis was done using the analysis of variance (ANOVA) with the SYSTAT 9.0 program (Sokal and Rholf, 1995). The level of significance was defined as 95 percent ($\alpha = 0.05$). Therefore, p values less than 0.05 generated by ANOVA are considered to be statistically significant.

Results

Water temperature ranged between 14.5 and 26.0^{o}C with a mean of 21^{o}C; pH ranged from 6.8 and 8.9 with a mean of 7.6. Dissolved oxygen levels were close to saturation in most of the samples with a mean of 8.3 mg/l. Turbidity ranged between <0.01 to 57.4 NTU with a mean of 9.3 NTU, and flow lineal velocity variation in the canals was between 0.0 and 1.63 m/s with a mean of 0.43 m/s (Table 2).

Total coliforms ranged from 1,140 and 68,670 MPN/100 ml with a mean of 11,416 MPN/100 ml. *E .coli* ranged between 1 and 22,325 MPN/100 ml with a mean of 291 MPN/100 ml. Coliphage ranged from <5 and 8700 PFU/100 ml with a mean of 141 PFU/100 ml (Table 2). All the samples were greater than 1,000 MPN/100 ml for total coliforms. While that 98 percent of the samples were less than 1,000 MPN/100 ml for E. coli. Also for coliphages approximately 95 percent of the samples were less than 1000 PFU/100 ml (Figure 2).

Enteroviruses varied between 0.016 and 0.12 MPN/100 ml with a mean of 0.05 MPN/100 ml. *Cryptosporidium* ranged between < 0.8 and 5.8 oocysts/100 ml with a mean of 1.8 oocysts/100 ml, and *Giardia* ranged from < 0.8 and 9.1 cysts/100 ml with a mean of 3.5 cysts/100 ml (Table 2).

Table 3 show water quality results analyzed by canal system (upper or lower). Physicochemical parameters and total coliforms values were similar in both canal systems. Although fecal coliforms, *Clostridium* , coliphages, *Crypstosporidium*, *Giardia*, and Enteroviruses levels were slightly lower in the upper system than the lower one (Figure 3), they were not significantly different ($p < 0.05$). There was a tendency for indicator organisms to increase in the smaller canals, but the differences were not significant (Figure 4). The number of samples, however, that were collected in the smallest (tertiary) canals was somewhat limited and may have impacted the results.

Based on statistical analysis there was a significant difference in the concentration of indicators on travel distance of the water from the dam. This variation is more significant for *E. coli* ($p< 0.011$) and total coliforms ($p < 0.022$). Ciudad Obregon is a

city with approximately 300,000 inhabitants and activity in this area (i.e. urban runoff) could adversely impact the quality of the water in the canals. Concentrations of all microbial indicators were greater for the sampling sites close to populated (towns and villages) areas than those more distant (Figure 5). However, this difference was not found to be significant ($P < 0.05$).

All microbial indicators were in relatively greater concentrations at sites considered close to the source (City of Obregón) than those at a greater distance from the source (Figure 6). However, this difference was not found to be significant ($P < 0.05$).

Discussion

Around ninety eight percent of the samples contained fecal coliform levels less than that recommended by WHO (Gerba and Rose, 2003) and Mexican guidelines (Secretaría del Medio Ambiente Recursos Naturales y Pesca, 1997) for irrigation water. Irrigation systems are a complex set of canals designed to deliver water to fields for crop production. No previous studies have been conducted on factors which may influence the occurrence of indicators of microbial quality. In addition, few studies have been conducted on the quality of irrigation waters in general. Existing studies deal with sewage contaminated source water (Ceballos *et al.*, 2003; Pianetti *et al.,* 2004; Okafo *et al.,* 2003). Geldrich and Bordner (1971) conducted a study in the 1960's of irrigation canal waters in the western United States. At that time sewage was still being discharged into irrigation waters, as noted by the authors. Based on their observations on the occurrence of *Salmonella* they recommended that irrigation waters should contain not

more than 1,000 fecal coliforms/100 ml. Most other studies have dealt with standands for the use of reclaimed sewage effluents for irrigation (Gerba and Rose, 2003).

Although not statistically significant, it was observed in this study what tended to decrease with decreasing canal size. There was significant increase in water quality (i.e. lower concentrations of *E. coli* and coliforms) the greater the travel distance from the city of Obregón. This suggests that passage of the canals near the city may have resulted in some microbial contamination. Die-off of the indicator bacteria and coliphage from UV light and predation may have caused this observed decrease.

During this study, it was possible to collect only a small number of samples for virus and protozoan parasites. The data from these samples demonstrated that some contamination by enteric viruses and protozoan parasites had taken place. Additional studies would be necessary to assess the significance of these results. However, the results do demonstrate that the *E. coli* and coliphage detected in the irrigation canals are reflective of the presence of waterborne enteric pathogens.

There are no generally accepted standards for irrigation waters used for produce production, except when reclaimed water is used as the source. Reclaimed water standards are also linked to required advanced treatment of the wastewater (Gerba and Rose, 2003). Suggested fecal coliform standards for crop irrigation range from <1 to 1,000 per 100 mL (Gerba and Rose, 2003). Currently there are no suggested standards for *E. coli*, which is a member of the fecal coliform group.

Conclusions

There was not a significant variation in the water quality with respect to canal-zone, canal type, stream location, and proximity to populated areas. However, results suggest that microbial indicator levels slightly increase as the water flowed through smaller canals. In addition, the denser the human habitation resulted in greater average concentration of indicator organisms.

There was a significant variation in the water quality with respect to the water travel distance. However, this variation was not easily defined because some times microbial levels decrease and suddenly increase and again decrease in all the water travel distance. This behavior could have been due to non point source of microbial contaminants discharging to irrigation canals.

Acknowledgments

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Table 1. Sample locations by canal-system, size, distance from sources, and communities.

FACTOR	Upper Main Canal System	Lower Main Canal System	TOTAL
	Number of Samples Collected		
CANAL SIZE			
Main	9	9	18
Primary	32	25	57
Secondary	27	34	61
Tertiary	5	5	10
TOTAL	73	73	146
TRAVEL DISTANCE			
1 km	29	14	43
20 km	17	23	40
50 km	27	36	63
TOTAL	73	73	146
DISTANCE TO NEAREST COMMUNITY			
Close (within 2 Km)	23	21	44
Far away (out 2 Km)	50	52	102
TOTAL	73	73	146

Table 2. Water quality in El Valle del Yaqui irrigation system

Parameter	Number of Samples	Range	Arithmetic Mean
Temperature (^{o}C)	103	14.5 - 26	21
pH	144	6.8 - 8.9	7.7
Turbidity (NTU)	141	0 - 57.4	9.3
Flow velocity (m/sec)	52	0 – 1.6	0.43
Dissolved Oxygen (mg/l)	91	4.2 - 11.5	8.3
Total coliforms (MPN/100 ml)	146	1,140 -68,670	11,416
E. coli (MPN/100 ml)	144	1 – 22,325	291
Coliphages (PFU/100 ml)	145	5 – 8,700	141
Enteroviruses (MPN/100 ml)	6	0.016 -0 .012	0.05
Cryptosporidium (Oocysts/100 ml)	6	<0.08 - 5.8	1.8
Giardia (Cysts/100 ml)	6	<0.08 - 9.1	3.5

Table 3. Variation of water quality in El Valle del Yaqui irrigation system by main canal system (upper and lower).

Parameter	Samples		Range		Mean	
	Upper	lower	Upper	Lower	Upper	Lower
Temperature (^{o}C)	51	52	15.4 - 26	14.5 - 25.5	20.8	21
pH	72	72	6.8 - 8.8	6.8 - 8.9	7.8	7.5
Turbidity (NTU)	68	73	1 - 35.8	0 - 57.4	8.1	10.5
Flow velocity (m/s)	23	26	0.05 - 1.0	0 - 2.3	0.4	0.4
Dissolved Oxygen (mg/l)	43	48	4.9 -11.5	4.2 - 11.4	8.4	8.1
Total coliforms (MPN/100 ml)	73	73	1,779 – 68,670	1,140 – 54,750	11,860	10,970
E. coli (MPN/100 ml)	71	73	1 – 1,300	1 – 22,325	70	506
Coliphages (PFU/100 ml)	72	73	5 – 95	5 – 8,700	9.3	271

