POTENTIAL MICROBIAL RISKS ASSOCIATED WITH UTILIZATION OF TREATED EFFLUENT FOR IRRIGATION

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ABSTRACT

Scarcity of water in arid and semi-arid regions throughout the world makes treated urban wastewater an unavoidable alternative water source for irrigation. The use of treated wastewater for agricultural irrigation may result in human exposure to pathogens, creating potential public health problems. Outbreaks of foodborne illnesses are increasingly linked to consumption of contaminated fruits and vegetables and irrigation with wastewaters. Various human pathogens are present in raw sewage water. Several bacterial pathogens, introduced through contaminated irrigation water, were demonstrated to survive long periods in soil and water, where they have the potential to contaminate crops in the field. Therefore, there is a risk of direct contamination of crops by human pathogens from the treated effluents used for irrigation, as well as a risk of indirect contamination of the crops from contaminated soil at the agricultural site. Until recently, it was generally recognized that potential health risks to consumers from edible agricultural produce irrigated with contaminated water were associated with the application of contaminated water to the aboveground, edible plant organs. However, recent studies have demonstrated that human pathogens can, to a limited extent, also enter the plants through their roots, translocate and survive in edible, aerial plant tissues. The practical implications of these new findings for food safety are still not clear, and, no doubt rely on the pathogenic microorganisms' ability to survive and multiply in water, irrigated soil, and the harvested edible crop, as well as on their ability to penetrate or adhere to plant tissue, and persist in the crop through the marketing chain.

INTRODUCTION

Water availability is the most limiting factor for the increase in extensive agricultural production required to support population growth in arid and semi-arid regions of the world. Therefore, maintained or increased production requires the utilization of marginal water for irrigation. Treated sewage effluents are becoming the main source of alternative marginal water for agricultural irrigation, due to their availability and relatively low cost. Utilization of treated wastewater for irrigation is, therefore, increasing steadily worldwide (Scott et al., 2004).

Pathogenic microorganisms present in the treated wastewaters can pose a health risk to farmers, contaminate the irrigated crops and/or be carried along to the consumers. The sanitation quality of treated wastewater is therefore a key issue in its reuse for agricultural irrigation. In addition to
public health risks, treated effluents may also have detrimental effects on the irrigated crops (Feigin et al., 1991). In particular, the high salinity levels in the effluents can restrict plant growth (Lazof and Bernstein, 1998; Bernstein and Kafkafi, 2000), decrease biomass production (Neves-Piestun and Bernstein, 1991; Bernstein et al., 1993a, b; Neves-Piestun and Bernstein, 1995) and reduce yield quality (Bernstein et al., 2006). Nevertheless, many successful agricultural production systems that utilize this water have been developed (Feigin et al., 1991; Bernstein et al., 2006).

Since outbreaks of food-borne illnesses are increasingly linked to consumption of contaminated fruits and vegetables and irrigation with treated wastewater (Blumenthal et al., 1989), the potential transmission of infectious diseases by pathogenic agents is the most common concern associated with the agricultural use of treated wastewaters.

**REGULATIONS CONCERNING UTILIZATION OF EFFLUENTS FOR IRRIGATION**

Several regulations and recommendations have been developed around the world for sanitation quality of effluents to be used for irrigation of food crops. For example, the World Health Organization has recommended a guideline of no more than 1000 fecal coliforms (FCs) per 100 mL for unrestricted irrigation of all crops (WHO, 1989). The U.S. Environmental Protection Agency guidelines require that there be no detectable FCs per 100 mL (US EPA, 1973). California's wastewater reclamation standard is 2.2 coliforms/100 mL, and the Israeli regulation of effluents for —unrestricted irrigation— in agriculture is ≤ 10 FCs/100 mL (IMH, 2001).

Different countries have developed various approaches for the sanitation of effluents prior to their use in agricultural irrigation. While most developed countries have adopted conservative, low-risk standards based on a high technology/ high cost approach, a number of developing countries have developed a low technology/ low cost approach based on the WHO recommendations (US EPA, 2004). The notion that better health protection can be achieved not only by employing strict water quality limits, but also by adopting other practices that could provide additional barriers against crop exposure to the pathogens has been gaining acceptance (Fine et al., 2006). An example of such an approach is the standard issued by the Israeli Ministry of Health (IMH, 2001). These standards set a low coliform limit of less than 10 E. coli/100 mL for reclaimed water that can be used for irrigation of vegetables that will be eaten raw, in the absence of any additional barriers.

