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Faecal Contamination and Health Aspects of Processing Tomatoes (*Solanum lycopersicum*) Irrigated with Wastewater Treated by Decentralised Wastewater Treatment Technologies

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Abstract

Direct or indirect water reuse involves several aspects including faecal microbial contamination. The challenge is to apply new strategies and technologies which allow using the lowest irrigation water quality without jeopardising food safety and health of farmers. The EU project SAFIR aims to develop flexible water treatment technologies to solve problems with low quality water and decreased access to water resources. Wastewater produced by small communities (≤ 2000 EI) was treated by Membrane Bio Reactor (MBR) technology and gravel filter during three cropping seasons in Italy. Treated wastewater, soil and processing tomatoes were analysed for the faecal indicator bacterium *E. coli* and helminth eggs. The study found processing tomatoes free of *E. coli* and low levels of *E. coli* in soil (95 cfu g⁻¹) even though elevated concentrations were detected in irrigation water (1677 cfu per 100 ml). A quantitative microbial risk assessment model adopted by the World Health Organisation (WHO) deemed the consumption of tomatoes to be safe. The accidental ingestion of wastewater irrigated soil by farmers was associated with risk that exceeded permissible risk as proposed by the WHO (1×10^{-3} disease risk per person per year) even for soils irrigated with tap water free of *E. coli*. This result in conjunction with no identical DNA fingerprint of *E. coli* isolated from water and soil by Pulse Field Gel Electrophoresis (PFGE) highlights limitation of the WHO QMRA and indicates other sources of faecal contamination, ex. wildlife.

INTRODUCTION

Sustainability of cropping systems is a worldwide overriding concern. Scarcity of clean water resources is already threatening food safety and quality even in the western countries. An increasing demand for water combined with decreasingly water availability are urging the European Commission to bring pressure on agriculture to significantly reduce the use of irrigation water. The use of treated urban wastewater (TWW) in agriculture has often been propagated as a way to overcome water scarcity. In several Mediterranean countries TWW has been incorporated as a resource into integrated water resource management programmes, with Israel currently at the forefront (Haruvy et al., 1999). The European Water Framework Directive (2000/60/EC) advocates a similar approach and specifies that TWW should be used in agriculture where and whenever appropriate (EU, 2000). Therefore, crops that are cooked or processed before be consumed, like processing tomato, will be preferably irrigated with reused water.

However, human pathogens found in urban wastewater are a matter of concern for both farmers health and food safety. Urban wastewater can contain high numbers of faecal microorganisms including disease-causing pathogens like *Salmonella*, *Shigella*, enteric viruses, protozoan parasites and helminth parasites (USEPA, 2004). To overcome public health concerns the World Health Organization (WHO) has developed water

quality guidelines for the safe use of wastewater in agriculture (WHO, 2006). 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. 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 pathogens concentrations. 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 crop type, how it is processed, the time workers are exposed to pathogens in the field (labour intensive vs. mechanized) and the level of pathogen exposure through different irrigation methods. The pathogens most frequently linked to fruit and vegetable related outbreaks include bacteria (*Salmonella*, *E. 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). Greene et al. (2008) identified pond water used for irrigation as a source of contaminated tomatoes.

The objective of this study was to evaluate the microbiological treatment efficiency of domestic wastewater treated on-site by Membrane Bioreactor technology (MBR) and gravel filter. Further, we aimed to assess the human health risk for farm workers and food safety for consumers eating tomato derivatives associated with the use of treated wastewater for subsurface drip irrigation or sprinkler irrigation.