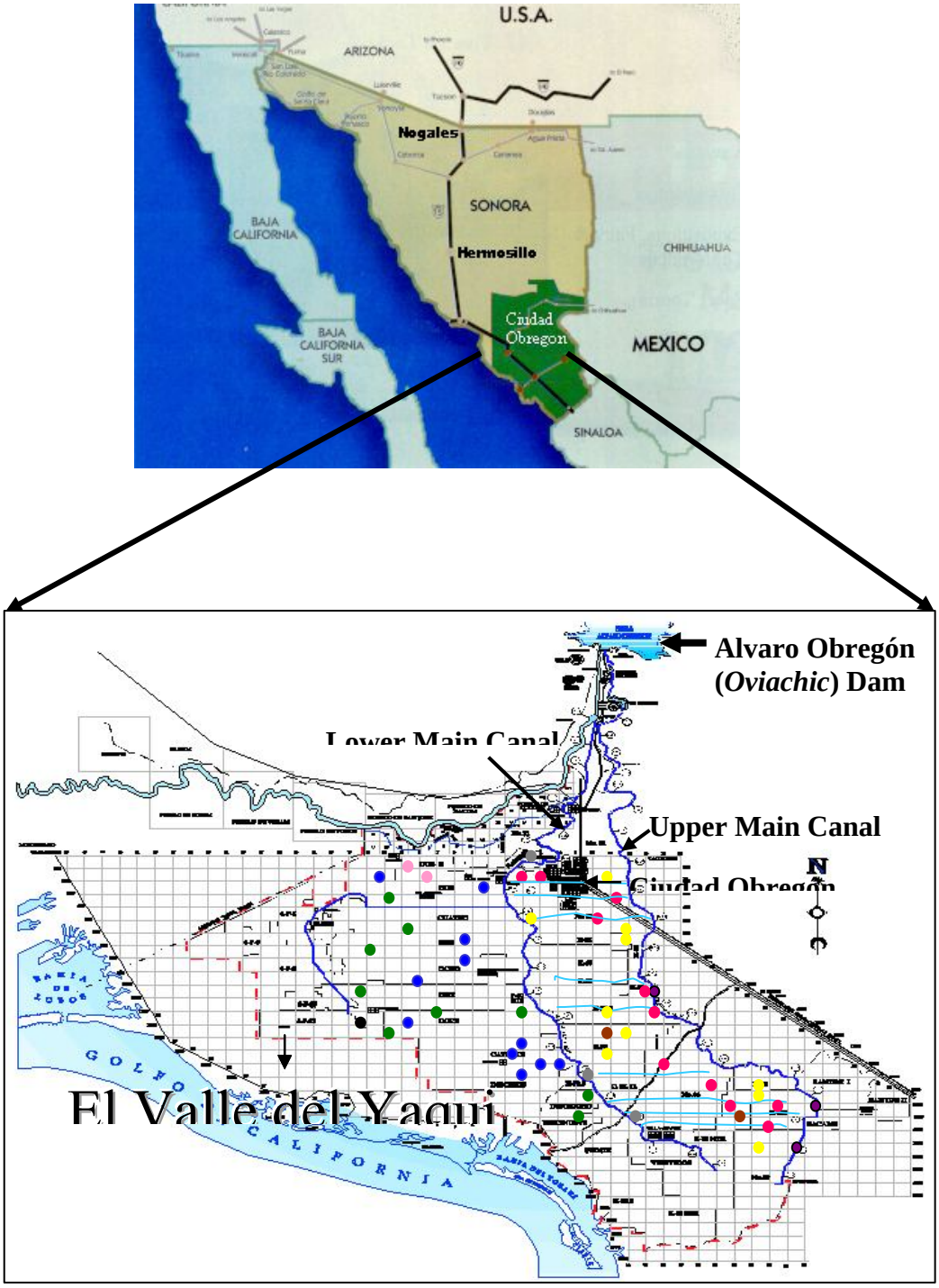


Figure 1. El Valle del Yaqui location.

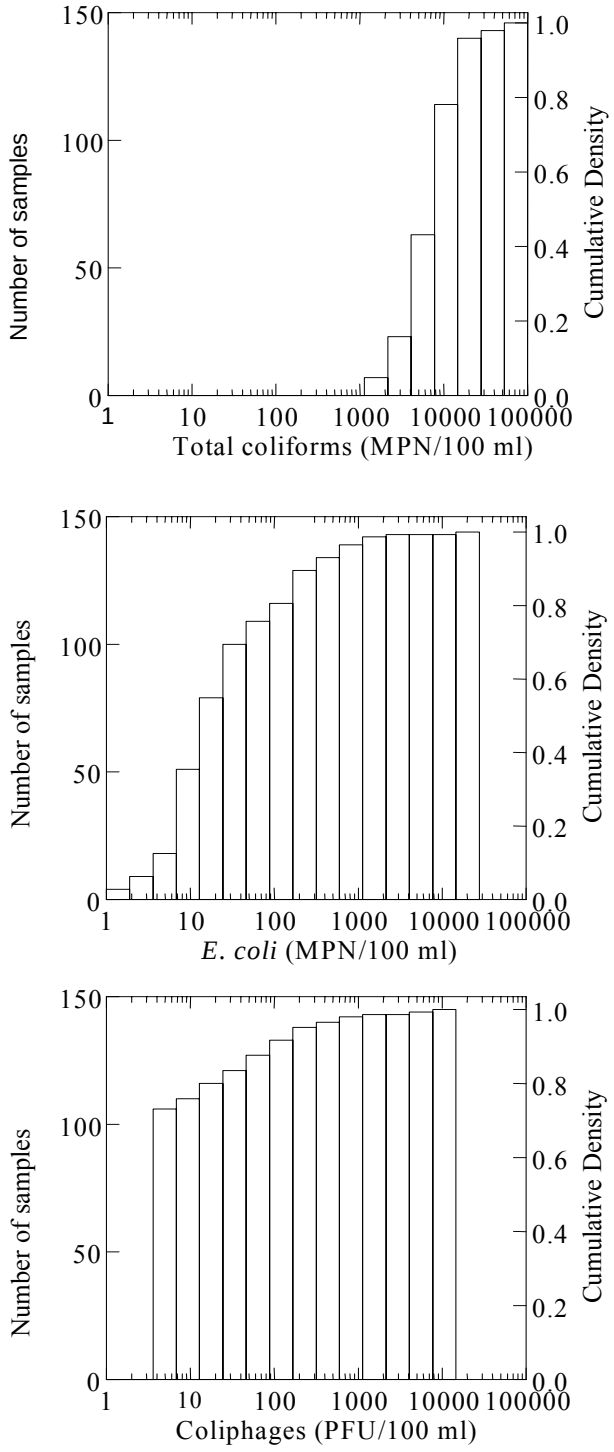


Figure 2. Microbial distribution in the overall irrigation system (It is showed the cumulative distribution related to the number of samples)

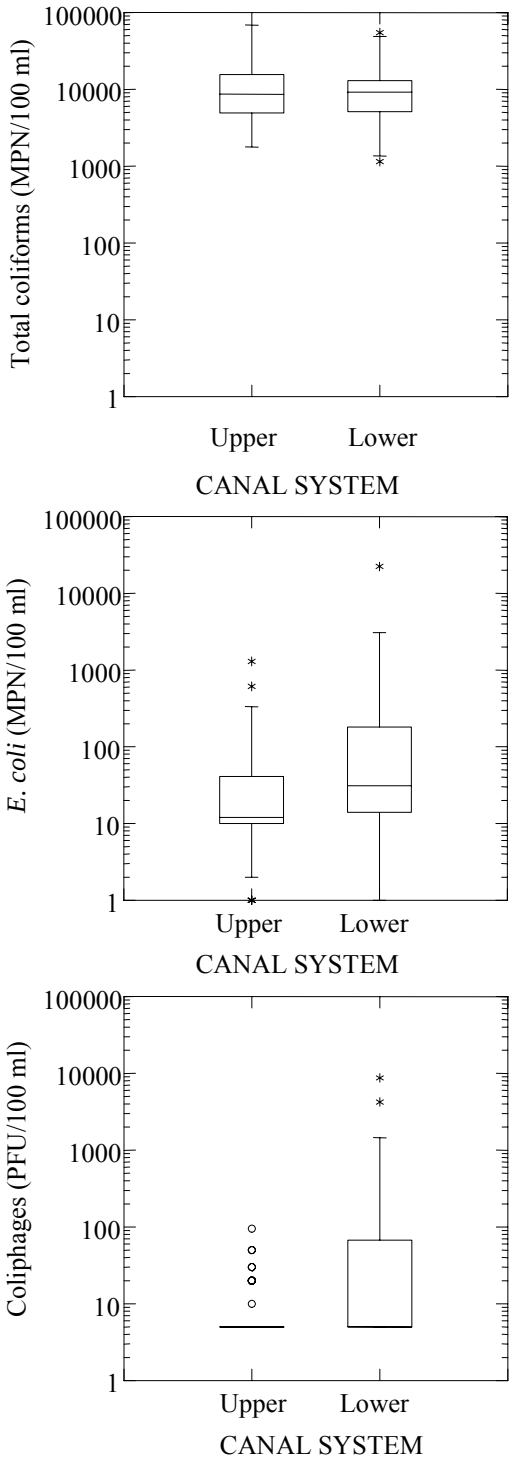
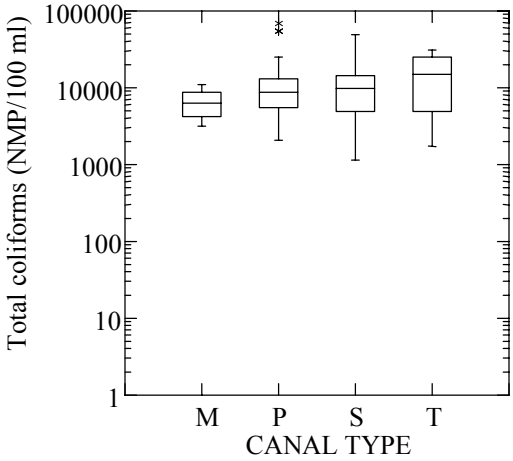
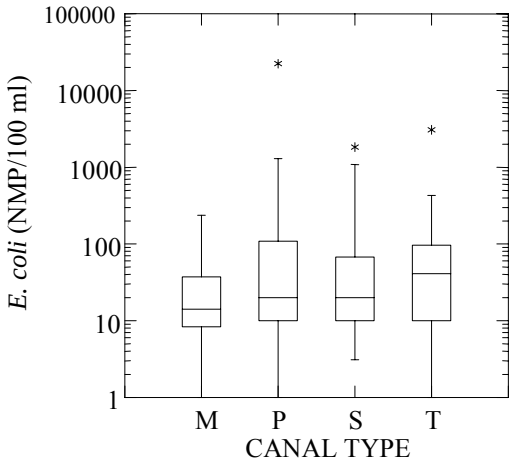


Figure 3. Variation in microbial numbers in the canal systems.