At the same time, additional barriers, such as buffer zones between the treated wastewater and the aboveground part of the plants are required if water of a lower quality is to be utilized for irrigation. The development of the physical barrier concept relies on the accepted notion that potential health risks to consumers from the consumption of agricultural produce irrigated with contaminated water stem primarily from the attachment of human pathogens to plants via the plant's aboveground organs, and not internalization via the root system. However, recent studies
suggest that human pathogens can also penetrate internal plant tissues via the root (Bernstein et al., 2007a, c; Guo et al., 2002; Solomon et al., 2002; further discussed below). The practical implications of these new findings for food safety are not yet known, but no doubt reflect the pathogenic microorganisms' ability to penetrate the plant roots, translocate to aboveground parts, and survive and multiply in water, soil, and the harvested edible crop through the marketing chain.

**SURVIVAL OF HUMAN PATHOGENIC MICROORGANISMS IN SOIL AND WATER**

The ability of a range of human pathogenic microorganisms to survive for extended periods of time in soils has been well-documented (Randall et al. 1999). Reddy et al. (1981) developed a first order rate constant to describe the die-off rates of several indicator organisms in soil systems. The first order die-off rate constants were 1.14 d⁻¹ for FC and 0.41 d⁻¹ for fecal *Salmonella* (FS). The average rate constants for specific pathogens were 0.68 d⁻¹ for *Shigella* and 1.33 d⁻¹ for *Salmonella*. Two to four month long survival periods for enteric bacteria in soil were reported in a review by Gerba et al. (1975). Sjogren (1994) estimated the survival times of *E. coli* to about 23 months. Since longer survival periods were demonstrated for the indicator organisms, in comparison to those of specific pathogens (Mubiru et al., 2000) more studies are needed to evaluate the persistence and fate of specific pathogenic bacteria in agricultural soils. The major factors that control the persistence of enteric bacteria in the soil environment are temperature, moisture content, pH, organic matter, bacteria type, and the presence of antagonistic bacteria (reviewed by Jamieson et al., 2002). Survival of bacterial populations may, therefore, vary in different soil and environmental conditions.

In a recent study, we have evaluated the effects of three irrigation regimes, —no-irrigation! and irrigation with or without generation of leachate, on the capacity of *S. enterica* serovar Newport to survive in a potting medium (Bernstein et al., 2007b). The duration of bacterial survival varied under the irrigation regimes employed, ranging from 4.7 to 10 weeks and was reduced by leaching. Survival duration in soils ranging 2-14 weeks (Baloda et al., 2001; Natvig et al., 2002; Cote and Quessy, 2005; Franz et al., 2005) and up to 29 weeks (Islam et al., 2004) was previously reported for *Salmonella*. A similar range of survival periods in manure-amended soils was reported for *E. coli* (Franz et al., 2005). The survival period of *S. enterica* Newport in contaminated potting media irrigated with clean water without generation of leachate was longer than when the volume of irrigation allowed generation of leachate (Bernstein et al., 2007b). Leaching reduced the concentration of *Salmonella* in the soil media, presumably due to a washing effect and, consequently, the bacteria's survival period was shortened from 70 to 33 days. In the irrigated medium *Salmonella* survived was longer than in drying medium (Bernstein et al., 2007b). The observed dependency of *Salmonella* viability upon irrigation schemes points to the
need for consideration of local irrigation regimes when evaluating the health hazards associated with the utilization of effluents in agronomic production systems.

Variations in soil characteristics may account for differences in the survival of coliforms in two different soil-less media (Bernstein et al., 2006). Specifically, the high organic matter content of a coconut fiber media was suggested to facilitate the development of coliform populations, more so than the inorganic medium, perlite. The high ionic absorption capacity of the coconut fiber medium, as compared to perlite, probably allowed better bacterial sorption and adherence to the media, and enhanced development of soil-associated populations. Moreover, the physical properties of perlite, which allow better aeration, as compared with the coconut fiber media, might selectively affect population development (Bernstein et al., 2006).

