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Escherichia coli contamination and health aspects of soil and tomatoes (*Solanum lycopersicum* L.) subsurface drip irrigated with on-site treated domestic wastewater

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ABSTRACT

Faecal contamination of soil and tomatoes irrigated by sprinkler as well as surface and subsurface drip irrigation with treated domestic wastewater were compared in 2007 and 2008 at experimental sites in Crete and Italy. Wastewater was treated by Membrane Bio Reactor (MBR) technology, gravel filtration or UV-treatment before used for irrigation. Irrigation water, soil and tomato samples were collected during two cropping seasons and enumerated for the faecal indicator bacterium *Escherichia coli* and helminth eggs. The study found elevated levels of *E. coli* in irrigation water (mean: Italy 1753 cell forming unit (cfu) per 100 ml and Crete 488 cfu per 100 ml) and low concentrations of *E. coli* in soil (mean: Italy 95 cfu g^{-1} and Crete 33 cfu g^{-1}). Only two out of 84 tomato samples in Crete contained *E. coli* (mean: 2700 cfu g^{-1}) while tomatoes from Italy were free of *E. coli*. No helminth eggs were found in the irrigation water or on the tomatoes from Crete. Two tomato samples out of 36 from Italy were contaminated by helminth eggs (mean: 0.18 eggs g^{-1}) and had been irrigated with treated wastewater and tap water, respectively. Pulsed Field Gel Electrophoresis DNA fingerprints of *E. coli* collected during 2008 showed no identical pattern between water and soil isolates which indicates contribution from other environmental sources with *E. coli*, e.g. wildlife. A quantitative microbial risk assessment (QMRA) model with Monte Carlo simulations adopted by the World Health Organization (WHO) found the use of tap water and treated wastewater to be associated with risks that exceed permissible limits as proposed by the WHO (1.0×10^{-3} disease risk per person per year) for the accidental ingestion of irrigated soil by farmers (Crete: 0.67 pppy and Italy: 1.0 pppy). The QMRA found that the consumption of tomatoes in Italy was deemed to be safe while permissible limits

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were exceeded in Crete (1.0 pppy). Overall the quality of tomatoes was safe for human consumption since the disease risk found on Crete was based on only two contaminated tomato samples. It is a fundamental limitation of the WHO QMRA model that it is not based on actual pathogen numbers, but rather on numbers of *E. coli* converted to estimated pathogen numbers, since it is widely accepted that there is poor correlation between *E. coli* and viral and parasite pathogens. Our findings also stress the importance of the external environment, typically wildlife, as sources of faecal contamination.

1. Introduction

Since the beginning of the 20th century the demand for water by European agriculture has more than doubled as a result of rapid population growth and urbanization (Lavalle et al., 2009). Imbalances in water supply and availability are experienced by many European Union (EU) member countries around the Mediterranean, particularly in the summer months mainly as a result of low precipitation and peak demands for irrigation water by agriculture and the tourist industry (Angelakis et al., 1999). The use of urban wastewater in agriculture has often been propagated as a way to overcome water scarcity and to protect aquatic ecosystems from contamination. In several countries around the Mediterranean treated urban wastewater has been incorporated as a resource into integrated water resource management programmes, especially in Israel, where wastewater reuse has been practiced since 1955 (Camp et al., 2000; Haruvy et al., 1999). The European Water Framework Directive (2000/60/EC), advocates a similar approach and specifies that treated wastewater should be used in agriculture where and whenever appropriate (EU, 2000). In addition, the growing water scarcity emphasizes the need for efficient use of water for crop irrigation (Hsiao et al., 2007). Subsurface drip irrigation utilizes less water than most other types of irrigation, mainly through reduced soil evaporation, but also as the water requirements of plants can be met more precisely (Ayars et al., 1999; Battilani et al., 2009a; Shahnazari et al., 2007). An additional advantage of subsurface drip irrigation is the reduced risk of crop contamination and reduced direct exposure to farm workers when wastewater is used for irrigation (Armon et al., 2002; Song et al., 2006).

Urban wastewater can contain high numbers of faecal microorganisms including disease-causing pathogens like *Salmonella*, *Campylobacter*, *Shigella*, enteric viruses, protozoan parasites and helminth parasites (Déportes et al., 1995; Girones, 2006; Steele and Odumeru, 2004; USEPA, 2004). Human pathogens, organic and inorganic pollutants, found in urban wastewater are a matter of concern for both farmer health and food safety (FDA, 2001; Toze, 2006). To overcome public health concerns the World Health Organization (WHO) has developed guidelines for the safe use of wastewater in agriculture. The WHO guidelines are based on health targets and the assumption that no additional cases of disease should occur as a result of exposure to wastewater or wastewater irrigated produce (WHO, 2006). The guidelines use a Quantitative Microbial Risk Assessment (QMRA) model based on a permissible annual disease risk (1.0×10^{-3} disease risk per person per year) which is used to calculate a required reduction in pathogen concentrations to achieve acceptable disease risks. The guidelines promote a multiple barrier approach and the required reduction in pathogen concentration is not expected to be met only through wastewater treatment, but will also depend on the type and processing of crop grown (crop consumed uncooked vs. crops consumed cooked and industrial crops), the level of human exposure when farmers work in the field (labour intensive vs. mechanized) and the level of exposure through different irrigation methods (basin vs. bed and furrow irrigation and sprinkler vs. surface or subsurface drip irrigation) (WHO, 2006).

The demand for fresh produce by European consumers has increased dramatically over the last century as a result of changing food habits and rapid population growth (Brandl, 2006) while at the same time the incidence of foodborne outbreaks caused by contaminated fresh fruit and vegetables has increased (Harris et al., 2003; Sivapalasingam et al., 2004). The pathogens most frequently linked to fruit- and vegetable-related disease outbreaks include bacteria (*Salmonella*, *Escherichia coli*), viruses (Norwalk-like, hepatitis A), and parasites (*Cryptosporidium*, *Cyclospora*) (Tauxe et al., 1997), with *Salmonella* and *E. coli* O157:H7 being the leading causes of produce-related outbreaks in the USA (Lynch et al., 2009). Tomatoes are the second most important vegetable in the world and the annual world production is approximately 130 million tons and the cropped area worldwide is approximately 29.9 million hectare per year (FAO, 2009). The crop water consumption at global scale can be roughly estimated to 1.8×10^{11} m^3 per year, at least half of this amount supplied as irrigation. Tomato for processing represent approximately 31% of the global production (40 million tons), cultivated on a surface of about 6.0 million hectare per year (FAO, 2011). Tomatoes have been involved in several human disease outbreaks and the faecal contamination of tomatoes has in many cases occurred post-harvest e.g. dirty wash water (Hanning et al., 2009), but pond water used for irrigation has also been identified as a source of contaminated tomatoes (Greene et al., 2008).

The objective of this study was to evaluate if the use of subsurface drip irrigation with domestic wastewater was associated with reduced human health risk for farm workers and consumers eating raw vegetables. At study sites in Chania, Crete and Bologna, Italy, tomatoes were irrigated with treated domestic wastewater using conventional irrigation techniques (sprinkler and surface drip irrigation) and subsurface drip irrigation. Irrigation water, soil and tomato samples were collected during two cropping seasons in the period from May 2007 to September 2008 and analysed for the