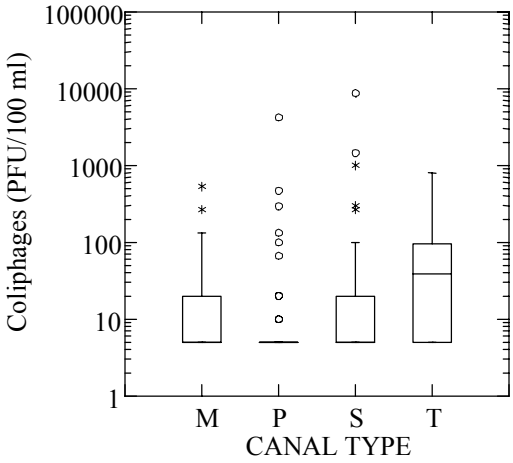


Figure 4. Variation in microbial numbers by canal type (M: Main, P: Primary, S: Secondary, and T: Tertiary).

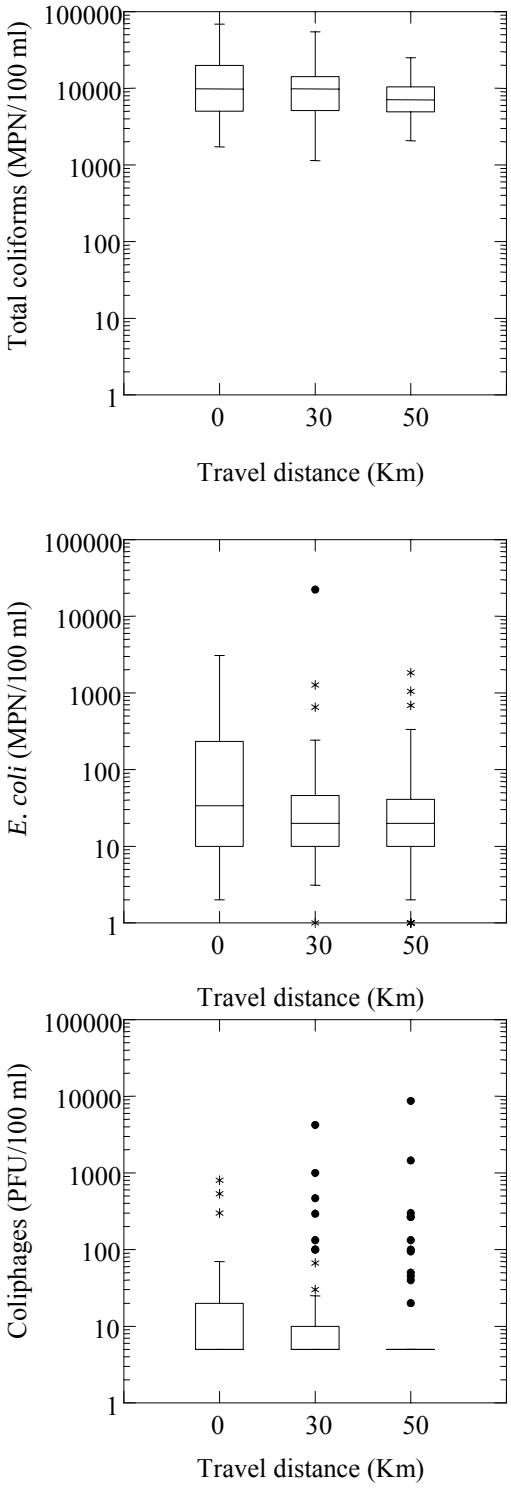


Figure 5. Microbial variation by travel distance from reservoir.

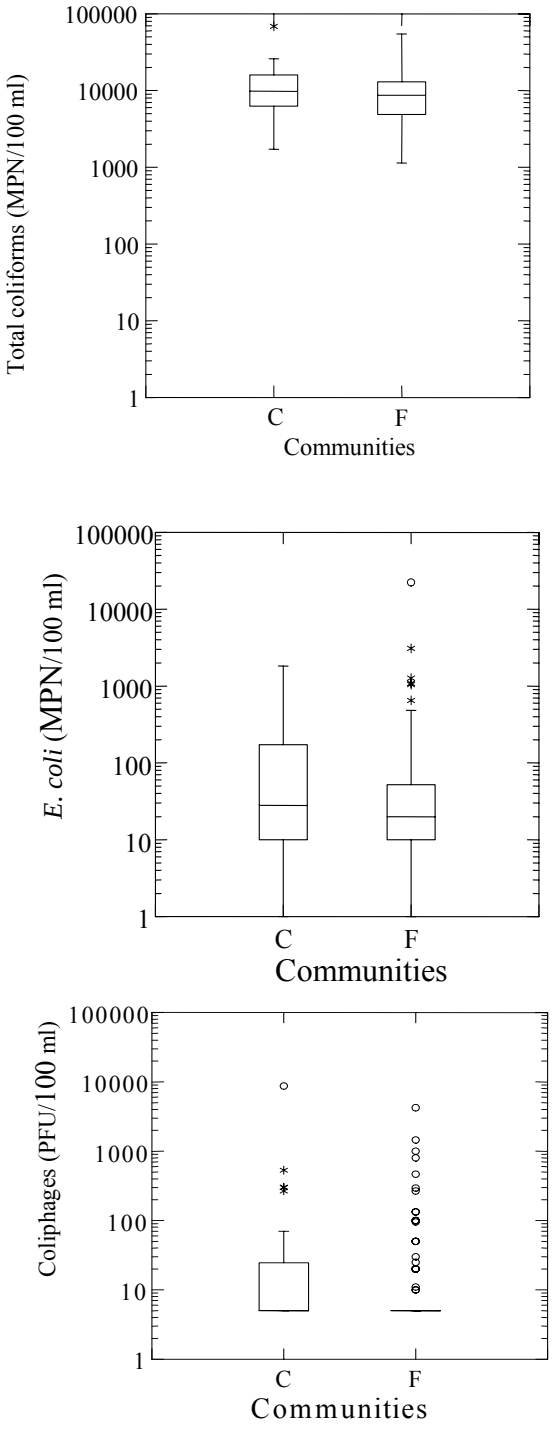


Figure 6. Microbial variation for population in the proximity to sampling sites. (C: Close (within 2 Km) to towns and F: Far to towns (out of 2 Km)

APPENDIX B:

ASSESSMENT OF AERATED LAGOONS FOR PRODUCTION OF IRRIGATION WATER

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Abstract

Treated wastewater is an important potential source of irrigation water in arid regions of the world. The reuse of treated wastewater for the irrigation of edible, non-edible crops and others plants depend on its physical, chemical, and microbiological quality. The World Health Organization has suggested treatment and microbial standards for reclaimed waste water to be used for food crop irrigation. The primary objective of this study was to determine if aerated lagoons used to treat municipal wastewater could meet these guidelines. In addition, to assess the impact of microbiological quality on vegetables, plots of carrots, lettuce, and tomatoes were established using furrow irrigation with both raw wastewater (RWW) and treated wastewater (TWW). Results indicate that the physical, chemical, and microbiological quality of treated wastewater is acceptable for agricultural irrigation as well as other uses. The microbiological quality of vegetables was not significantly different using treated or raw wastewater.

Key words

Wastewater, irrigation, reuse, vegetables, water quality, *E. coli*, *Giardia*, *Cryptosporidium*, enteroviruses, helminthes

Introduction

In many arid environments an alternative source of water is required to meet the growing demands of the communities, agriculture, and industry, thus wastewater reuse has become an increasingly important possible option (Rose, 1986) . The utilization of wastewater in arid regions in the world has been a common practice, especially in countries such as Australia, Israel, México, Saudi Arabia, South Africa, and the United Arab Emirates. Although the use of wastewater for agricultural irrigation has been practiced for centuries, it must be treated and carefully managed to prevent transmission of enteric pathogens and toxic chemical contaminants.

Public health risk due to the possible presence of enteric pathogenic organism is one of the major disadvantages for recycling wastewater (Rose, 1986). It is well established that, pathogenic bacteria, parasites, and viruses are found in sewage and they may survive treatment processes. Once in the environment, many of these are able to persist for prolonged periods of time. Sadowski *et al.* (1978) and Rose (1986) have demonstrated that crops directly irrigated with wastewater become contaminated with enteric microorganisms. Although enteric viruses do not grow on contaminated vegetables, they can survive long enough to cause disease in humans. Studies done by Tierney *et al.* (1977), and Ward and Irving (1987) in which enteric viruses were added to sewage effluent used for crop irrigation showed that viruses can remain viable from 3 to 5 weeks on crops. Furthermore, Badawy *et al.* (1985) found that enteroviruses and rotaviruses can survive 1-4 months on vegetables during commercial and household storage after harvesting.