Studies of bacterial transport in agricultural soil-less media (Bernstein et al., 2007b) and results of field studies (Gagliardi and Karns, 2000) demonstrated significant transport of enteric bacteria in the soil profile. The high volume of leachate (i.e., 20-50% of each irrigation event) routinely practiced in many experimental set-ups utilizing recycled effluents, which is intended to minimize salt build-up, is probably instrumental in the transport of bacteria from the irrigation water through the soil to the leachates (discussed by Bernstein et al., 2006). The ability of bacteria to be transported through soil with the mass flow of water is therefore considered to be of major importance for contamination of roots and surface- and groundwater (Jamieson et al., 2002).

In a study of an agricultural soil-less production system, we recently demonstrated that in both organic (coconut fibers) and mineral (perlite) soil-less media, the concentration of coliforms and fecal pollution indicators was low following prolonged periods of irrigation with secondary treated effluents. The irrigation water was chlorinated to the final concentration of 0.5 ppm chlorine, in accordance with the guidelines set by the Israeli Ministry of Health (Bernstein et al., 2006). The concentrations of fecal indicator bacteria in the leachates from the growing beds were not higher than those of the irrigation solutions, suggesting that specific cultures had not developed in the soil-less media. The high volume of leachate practiced in the project was probably instrumental in limiting population build-up in the soil-less medium, thereby limiting the risk of contamination of the greenhouse environment (Bernstein et al., 2006). The extent of pathogenic bacterial leaching from soil irrigated with contaminated effluents, in addition to affecting these microorganisms' survival in the agronomic land, should also be viewed in light of environmental risks associated with pathogen dispersal by drainage.

Recent evidence for uptake of bacterial human pathogens into crops via the root system, and potential contamination of the edible yield by bacteria present in the soil (detailed below) suggests that the implications of the prolonged persistence of specific pathogenic bacteria in soils may need to be considered in agricultural production systems that utilize treated effluents.
Pathogenic microorganisms associated with outbreaks of waterborne diseases throughout the world include bacteria, viruses, and parasites. Pathogens from these three groups are found in raw domestic sewage. For example, the bacteria *Shigella spp.*, *Salmonella spp.*, *Vibrio cholera*, various groups of *E. coli*, and *Campylobacter sp.*; the viruses *Hepatitis A, E, Calciviruses* (Norwalk-like and others), *Rotavirus, and Poliovirus*; the protozoa *Entamoeba histolytica, Giardia lamblia, Cryptosporidium sp.*, and *Balantium coli*; and the Helminths *Ascaris sp., Taenia sp., Necator americanus, and Trichuris trichuria* were all reported to be associated with contamination by raw domestic sewage and sewage solids (Kirby et al., 2003). Although the high numbers of human pathogens present in non-treated sewage decrease successively at each step of the wastewater reclamation process (Steen et al., 2000), the secondary treated effluents, which are still common for irrigation, contain fecal coliforms that may pose a threat to public health (Maynard et al., 1999; Armon et al., 2002). There is a risk of direct contamination of crops by human pathogens present in the treated effluents used for irrigation, as well as indirect contamination of crops through contaminated soil at the agricultural site. The risk of disease transmission from pathogenic microorganisms present in irrigation water is influenced by the level of contamination; the persistence of the pathogens in water, soil and on crops; and the route of exposure (reviewed by Steele and Odumeru, 2004).

Bacterial pathogens were shown to persist in water for long periods of time. The survival duration of individual pathogens in water and wastewater varies between pathogens (reviewed by Steele and Odumeru, 2004). For example, *Shigella spp.*, survive less than 30 days in water and sewage, *E. histolytica* cysts survive less than 15 days and enteroviruses less than 50 days, whereas *A. lumbricoides* eggs can survive many months. Viruses have been reported to survive and remain infective for up to 120 days in fresh water and sewage (Fong and Lipp, 2005).

Numerous disease outbreaks were associated with irrigation with untreated wastewater (for example (Cifuentes, 2000), and contaminated irrigation water was shown to be linked to outbreaks associated with the consumption of fresh produce (for example Wachtel et al., 2002b). At the same time, adherence to public health safety guidelines for the appropriate use of treated effluents in agricultural production systems has been shown to allow for the production of microbiologically safe produce (Bernstein et al., 2006).

