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Leaching of human pathogens in repacked soil lysimeters and contamination of potato tubers under subsurface drip irrigation in Denmark

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ABSTRACT

The risk for contamination of potatoes and groundwater through subsurface drip irrigation with low quality water was explored in 30 large-scale lysimeters containing repacked coarse sand and sandy loam soils. The human pathogens, *Salmonella* Senftenberg, *Campylobacter jejuni* and *Escherichia coli* O157:H7, and the virus indicator *Salmonella* Typhimurium bacteriophage 28B, were added weekly through irrigation tubes for one month with low irrigation rates (8 mm per week). In the following six months lysimeters were irrigated with groundwater free of pathogens. Two weeks after irrigation was started, phage 28B was detected in low concentrations (2 pfu ml^{-1}) in leachate from both sandy loam soil and coarse sand lysimeters. After 27 days, phage 28B continued to be present in similar concentrations in leachate from lysimeters containing coarse sand, while no phage were found in lysimeters with sandy loam soil. The added bacterial pathogens were not found in any leachate samples during the entire study period of 212 days. Under the study conditions with repacked soil, limited macropores and low water velocity, bacterial pathogens seemed to be retained in the soil matrix and died-off before leaching to groundwater. However, viruses may leach to groundwater and represent a health risk as for some viruses only few virus particles are needed to cause human disease. The bacterial pathogens and the phage 28B were found on the potato samples harvested just after the application of microbial tracers was terminated. The findings of bacterial pathogens and phage 28 on all potato samples suggest that the main risk associated with subsurface drip irrigation with low quality water is faecal contamination of root crops, in particular those consumed raw.

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1. Introduction

Clean freshwater is a limited resource and its use for crop irrigation is in competition with the demand for household and industrial consumption. Further, water availability is of importance to the external environment, e.g. groundwater sources, as stated by the European Water Framework Directive (EC, 2000). On top of this, the problem with limited clean freshwater resources will be amplified by changes in climate and precipitation patterns reducing groundwater recharge as a consequence of decreased precipitation across Europe (IPCC, 2007). Lack of clean freshwater have already forced agriculture, especially in Central Europe and the Mediterranean area, to search for alternative water sources and irrigation strategies to sustain food production. Even in humid areas irrigated agriculture may foresee reduction in water availability as climate change scenarios forecast a decrease in summer precipitation (IPCC, 2007). Hence, low quality water, e.g. treated/untreated wastewater or surface water run-off, will be increasingly used for irrigation in agriculture. Already today, low quality waters are used to irrigate food crops in Australia, Mediterranean countries and elsewhere, e.g. Israel has for decades used treated wastewater in irrigated agriculture (Lazarova et al., 2000).

Irrigation of agricultural land with low quality water, in particular subsurface irrigation, can potentially also lead to contamination of groundwater when irrigation water contains high numbers of faecal microorganisms and human pathogens like *Salmonella*, *Campylobacter*, *Shigella*, enteric viruses, and protozoan parasites (Calci et al., 1998; Nwachuku and Gerba, 2008; U.S. EPA, 2004). Waterborne illness associated with consumption of contaminated groundwater is common in United States and Europe, but often these outbreaks are related to faecal contamination in the distribution system or from surface run-off water, e.g. contamination of wells (Abbaszadegan et al., 2003; Craun et al., 2006; Kramer et al., 2001). It is unknown to what extent groundwater aquifers are contaminated due to irrigation with faecal contaminated water and the subsequent transport of pathogens through the soil to the groundwater.

Water scarcity requires different measures to save water and increase productivity in irrigated agriculture. Therefore, there is a need for water-saving irrigation practices to be explored. The efficiency of crops to take up water is significantly increased by the use of subsurface drip irrigation, mainly due to reduced soil evaporation, but also because the requirements of plants for water can be met more precisely (Ayars et al., 1999; Shahnazari et al., 2007). In Denmark, surface drip irrigation is presently used for irrigation of one-third of strawberries fields and more than 50% of apple and pear orchards (approx. 3000 ha), but farmers' advisory service has recently initiated experiments on subsurface drip irrigation of potatoes and lettuce as quotas on irrigation water are under implementation. However, subsurface soil application of treated wastewater, which often still contains human pathogens, may potentially increase pathogen survival by preventing their exposure to the harmful effects of UV-light and desiccation. Pathogens in protected soil environments may subsequently be transferred to root crops and could therefore pose a food safety risk for consumers, in particular when such products are consumed raw, e.g. radishes and other vegetables (Natvig et al., 2002). In 2006, illness associated with the consumption of fruits and vegetables due to contamination with bacteria and viruses accounted for 8% of reported cases of illness in United States (CDC, 2009). The farm environment, including irrigation water, has been the likely source of contamination with *Escherichia coli* O157:H7 (Söderström et al., 2008; Wendel et al., 2006) and hepatitis A (Hernández et al., 1997) in disease outbreaks associated with consumption of spinach and lettuce.

The survival of microorganisms in soil depends on parameters such as temperature, moisture content, pH, soil composition and inhibitory competition from the indigenous microflora (Abu-Ashour et al., 1994; Chu et al., 2003; Mawdsley et al., 1995), as well as the time the microorganisms are able to survive outside a natural host. Pathogen numbers will show a temporal decrease even at low temperatures, if the conditions are unfavorable (Maule, 1999). Survival of bacterial pathogens in soil have been reported for up to one month after they were applied to grassland soils (Nicholson et al., 2005), while several studies have reported prolonged survival of viruses in soil (Feachem et al., 1983; Rzezutka and Cook, 2004).

In the present study human bacterial pathogens were studied rather than faecal indicators like thermotolerant coliforms and *E. coli* as few studies have examined their fate and transport in natural soil system (Bech et al., 2010). This was done as differences in cell surface, but also other properties of microorganisms may affect their transport through and survival in soil and water (Castro and Tufenkji, 2007; Long et al., 2009). Bolster et al. (2006) observed a greater transport of *Campylobacter jejuni* compared to *E. coli*. Even though *E. coli* is used as an indicator for the presence of pathogenic bacteria, *Salmonella* has shown better survival in the soil environment compared to *E. coli* (Winfield and Groisman, 2003). Bacteriophages have been suggested as model organisms for virus transport to predict human enteric viral behavior and risks for their environmental transmission (Havelaar, 1991). *Salmonella* Typhimurium bacteriophage 28B was used in this study and it has previously been used as a surrogate for human enteric viruses, like adenovirus and rotavirus (Leclerc et al., 2000).

The current investigation was carried out in repacked soil lysimeters with coarse sand and sandy loam soils where potatoes were irrigated with water spiked with the microbial tracers *Salmonella*, *Campylobacter*, pathogenic *E. coli* and bacteriophage 28B (a virus indicator). The objective of the study was to determine the occurrence of the microbial tracers on potatoes and in leachate following subsurface drip irrigation with artificially contaminated water at low irrigation rate.

2. Materials and methods

2.1. Study site and climatic conditions

The study was carried out in Jutland, Denmark at the Research Centre Foulum (56°30′N, 9°35′E). The irrigation with microbial tracers was initiated in August 2007 and the study terminated in March 2008. Details on the irrigation strategy and frequency