Before 1990, only 10 percent of municipal wastewater in Mexico was treated. Currently, between 20 and 30 percent of the municipal wastewater receives some treatment (Monroy *et al.* , 2000). In 1994, the reuse of raw wastewater (RWW) was forbidden (Mexican Clean Water Program) and an arrangement to increase the volume of treated municipal wastewater was implemented by the Mexican Government. In 1996, two municipal WW treatment plants started operations in Ciudad Obregón, Sonora. These plants include primary (screen and sedimentation) and secondary treatment. The secondary treatment consists of three sets of three lagoons each, working sequentially. Every set includes an aerated pond, one facultative pond and finally an anaerobic-sedimentation lagoon. After the secondary treatment, the effluent is chlorinated. In total, both plants are treating around 1.0 cubic meter per second. Presently, this treated water is not being used and is discharged to an open canal which travels 60 km to the Gulf of Mexico.

Severe drought conditions have occurred in recent years in Sonora, Mexico. Therefore, the reuse of wastewater represents a potential alternative water supply for irrigated agriculture. However, before recycling treated wastewater is allowed additional information is needed on the quality of the treated wastewater. Hence, the goal of this study was to determine the physical, chemical, and microbiological quality of treated wastewater in Ciudad Obregón, Sonora and to assess the microbiological impact on agricultural products furrow irrigated using treated wastewater.

Methods

The study was conducted at El Instituto Tecnológico de Sonora in Ciudad Obregón, Sonora, Mexico, between May 1999 and April 2001. The project was carried out in two main parts: One of them related to the effluent quality evaluation and another to assess the microbiological impact on vegetables irrigated by treated wastewater

Chemical water quality

Wastewater quality parameters studied included: pH, temperature (T), electrical conductivity (EC), total dissolved solids (TDS), total nitrogen (TN), free ammonia (NH_3), hardness (H), alkalinity (ALK), setteable solids (SS), phosphate (PO_4), chloride (Cl), biological oxygen demand (BOD), chemical oxygen demand (COD), detergents (DET) greases (G), fecal coliforms (FC), cyanide (CN) and heavy metals (As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, and Zn). Analyses were carried out according to Standards Methods for Examination of Water and Wastewater (APHA, 1998).

Irrigation plots

Three vegetables, lettuce (leaf), carrots (root), and tomatoes (fruiting body), were planted in a total of six-344 m^2 plots. Three plots, one for each vegetable, were irrigated using treated wastewater (TWW); other three plots were supplied with raw municipal wastewater (RWW), as a control. There were five irrigation events, with each application being 20 cm of water supplied to each plot by furrow irrigation. Water, soil, and vegetable samples were analyzed for the presence of several organisms.

During each irrigation event, samples of RWW and TWW were collected for microbial assay. One-liter samples of both TWW and RWW were collected for bacteria assays according to the recommendations of APHA (American Public Health Association *et al.*., 1998). One hundred-ninety liters of TWW were processed for parasites and enteroviruses, according to the ICR Microbial Laboratory Manual (USEPA, 1996). Five hundreds milliliter RWW samples were collected for parasites and viruses. Fecal coliforms were detected using the membrane filter technique, using FC media as described in Standard Methods (APHA, 1998). Coliphages were assayed by the double-layer agar method described by Pepper *et al.* (1995). *Cryptosporidium* oocysts and *Giardia* cysts were assayed according to the ICR method (USEPA, 1996). Enzys Hydrofluor-Combo kit was used to detect *Giardia* cysts and *Cryptosporidium* oocysts. Enteroviruses were detected by production of cytopathogenic effects on the BGM cell line (APHA, 1998). The 9510C protocol was followed to elute and concentrate the treated wastewater samples (TWW). While the 9510D protocol was used to concentrate RWW samples. Both TWW and RWW samples were assayed via the cell culture technique as described in the 9510G protocol. Helminthes eggs were assayed as maintained by the Mexican Standard recommendation NMX-AA113-SCFI-1999 (SECOFI, 1999).

Soil Samples

Soil samples were collected and processed according to the recommendations given by Wollum (1994). Two composite soil samples were collected from each plot before wastewater was applied to irrigate vegetable plants, and after vegetables were

harvested. Eight samples were randomly taken from the top 20 cm of soil and mixed to make each composite sample. Ten grams of each composite soil sample were weighed and mixed with 95-ml of phosphate buffered solution (PBS) containing sodium dodecyl sulfate (SDS) in 250-ml previously sterilized bottles. The bottles were shaken by hand for 30 to 60 seconds and then placed on a horizontal shaker (rotary Lab Line shaker) and shaken for 20 minutes. After shaking, the bottles were allowed to stand for approximately 30 seconds. The soil extract was used to determine *Cryptosporidium* oocysts and *Giardia* cysts following the same procedure as outlined by Karim *et al.* (2004) for sediment samples.

Vegetable Samples

Five samples from each plot of lettuce, carrot, and tomatoes were randomly collected. All harvesting were conducted between four and fourteen days after an irrigation event. Vegetable samples were placed in sterile containers and transported in a cooler with ice to the laboratory. Approximately 250 grams of each vegetable were placed into sealable plastic bags. The vegetables were eluted by shaking at 150 rpm on a flat lab shaker for 10 minutes in a phosphate buffered saline solution (0.85 % NaCl in 0.02 M K_2HPO_4, pH 7.4 ± 0.2) at ratio of 1:2 (W/V, weight of vegetables to volume of rinse solution). This solution was then tested for the same organisms as the water samples. A similar volume of eluate was mixed with 500 ml beef extract and flocculated to concentrate enteroviruses to concentrate the eluate to a final volume of 20 – 25 ml before assay on the BGM wells. This procedure follows the method protocol 9510 c in

APHA (1998). The cysts and oocysts were concentrated from 250 ml of this eluate to a volume of 10 – 25 ml by low speed centrifugation and then assayed as the water samples.

Results and Discussion

Chemical water quality

Table 1 shows physical and chemical results and Table 2 shows the microbiological results. Effluent quality is similar as reported in other studies (Rose, 1986; Sadowsky, 1978; Metcalf and Eddy, 1994). Comparison of these results as well as between Mexican and international guidelines is shown in Table 3. Data for treated wastewater appear to indicate that the treated wastewater effluent can be used in agricultural to irrigate restricted crops. Although an exhaustive screening of all the parameters that must be satisfied to demonstrate the reuse of effluent would be recommended in other activities such as industrial, recreational, and aquifer recharge was not carried out as part of the present study, several parameters studied in treated wastewater met some of those requirements. Parasites such as *Cryptosporidium* and *Giardia* are not included in Mexican and International guidelines for irrigation water or reuse of treated wastewater these are of concern particularly because of their concentration levels are close to the infective doses (Bitton, 1994; Blumenthal *et al.*., 2000).

The level of *Cryptosporidium*, 18 ooysts/100 ml, and *Giardia*, 1200 cyst/100 ml is greater than the infective dose levels. Therefore, precautions must be taken if the effluent is used to grow fresh-food or activities where direct human contact is expected. Results from this study confirm that fecal coliform (99.998 %), coliphages (99.4%) and enteric viruses (99.1 %) were efficiently removed by the wastewater treatment processes. However, lower removals were observed for BOD (60 %) or COD (57%), as well as

Helminthes (> 84.6 %), *Cryptosporidium* oocysts (68.4 %), and *Giardia* cysts (50.0 %). The relatively low removal efficiency for organic matter and helminthes, nevertheless, was sufficient to guarantee that the treated wastewater met the Mexican and International guidelines to be used in agriculture (SEMARNAP, 1997; SEMARNAP, 1998).

Treated wastewater reuse

Results of microbiological quality of vegetables irrigated by TWW and RWW are shown in Table 4. Organisms were found at relatively low concentrations whether the vegetables were irrigated with RWW or TWW. The concentrations of all organisms studied were higher in RWW than in TWW, however, no significant difference was observed on the microbiological quality of vegetables regardless of the type of irrigation water used. *Cryptosporidium* and *Giardia* were found to accumulate on the soil irrigated with RWW or TWW (Figure 1). Parasite accumulation in soil is due to size of *Cryptosporidium* oocysts (2 – 4 μ) and *Giardia* cysts (6-10 μ) which is greater than soil pore size (1 – 2 μ).

Conclusions

Data for treated wastewater appear to indicate that the treated wastewater effluent can be used in agricultural to irrigate restricted crops. However, due to the level of *Cryptosporidium*, 18 ooysts/100 ml, and *Giardia*, 1200 cyst/100 ml is greater than the infective dose levels, precautions must be taken if the effluent is used to grow fresh-food or activities where direct human contact is expected.

Even though the RWW contained much greater concentrations of indicators bacteria, parasites, and viruses no significant difference was found in the microbial quality of vegetables irrigated with raw wastewater or with treated wastewater.