**CONTAMINATION OF CROPS BY INTERNALIZATION OF BACTERIAL HUMAN PATHOGENS INTO ROOTS**

Until recently, it was generally accepted that potential health risks to consumers from edible agricultural produce irrigated with contaminated water, source primarily from the direct attachment of human pathogens to the aboveground parts of plants, and not to the root system. However, recent studies suggest that human pathogens may also be associated with underground
plant organs (Natvig et al., 2002); may internalize plant tissues through roots (Guo et al., 2002; Solomon et al., 2002; Bernstein et al., 2006; Franz et al., 2006; Bernstein et al., 2007) or seeds (Natvig et al., 2002; Warriner et al., 2003; Islam et al., 2004); and be translocated to the edible, aerial plant organs, where they can persist (Samish et al., 1962; Guo et al., 2002). The use of treated effluents for irrigation may introduce human pathogens to the roots of agricultural crops. A range of human pathogens are capable of surviving extended periods of time in soils and water (discussed above), where they can act as inoculum for the contamination of crop roots. 

*E. Coli* was reported to internalize roots of several dicotyledonous plants, including lettuce (Solomon et al., 2002; Wachtel et al., 2002a), tomato (Guo et al., 2002) and *Arabidopsis thaliana* (Cooley et al., 2003). In lettuce (Solomon et al., 2002) and spinach (Warriner et al., 2003), *E. Coli* cells were found to penetrate the vascular system, probably facilitating their long-distance transport in the plant. Fewer studies have investigated the uptake of *Salmonella* by roots. The studies available report internalization of *S. enterica* into the roots of lettuce (Bernstein et al., 2007a) and tomato (Guo et al., 2002). We have recently reported internalization of *E. coli* into a monocot plant as well (maize: Bernstein et al., 2007c).

Not all studies of root internalization have identified bacterial penetration and some of the available reports are contradictory. For example, internalization of *S. enterica* was reported in hydroponically-grown tomato (Guo et al., 2002), while in soil-grown lettuce no internalization of the bacterium was observed after 21 days of exposure to contaminated soil (Franz et al., 2005). Johansen et al. (2005), also working with lettuce and *E. coli* O157:H7, was unable to identify root penetration when the pathogen was introduced at the crop's seedling stage. In a recent study with lettuce grown in a potting medium, we observed internalization of *S. enterica* via the root and its spread to aboveground plant organs in 33-day-old plants, but not in 17- or 20-day-old plants (Bernstein et al., 2007a). The observed differences in penetration of the plant roots could be due to variations in developmental stages of the plants, growth media characteristics, concentration of the pathogen, genetic background of the specific strain used, as well as interactions with the rhizosphere community. Contamination through the root system was found to be dose-dependent (Wachtel et al., 2002b) and results of some studies suggest that the potential for root penetration is bacteria-specific (Kutter et al., 2006; Jablasone et al., 2005).

Very little information is currently available concerning the factors that affect the interactions between human pathogens and plants which result in uptake of bacteria by roots and their subsequent translocation in the plant. Similarly unknown is the variability within the root population of a single plant, in terms of permeability to enteric pathogens and resistance to bacterial loading and transport in the apoplast. Therefore, reported inconsistencies regarding contamination of vegetables by foodborne pathogens via the root system may be due to variability.
in the physiological traits of the different roots existing in the plant at different developmental stages, bacterial physiology, pathogen specificity or specific conditions in the rhizosphere.

CONCLUSIONS

Due to the long survival durations of pathogenic microorganisms in water and soils, the introduction of pathogenic bacteria into agronomic soils, by irrigation with contaminated effluents, might have long-term implications for food safety on crops in following years.

No data is available of human pathogens internalization of roots under agronomic conditions in the field to an extent that might affect public health. Many of the studies reporting internalization of pathogens into roots were conducted in controlled environments, which are characterized by conditions different from those typical of agronomic environments. Therefore, the potential for internalization in open field agricultural systems irrigated with effluents and hence for crop contamination may vary from that of controlled artificial media.

Several topics need to be further investigated to facilitate evaluation of the health risks associated with irrigation with treated effluents. Among these are survival of different pathogenic microorganisms present in the treated effluents in the agricultural soils, factors affecting the internalization of human pathogens into plant roots, short and long-distance translocation of the pathogens in the plant, adherence of human pathogens to aboveground plant parts and the survival and possible reproduction of pathogenic bacteria on and in the plant tissues.

REFERENCES