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Table 1. Physical and chemical water quality

	RWW			TWW			REMOVAL
	Samples Number	Range	Mean	Samples Number	Range	Mean	%
T (^oC)	655	19.0 – 34.0	27.4	655	18.0 – 35.0	27.3	
PH	700	6.8 – 8.0	7.1	698	6.7 – 8.0	7.0	
EC	241	538.0 – 1131	684.5	241	587.0 – 995.0	689.0	
ALK	57	168.0 – 280.0	228.0	57	175.0 – 282.0	222.2	2.5
HARD	57	102.0 – 157.0	122.5	57	104.0 – 163.0	126.9	
Cl	57	42.0 – 93.0	59.8	57	50.0 – 135.0	65.1	
TS	53	452.0 – 627.0	542.2	52	315.0 – 592.0	474.0	12.6
TSS	614	105 – 690	178.7	548	46.0 – 165.0	71.4	60.0
TDS	53	308.0 – 464.0	385.1	52	226.0 – 523.0	401.7	
SS	604	0.7 – 4.0	2.0	241		< 0.1	
$N\text{-}NH_3$	6.	18.8 – 22.4	20.8	8	17.0 – 20.1	18.1	13.0
N_T	20	25.7 – 33.3	29.9	29	21.3 – 35.7	26.3	12.
PO_4	27	4.4 – 6.3	5.2	28	3.6 – 5.9	4.6	11.5
BOD	162	107.0 – 263.9	169.1	257	40.4 – 94.1	66.9	60.4
COD	501	234.7 – 640.0	433.4	508	111.1 – 244.2	186.6	56.9
DET	6	5.4 – 9.6	7.2	7	1.3 – 2.9	2.4	66.7
G	3	10.4 – 12.1	11.4	38	3.1 – 23.6	6.5	43.0

Units are given in mg/ml except pH, temperature (^oC), and EC (dS/cm). Cyanide and heavy metals were below detection limits (< 0.001 for Hg, < 0.002 for CN, < 0.01 for As and Ni, < 0.02 for Cd, < 0.03 for Cu, and < 0.1 for Cr, Pb, and Zn). Removal percent was estimated using the mean values

Table 2. Microbial water quality.

Organism	Units	Influent			Effluent			Rem
		Samples	Range	Mean	Samples	Range	Mean	(%)
Fecal coliforms	(MPN/ 100 ml)	6	8.0E+06–2.6E+07	1.7E+07	102	1.2E+01–1.2E+02	2.2E+02	99.9987
Coliphages	(PFU/100 ml)	5	6.7E+00–6.6E+07	2.2E+07	5	3.0E+00–1.8E+05	1.3E+05	99.4
Enteric viruses	(MPN/ 100 ml)	3	5.5E+000–4.5E+01	2.5E+01	9	1.9E-02– 9E+00	2.3E-01	99.1
Helminthes	(eggs/liter)	12	6.0E-01–2.6E+00	1.3E 00	12	< 0.2		>84.6
Cryptosporidium	(Oocysts/100 ml)	8	1.0E+01–4.8E+02	5.7E+01	12	4.4E+00–2.5E+02	3.9E+01	68.4
Giardia	(Cysts/100 ml)	8	5.7E+01–6.0E+03	2.4E+03	13	6.6E+01–2.6E+03	1.2E+03	50.0

Rem (%): Removal percent, which was estimated using the mean values

Table 3. Comparison between this study results and water quality guidelines for different reuses of treated wastewater.

Water Parameter	Mexican Standards Guidelines				Results This Study
	Irrigation	Public Services		Others	
		Direct Contact	Indirect or casual contact		
pH	5.0 – 10.0	5.0 – 10.0	5.0 – 10.0	6.5 – 8.4[e]	7.0
T (^{o}C)	40.0				27.3
G	15.0	15	15		6.5
FM	Absent	Absent	Absent		Absent
SS	1.0				< 0.1
TS					474
TSS	75.0	20	20		71.4
EC (ds/cm)				0.0 - < 3.0[e]	0.69
TDS				0.0 - < 2000.0[e]	401.7
Cl				0.0 – 355.00[e]	65.1
BOD	75.0	20	30		66.9
TN	40.0				18.1
TP	20.0	20.0	20.0		4.6
As	0.2 – 0.4	0.2 – 0.4	0.2 – 0.4	0.1[e]	< 0.01
Cd	0.2 – 0.4	0.2 – 0.4	0.2 – 0.4	0.01[e]	< 0.02
Cn	2.0 – 3.0	2.0 – 3.0	2.0 – 3.0		< 0.002
Cu	4.0 – 6.0	4.0 – 6.0	4.0 – 6.0	0.20[e]	< 0.03
Cr	1.0 – 1.5	1.0 – 1.5	1.0 – 1.5	0.1	< 0.1
Hg	0.01	0.01	0.01		< 0.001
Ni	2.0 – 4.0	2.0 – 4.0	2.0 – 4.0	0.2[e]	< 0.01
Pb	0.5 – 1.0	0.5 – 1.0	0.5 – 1.0	5.0[e]	< 0.1
Zn	10.0 - 20.0	10.0 - 20.0	10.0 - 20.0	2.0[e]	< 0.1
FC (MPN/100 ml)	1,000	240	1,000	1,000[f] – 100,000[g]	220
HE (Eggs/Liter)	≤ 1.0[c] – ≤ 5.0[d]	≤ 1.0	≤ 5.0	≤ 5.0[f]	< 0.2

All units are given in mg/Liter except for pH and when other units are annotated

a: water quality for agriculture activities (SEMARNAP, 1997).

b: According to the Official Mexican Standard NOM-SEMARNAP-003 (SEMARNAP, 1998)

c: For non-restricted crops. All crops may be seed, growth, and harvested, including grains, vegetables

d: For restricted crops: non for fresh food vegetables

e: From Ayers and Westcot (1989)

f: For unrestricted irrigation, from Blumenthal *et al.* (2000)

g: For restricted irrigation, from Blumenthal *et al.* (2000)

Table 4. Microbiological Quality of vegetables irrigated with Raw Wastewater (RWW) and Treated wastewater (TWW).

Organism	Carrots		Lettuce		Tomatoes	
	RWW	TWW	RWW	TWW	RWW	TWW
FC (MPN/g)	< 1 to 30	< 1 to 3	< 1 to 20	< 1 to 2	< 1 to 10	< 1 to 2
CPH (PFU/g)	< 1	< 1	< 1	< 1	< 1	< 1
GC (cyst/g)	<0.01 to 0.02	< 0.01 to 0.02	< 0.01 to 0.04	< 0.01 to 0.02	< .01 to .02	< .01 to .02
CO (oocyst/g)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

FC: Fecal coliforms, CPH: Coliphages, GC: *Giardia* cysts, CO: *Cryptosporidium* oocyst organism

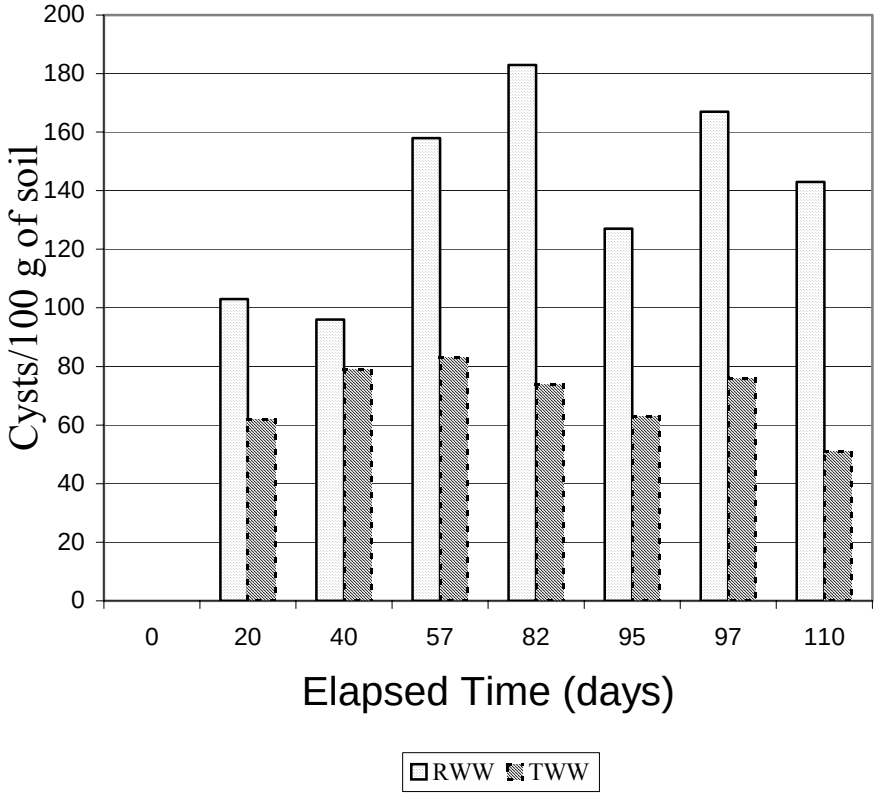

Figure 1. *Giardia* cysts accumulation in soli.

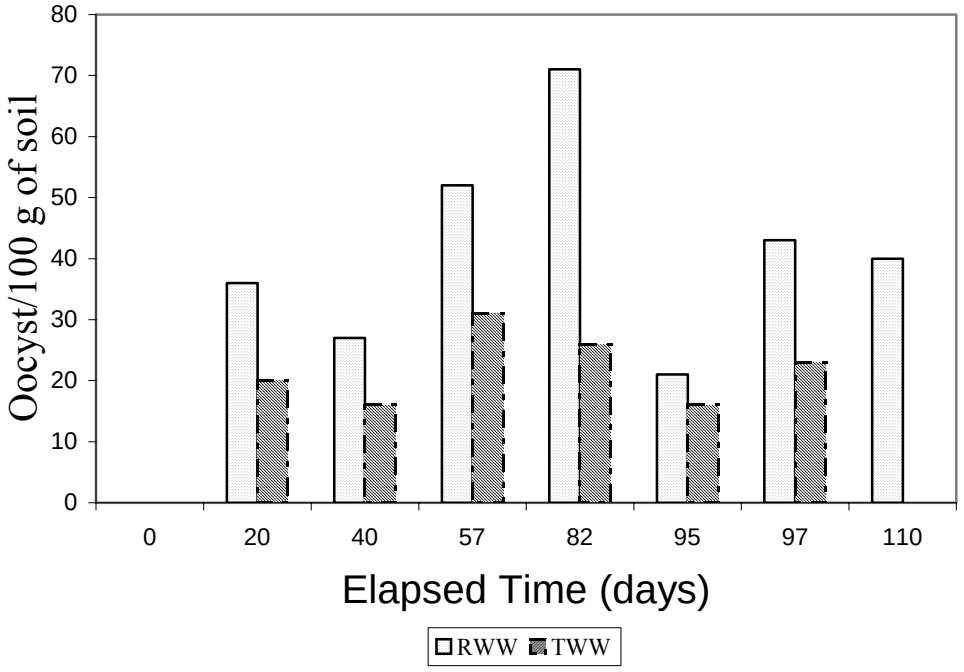

Figure 2. *Cryptosporidiumiardia oo*cysts accumulation in soli.

APPENDIX C:

THE IMPACT OF IRRIGATION WATER QUALITY ON MICROBIAL CONTAMINATION OF PRODUCE

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Abstract

The goal of this study was to assess the potential for contamination of vegetables during irrigation with untreated wastewater and surface water. Untreated municipal wastewater (WW) from a small town (El Tobarito) in El Valle del Yaqui, Sonora, Mexico and surface irrigation water (IW) from storage dams were used to irrigate three different vegetables: lettuce, carrot, and green pepper. Samples of each vegetable, as well as WW and IW, were examined for: total coliforms, *Escherichia coli*, *Clostridium perfringens*, *Cryptosporidium* oocysts, *Giardia* cysts, coliphages, and enteroviruses. All of the studied microorganisms were present in the untreated wastewater, but only total coliforms; *E. coli* and *C. perfringens* were present in the surface irrigation water. Total coliforms, *E. coli*, and *C. perfringens* were detected on all vegetables regardless of irrigation source water. The highest concentration of these organisms was found on carrots followed by lettuce and green peppers. *E. coli* levels were 10 to 100 times on produce irrigated with untreated wastewater vs. surface water.

Key Words

Treated wastewater, untreated wastewater, water quality, vegetables, *E. coli*, *Giardia*, *Cryptosporidium*, enteroviruses, helminthes

Introduction

Minimally processed and refrigerated (MPR) fruits and vegetables are important sources of fresh produce and their per capita consumption is increasing. In most cases, MPR foods are consumed raw, making the presence of pathogens a concern. Han *et al.* (2000) noted that pathogens can reach MPR food from several sources including, but not limited, irrigation or wash water, infected operators, animal waste and municipal biosolids used as fertilizers, and operational facilities with poor sanitation. Each year food-borne illness affects 6 to 80 million people, cause 9,000 deaths, and cost an estimated five billion dollars in the USA (Altekruse *et al.*, 1997). Fresh fruit and vegetable consumption in the USA has increased approximately 60 percent during the last 30 years (Forrest and Gushulak, 1997; Majkowski, 1997; Miller and Paige, 1998).

In many arid environments additional sources of water are needed to meet the growing demands of municipalities, agriculture, and industry. Wastewater as one of the few growing sources of supplies has increasingly become a viable supply option (Rose, 1986). The use of wastewater in arid regions in the world has been a common practice, especially in countries such as Australia, Israel, Mexico, Saudi Arabia, South Africa, and the United Arab Emirates (Rowe and Abdel-Magids, 1995).

Several studies have demonstrated that crops directly irrigated with wastewater become contaminated with enteric pathogens including viruses (Sadovski *et al*., 1974; Sadovski *et al*., 1978a; Sadovski *et al*., 1978b; Rose, 1986). Although enteric viruses cannot grow on contaminated vegetables, they can survive long enough to cause disease in humans (Rowe and Abdel-Magids, 1995). Studies conducted by Tierney *et al*. (1977)

and Ward and Irving (1987), in which enteric viruses were added to sewage effluent used for crop irrigation, have shown that viruses can remain viable from 3 to 5 weeks under growing conditions. Furthermore, Badawy *et al*. (1985, 1990) affirm that enteroviruses and rotaviruses can survive 1-4 months on vegetables during commercial and household storage after harvesting.

In Mexico, particularly in arid regions like the state of Sonora, wastewater recycling has been an important potential alternative source to satisfy the growing demand for water (Jimenez and Chavez-Mejia, 1997; Jimenez *et al*., 1999). In recent years, several projects have been conducted in El Valle del Yaqui, Sonora, Mexico, to evaluate water quality, treatment, and reuse of wastewater and sewage for irrigation (Gortares, 1992; 1993; 1997;1998; Gortares and Castro, 1993), as well as to assess the level of enteric organisms on vegetables sold in supermarkets (Hirotani *et al*., 2002).

The goal of this study was to assess the microbiological water quality of both surface irrigation water and untreated municipal wastewater and their potential for contamination of vegetables irrigated with these waters.

Methods

Location of Study

The study was conducted at El Instituto Tecnológico de Sonora in Ciudad Obregón, Sonora, Mexico, located in El Valle del Yaqui (The Yaqui Valley) (Figure 1), which occupies five counties including Bacum, Guaymas, Navajoa, Etchojoa, and Cajeme, with an overall population of around 700,000 inhabitants. Cuidad Obregon,

which is located in Cajem County has 400,000 inhabitants and it is the major city in El Valle del Yaqui. The Yaqui Valley is widely known as one of the most developed agricultural regions in Mexico.

Irrigation and Plot Design

Untreated domestic wastewater (WW) from El Tobarito, a small town close to the experimental field, and surface irrigation water (IW) were used to irrigate the experimental plants. The surface irrigation water originates from a dam on the Yaqui River and does not receive sewage discharges. Three vegetables, lettuce (leaf), carrots (root), and green peppers (fruiting body), were planted in six-156 m^2 experimental plots. Distribution and plot size are shown in Figure 2. Seeds of the selected vegetables were planted at the end of November 2000, and only IW was used for all plots for the first two months. After, one plot of each vegetable was irrigated, every 10-15 days, using WW and the control plots continued to be supplied with IW. Furrow irrigation was used to deliver a total of 16,125 gallons (61,036 liters) to each plot during seven irrigation events. Water, soil, and vegetable samples were analyzed for several organisms according to the methodology described below.

Irrigation Water Analysis

To establish background concentrations, both WW and IW were analyzed for the presence of the following organisms: Total coliforms, *Escherichia coli*, *Listeria monocytogenes*, *Clostridium perfringens*, *Cryptosporidium* oocysts, *Giardia* cyst,

coliphages, and enteroviruses. During each irrigation event, three one liter-samples of both types of water (IW and WW) were collected for bacteria and coliphages, but only one sample was collected for parasites and enteroviruses. In the case of IW, 190 liters were collected for parasites and enteroviruses, according to Standard Methods for the Examination of Water and Wastewater (APHA, 1998). Positively charged filters 1MDS filter cartridges (Cuno Inc., Meriden, CT) were used to collect virus samples and Filterite polypropylene cartridges (Filterite Corporation, Timorium, MD) were used to collect parasite samples. In contrast only five hundred milliliters of unfiltered WW samples were collected for parasites and enteroviruses .

Simultaneous enumeration of total coliforms and *E. coli* was performed using the Colilert Quanti-tray system (IDEXX Laboratories, Inc., West Brook, MA). Results were reported as Most Probable Number (MPN) per ml.

Membrane filtration was used to detect *C. perfringens* as described by Bisson and Cabelli (1979). The samples were heat shocked at 65-70^{o}C in a water bath to stimulate sporulation of vegetative cells. Heat shocked water samples were then filtered through a 0.45 μm pore size membrane filter and transferred to mCP media (Acumedia, Baltimore, MD) and incubated in an anaerobic jar for 24 hours at 42^{o}C. Yellow colonies turning pink to red after exposure to ammonium hydroxide vapor were considered as presumptive *Clostridium* colonies.

The double-layer agar method described by Peppers *et al*. (1995) was used to detect coliphages using *E. coli*, strain ATCC 15597, four replicates of five and one milliliter aliquots of both types of waters were assayed for each sample.

Cryptosporidium and *Giardia* were concentrated by density gradient centrifugation and detected by use of an indirect fluorescent antibody (APHA, 1998). The Ensys Hydrofluor-Combo II kit (Product Number 7080500 from Strategic Diagnostic Inc., Newark, DE) for the simultaneous detection of *Giardia* cysts and *Cryptosporidium oocysts* was used.

Enteric viruses were analyzed according to Standard Methods for the Examination of Water and Wastewater (APHA, 1998). Protocol 9510C was followed to elute and concentrate the irrigation water samples (IW). Five hundred milliliters samples of WW were concentrated following the 9510D protocol. Both IW and WW samples were assayed using the cell culture technique as described in protocol 9510G, in which cytopathic effects (CPE) on BGM cells is used for virus detection. Cell cultures were observed for CPE during 14 days. If no CPE was observed, they were passed a second fourteen-day period.

Soil Analysis

Soil samples were collected and processed according to Wollum (1994), Scott (1994), and Zuberer (1994). Two composite soil samples were collected from each plot before and after the application of WW. Eight soil simple samples (around 50 to 100 grams each) were randomly taken from the top 20 cm of soil and mixed to make each composite sample. Ten grams of each composite soil sample were weighed and mixed with 96-ml of peptone water (0.1%) in 250-ml sterile bottles. The bottles were shaken by hand for 30 to 60 seconds and then placed on a horizontal rotary shaker and shaken for 20

minutes. After shaking, the bottles were allowed to stand for approximately 30 seconds. The soil extract was used directly or after decimal dilutions with peptone water (0.1%) to determine *Escherichia coli*, *Clostridium perfringens*, and coliphage concentrations. *E. coli* and coliforms were detected according to the procedure described by Turco (1994), while *Clostridium perfringens* and coliphage were assayed using the same technique used for water samples.

Vegetable Analysis

Twenty-four samples were randomly collected from each plot of lettuce and carrots, while twelve samples were randomly collected from each plot of green peppers. All harvesting was conducted between ten and fourteen days after an irrigation event. Vegetable samples were placed in sterile containers and transported on ice to the laboratory. Each lettuce sample (approximately 500 grams) consisted of the first two outer leaves of four heads of lettuce. The surface area of lettuce was estimated assuming that this vegetable has a somewhat spherical shape. The diameter of each head sampled was measured and the surface area was estimated using the sphere surface equation. ($a = \pi r^2$, where a is the surface area, and r is the radius)

Approximately 500 grams samples of carrot and green pepper were used for microbial assays. The surface area of each individual carrot and green pepper was estimated. Since these vegetables are somewhat conical in shape, the length and top diameter of each carrot and each green pepper was measured and the surface area was determined using the circular cone surface equation. ($a = 2\pi r*(r^2 + h^2)^{1/2}$, where a is the

lateral surface area, r is the radius and h is the length). Each carrots sample consisted of around 6 to 10 individual carrots and each green peppers sample consisted between 8 and 15 individual green pepper.

Vegetable samples were placed into Ziploc clear plastic bags, which were collected inside four-liter beakers. Vegetable samples were eluted by shaking at 150 revolutions per minute on a water bath shaker (New Brunswick Scientific, Edison, NJ, USA) for 10 minutes in a phosphate buffered saline solution (0.85% NaC1 in 0.02 M K_2HPO_4, pH 7.4 to 7.8) a ratio of 1:2 (W/V, weight of vegetables to volume of rinse solution). The elution obtained from the shaken vegetable samples was analyzed for the same organisms as the water samples. All vegetable samples were analyzed for bacteria and bacteriophages but only eight samples from each plot of lettuce and carrot and four samples from each plot of green peppers were analyzed for parasites and enteroviruses.

The same techniques as described for water samples were used to determine the concentration of total coliforms, *E. coli*, *C. perfringens*, and coliphage.

Cryptosporidium oocysts and *Giardia* cysts were concentrated and detected from 250 ml volumes of the vegetable elution using the same procedure like water samples Enteroviruses were concentrated from 250 ml aliquots of the vegetable elution by addition of 500 ml of 10% beef extract and concentrated to a final volume of 20 to 25 ml by flocculation (protocol 9510C from APHA, 1998).

The recovery efficiency for each organism assayed in this study was determined previously using saline solution to elute organisms from vegetables and according to the technique indicated in the part of water samples for each organism. Values of recovery

efficiency are given on Table 1. Lettuce and green peppers were used to carry out the assessment of two methods for the recovery of organism from vegetables surfaces. Particularly, lettuce was selected for this purpose based on the morphological complexity of wax structure present on its leaves. It was assumed that if saline solution could be used to recover organism from lettuce leaves this option would likely work better with both green peppers and carrots.

Results

Water quality

Irrigation Water

The results of the microbial analysis of IW and WW are shown in Table 2. The number of fecal coliform (*E. coli*) found in the IW ranged from 1.04 and 3.70 with a geometric mean of 2.41 $\log_{10}$ MPN/100 ml. This geometric mean value was found to be below both the Mexican and International Standards (1000 MPN/100 ml) for irrigation water (Secretaria del Medio Ambiente Recursos Naturales y Pesca, 1997; WHO, 1989; Blumenthal *et al*., 2000). No enteroviruses or protozoan parasite were detected in the IW. Coliphage concentrations ranged from 2.00 to 2.94 $\log_{10}$ per liter.

Cryptosporidium oocysts and *Giardia* cysts were below the detection limit in this study. *Clostridium perfringens* concentrations ranged from 2.0 to 5.58 $\log_{10}$ per liter. In contrast, *L. monocytogenes* was not detected.

Wastewater

The concentrations of the organisms studied in the WW are similar to those found in typical municipal wastewater (Rose, 1986). All the organisms evaluated were detected at concentrations 100 to 1,000 times greater in WW than in IW. *E. coli* ranged between 6.30 and 7.21 $\log_{10}$ MPN/100 ml with a geometric mean of 7.69 $\log_{10}$ MPN/100 ml. Coliphages ranged from 5.20 and 6.55 $\log_{10}$ PFU/100 ml with a geometric mean of 6.62 $\log_{10}$ PFU/100 ml. *Cryptosporidium* oocyst values ranged between 2.8 and 3.78 $\log_{10}$/100 ml with a geometric mean of 3.16 $\log_{10}$ oocysts/100 ml and *Giardia* cysts between 3.84 and 4.67 $\log_{10}$/100 ml with a geometric mean of 4.21 $\log_{10}$ cysts/100 ml. Enteroviruses ranged from 0.94 and 3.18 $\log_{10}$/100 ml with a geometric mean of 1.91 $\log_{10}$ MPN/100 ml.

Soil quality

The results of the soil analysis are shown in Table 3. The concentration of total coliforms and *E. coli* were below the detection limit ($\leq$ 3.30 CFU/g) in soil samples collected prior to the application of wastewater. Post harvest sampling taken after several applications of wastewater contained a geometric mean of 120 coliforms per gram and 4 to 110 *E. coli* per gram. In contrast, *C. perfringens* did not increase in numbers after application of the wastewater. The geometric mean values obtained for soil samples were 321 and 145 CFU/g before and after wastewater application. Coliphages were not detected (less than the detection limit, $\leq$ 0.67 PFU/g) either in soil samples taken before or after wastewater application.

Vegetable quality

Microbiological results for vegetables are given in Tables 4, 5, and 6. *Listeria monocytogenes*, parasites, enteroviruses, and coliphages were not detected on the harvested lettuce, carrots, and green peppers irrigated with IW or WW. Only total coliforms and *E. coli* were detected on the harvested vegetables. Results of lettuce sample analysis are shown in Table 4. Total coliforms were greater than 48 MPN/g in all samples. *E. coli* was not detected on the vegetables irrigated with IW (≤ 0.02 MPN/g); however, when WW was used, 11 of 24 samples were positive (geometric mean of 0.05 MPN/g).

C. perfringens was found on all the vegetables whether irrigated with IW or WW. When IW water was used, values were between 0.2 and 3.75 MPN/g with a geometric mean of 0.9 MPN/g while WW irrigated lettuce had between 0.38 and 3.98 MPN/g with a geometric mean of 1.16 MPN/g for IW and WW, respectively. Coliphages, enteroviruses, *Giardia* cysts, and *Cryptosporidium* oocysts were not detected.

Results of carrot samples are shown in Table 5. All (24) samples were positive for total coliforms whether irrigated with IW or WW. The values ranged between 3.1 and 48.4 MPN/g with a geometric mean of 10.6 MPN/g and between 4.7 and 39.7 MPN/g with a geometric mean of 17.5 MPN/g for IW and WW, respectively. *E. coli* was detected in only two of 24 samples irrigated with IW and 22 of 24 samples when WW was applied (geometric mean of 0.17 MPN/g). *C. perfringens* was positive for all samples (24) whether the vegetables were irrigated with IW or WW. The level of

Clostridium was between 0.5 and 39.0 CFU/g with a geometric mean of 11.0 CFU/g on the carrots when IW was applied. Carrots irrigated with WW ranged from 2.0 and 22.0 CFU/g with a geometric mean of 8.6 CFU/g. All carrot samples analyzed for coliphages (24 samples), enteroviruses (8 samples), *Giardia* cysts (8 samples), and *Crytosporidium* oocysts (8 samples) were below the detection limits for these organisms.

Results of green pepper samples are shown in Table 6. Eleven of twelve green peppers samples were positive for total coliforms from plots irrigated using IW or WW. The level of coliforms, on green peppers samples, was between 0.5 and 4.7 MPN/g coliforms with a geometric mean of 1.2 MPN/g when IW was used and between 0.4 and 17.2 MPN/g with a geometric mean of 3.3 MPN/g for WW irrigated plants. *E. coli* was found in only one of twelve green pepper samples irrigated with IW while 9 of 12 samples from irrigated WW vegetables were positive with values between 0.02 and 5.5 MPN/g and a geometric mean of 0.16 MPN/g.

C. perfringens was detected in three of twelve green pepper samples when IW was used and only two of twelve samples when WW was used with a value of 0.05 CFU/g. No coliphages, enteroviruses, *Giardia* cysts, or *Cryptosporidium* oocysts were detected.

Although more positive samples were detected for *E. coli* and *C. perfringens* (Fig. 6), when WW was used for irrigation in comparison to IW for all three vegetables studied, the number of organisms per gram of vegetable was not significantly different between vegetables irrigated with IW and WW.

Discussion

No significant difference in the number of indicator organisms and enteric pathogens were found on vegetables irrigated with IW or WW. Geldreich and Bordner (1971) reported that when the amount of fecal coliforms in irrigation water is low (less than 630 MPN per 100 ml) no significant differences were observed in the number or organisms found on root crops and leafy vegetables. However, when the amount of fecal coliforms was around 53,000 MPN per 100 ml, they found a greater difference between root crops and leafy vegetables. The level of fecal coliforms, 269 CFU per 100 g for root crops and 16 CFU per 100 g of leafy vegetables, reported by Geldriech and Bordner (1971), is similar to levels of *E. coli* in this study, 17 CFU per 100 g of root crops (carrots) and 5 CFU per 100 g for leafy vegetables (lettuce).

The numbers of *E. coli*, fecal coliforms, and *Clostridium perfringens* detected in this study as well as those reported by Geldreich and Bordner (1971) are on the order of 0.1 and 2.0 $\log_{10}$ per 100 g of vegetables. In contrast, these values are much lower than those reported in other studies of vegetables (Ercolani, 1976; Beuchat, 1996; Francis *et al*., 1999; Garg *et al*., 1990; Hirotani *et al*., 2002; Monge and Arias, 1996; Monge and Chinchilla, 1996; Nguyen-the and Carlin, 1994). However, in most of these studies the vegetables were collected from supermarkets and restaurants and not from the field. Results obtained in our own study as well as the Geldriech and Bordner (1971) strongly *C. perfringens*, and parasites in the wastewater used in this study, only small numbers of these organisms were detected in both soil and vegetables. The low level of microbes

found in the soil and vegetables may be due to inactivation of the organism on the crops (Seo and Frank, 1999; Noy and Feinmesser, 1977) and the method used to elute the samples during analysis (Sadovski *et al*., 1978b; Jackson, 1990; Sadovski *et al*., 1978a). The vegetables were not harvested until 10-14 days after the last irrigation event which may have allowed for a significant decrease of the indicator organisms.

According to several studies (Brackett, 1992; Rowe and Abdel-Magids, 1995; Beuchat, 1996; Beuchat, 1998; Brackett, 1992), organisms can survive for varying periods of time in soils depending on different factors such as the physical and chemical properties of the soil, the type of microorganism, and environmental conditions. Some investigators (Sadovski *et al*., 1978b; Sadovski *et al*., 1978a; Shuval *et al*., 1989; Monge *et al*., 1996; Shuval *et al*., 1997) have suggested that environmental conditions such as sunlight, temperature, rainfall and others have an important effect on the ability of organisms to survive in the soil.

The Mexican and international microbiological water quality standards used for agriculture irrigation established that, in addition to fecal coliforms, the number of helminthes eggs is another indicator to be considered. Although helminthes eggs were not analyzed in this study, Martinez-Acuña (2002) demonstrated, in a study carried out in municipal wastewater from ciudad Obregón Sonora México, that these eggs are below that value (≤1 helminthe egg/liter) recommended for WHO (1989) and Mexican Standards (Secretaria del Medio Ambiente Recursos Naturales y Pesca, 1997) for unrestricted irrigation.

According to Blumenthal *et al*. (2000), the absence of fecal indicator bacteria in wastewater may be unnecessarily strict and could result in high costs per case of infection averted. Furthermore, they suggest that the high costs might be justifiable in industrialized countries with low levels of endemic enteric disease. However, that may not be justifiable in countries with higher levels of endemic enteric infections, where these diseases are more often transmitted through poor hygiene and sanitation than through wastewater reuse and where resources for preventive health care are limited. The authors recommend that other potential sources of crop contamination should be considered such as crop handling, transportation, and the sale of produce in unhygienic markets.

Conclusions

In spite of the great difference in the microbial water quality of IW and WW used to irrigate the vegetables tested in this study, no significant differences were found in the microbiological quality of field harvested vegetables after ten to fourteen days. The highest level of microbial contamination was found on carrots, followed by lettuce with even lower microbial concentrations on green peppers.

Although the differences in microbial numbers between IW and WW were around three to four logs, no significant differences were found in either the soil microbial populations or the vegetable. Lack of these differences may be due to indicator die-off since the last irrigation event (10-14 days), low level of crop exposure, and the sensitivity of the methods used to detect the enteric viruses and parasites.

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Table 1. Organism Recovery efficiency using saline solution

Organism	Recovery Efficiency (%)	
	Lettuce	Green Peppers
E. coli	75.7	23.0
L. monocytogenes	73.0	50
C. perfringens	NA	41.7
Coliphages	85.5	34.4
Enetroviruses	54.1	46.4
Cryptosporidium	40.1	68
Giardia	57.3	59.6

NA: Not assayed

Table 2. Microbiological Analysis of Irrigation Water (IW) and Wastewater (WW). All organism numbers are Log_{10}

Statistics	Total Coliform (MPN/1)		*E. coli* (MPN/1)		Coliphage (PFU/1)		*C. perfringens* (CFU/1)		Enteroviruses (MPN/1)		*Giardia* (Cysts/1)		*Cryptosporidium* (Oocysts/1)	
	IW	WW	IW	WW	IW	WW	IW	WW	IW	WW	IW	WW	IW	WW
Number of Samples	15	15	18	18	18	18	18	15	5	5	5	5	5	5
Minimum	3.91	7.38	2.04	7.30	2.00	6.20	2.00	4.71	NA	1.94	NA	4.84	NA	3.80
Maximum	5.89	8.62	4.70	8.21	2.94	7.55	3.53	5.58	NA	4.18	NA	5.67	NA	4.78
Median	4.67	8.05	3.61	7.62	2.00	6.62	2.81	5.37	<-1.65	3.01	<0.124	5.22	<0.124	4.10
Geometric Mean	4.71	8.02	3.41	7.69	2.12	6.73	2.75	5.20	<-1.65	2.91	<0.124	5.21	<0.124	4.16
Standard Deviation	0.56	0.40	0.92	0.28	0.29	0.36	0.46	0.33	NA	0.85	NA	0.34	NA	0.37

IW : Irrigation water
WW: Wastewater
NA: Not applicable because none detected

Table 3. Soil Microbiological Analysis before and after Irrigation with Wastewater

Statistics	Units	*C. perfringens*		Total Coliforms		*E. coli*	
		Before	Alter	Before	Alter	Before	After
No. of samples		12	12	12	8	12	6
Minimum	(CFU/g)	33	23	NED	2	NED	4.3
Maximum	(CFU/g)	1300	330	NED	120	NED	110
Median	(CFU/g)	152	97	NED	15	NED	12.0
Geometric Mean	(CFU/g)	321	145	NED	40	NED	42.2
Standard Deviation		392	111	NED	48.31	NED	52.6

NED: Not enough data, because all data were below detection limit (≤3.30 CFU/g)

Table 4. Microbiological Results for Lettuce

Statistic	Total Coliforms (MPN/g)		*E. coli* (MPN/g)		*C. perfringens* (CFU/g)	
	IW	WW	IW	WW	IW	WW
Number of Simples	24	24	24	24	24	24
Positive Simples	24[a]	24[a]	0[b]	13[c]	24	24
Arith. Mean	NED	NED	NED	0.09	0.90	1.2
Geometric Mean	NED	NED	NED	0.05	0.67	1.0
Median	NED	NED	NED	0.02	0.48	1.18
Maximum	NED	NED	NED	0.26	3.7	3.98
Minimum	NED	NED	NED	0.02	0.20	0.38
Standard Deviation	NED	NED	NED	0.09	0.80	0.72

a: All samples were greater the detection limit (48.38);
b: All samples were below the detection limit (0.02)
c: Thirteen samples were below the detection limit (0.02)
NED: Not Enough Data
IW = Irrigation Water
WW = Wastewater

Table 5. Microbiological results for carrots.

Statistic	Total Coliform (MPN/g)		*E. coli* (MPN/g)		*C. perfringens* (CFU/g)	
	IW	WW	IW	WW	IW	WW
Number of Samples	24	24	24	24	24	24
Positive Sammples	24[a]	24[a]	2[b]	22[c]	24	24
Arith. Mean	14.9	20.6	0.04	0.6	14.4	10.4
Geometric Mean	10.6	17.5	0.04	0.2	11.0	8.62
Median	9.8	4.5	0.04	0.1	10.8	8.34
Maximum	48.4	39.7	0.04	5.0	39.0	22.0
Minimum	3.1	4.7	0.04	0.02	0.5	2.0
Standard Deviation	12.9	11.3	0.00	1.1	10.5	5.8

a: Twelve samples were greater the detection limit (48.4)
b: twenty two samples were below the detection limit (0.02)
c: Two samples were below the detection limit (0.02)
IW = Irrigation Water
WW = Wastewater

Table 6. Microbiological results for Green Peppers

Statistic	Total Coliform (MPN/g)		*E. coli* (MPN/g)		*C. perfringens* (CFU/g)	
	IW	WW	IW	WW	IW	WW
Number of Samples	12	12	2	12	12	12
Positive Samples	11[a]	11[b]	1[c]	3[d]	3[e]	2[f]
Arith. Mean	1.6	6.3	0.02	1.8	0.07	0.05
Geometric Mean	1.2	3.3	NED	0.2	0.06	0.05
Median	0.6	3.0	NED	0.04	0.05	0.05
Maximum	4.7	17.2	NED	5.5	0.10	0.05
Minimum	0.5	0.42	NED	0.02	0.05	0.05
Standard Deviation	1.8	6.39	NED	3.1	0.029	0.0

a: Six samples were greater the detection limit (48.38) and one was below the detection limit (0.02)
b: Five samples were greater the detection limit (48.38)
c: Eleven samples were below the detection limit (0.02)
d: Nine samples were below the detection limit (0.02)
e: Nine samples were below the detection limit (0.05)
f: Nine samples were below the detection limit (0.05)
NED: Not enough data
IW = Irrigation Water
WW = Wastewater