MATERIAL AND METHODS

In 2006, 2007 and 2008, at a study site in Bologna, Po valley, northern Italy (44°34' N, 11°32' E), a total of 18 plots (two irrigation application types, three water qualities and three replicates) were cultivated with processing tomatoes (Perfect Peel variety). The soil was characterized as a silty-clay soil (24% sand, 41% silt, 35% clay). Each plot received the same type of irrigation water during the three seasons to allow any potential accumulation of contaminants. The plots were fertilized with only ammonium, nitrate and phosphoric acid. Three different water qualities were used for irrigation: tap water, primary treated wastewater (PTWW) and secondary treated wastewater (STWW). Municipal tap water (TW) was used for irrigation of control plots. PTWW and STWW were obtained from a small wastewater treatment plant (<2000 person equivalent) serving a nearby village. Large particles had been removed by screen filtration in PTWW. At the wastewater treatment plant, STWW had been treated by mechanical filtration, oxidation and in a sedimentation pool but without disinfection. At the study site, PTWW was further treated by MBR (Membrane Bio Reactor) technology (Grundfos, Bjerringbro, Denmark), referred to as MBR-water. STWW was further treated through a gravel filter (Battilani et al., 2010). Plots were irrigated by mini sprinkler or subsurface drip lines (Netafim Ltd, Israel). Subsurface drip lines were placed in each tomato bed and buried at 10 cm depth between twin rows. The Fertirrigere model (Battilani et al., 2006) was used to estimate the irrigation amount and timing. Irrigation period, amount of precipitation and irrigation water distributed by irrigation methods are reported in Table 1.

Irrigation water (totalling 118), soil (totalling 419) and tomato samples (totalling 54) were collected during the irrigation periods during the three years and analysed for the presence of helminth eggs and the bacterial indicator organisms *E. coli*. The samplings of soil were coordinated with irrigation events as soil samples were collected on the same day of irrigation or within one to three days after irrigation. *E. coli* isolated from water and soil samples ($n=137$) were analysed by Pulse Field Gel Electrophoresis (PFGE) DNA genotyping for discrimination between isolates and to determine the level of similarity of PFGE fingerprints as this may indicate to what extent the *E. coli* found in soil originated from irrigation water.

Impact on human health of the different irrigation practices were assessed using the QMRA model combined with Monte Carlo simulations set out in the WHO guidelines (2006) by comparing calculated disease risks with permissible disease risks. The

prevalence of *E. coli* in the various water types, soil fractions and on tomatoes was compared using Fischer's exact test. This test was used to test for significance since many of the 2×2 tables had expected cell counts less than 5. A p-value <0.05 were considered significant. Due to many samples where *E. coli* could not be detected, the statistical analysis of the quantitative measurements was done as described by Forslund et al. (2011). The data analysis was done in SAS®, v.9.2 (SAS Institute Inc., Cary, USA).

RESULTS

E. coli was not detected in any tap water samples. Overall, *E. coli* was detected in 12/28 (43%), 21/57 (37%) and 12/33 (37%) of irrigation water samples in 2006, 2007 and 2008, respectively. The strict Italian guidelines for direct wastewater reuse in agriculture (less than 0.1 *E. coli* ml⁻¹) were fulfilled only by 53% of the irrigation water samples in 2008 (Table 2). Nevertheless, 88% of samples were found to contain *E. coli* below the critical threshold of 1.0 *E. coli* ml⁻¹ which obliges to stop irrigation. Moreover, the MBR-water used in 2008 fulfilled the WHO (1989) standard for safe reuse of water and excreta in agriculture for irrigation of crops likely to be eaten uncooked (Table 3). A mean concentration of 2.0, 4.8 and 1.4 *E. coli* per ml was found in MBR-water in 2006, 2007 and 2008, respectively. The simple, widespread gravel filter, as used in agriculture, can reduce the *E. coli* load below the thresholds only in 24-30% of the samples (Table 2) and significantly more *E. coli* was found in STWW compared to MBR-water (p<0.0001). The mean concentration of *E. coli* in STWW was 118, 103 and 134 *E. coli* per ml in 2006, 2007 and 2008, respectively. Helminth eggs were not detected in any water samples.

Soil samples collected in 2006 did not contain *E. coli*. In 2007, soil samples taken before wastewater irrigation was initiated did not contain *E. coli* while 1/18 of the soil samples collected before wastewater irrigation in 2008 contained *E. coli* (Table 3). This single *E. coli*-positive soil sample was collected from a plot that the previous year had been irrigated with tap water. In 2008, *E. coli* was detected in 2/216 (1%) of the soil samples collected during the wastewater irrigation period and these two soil samples had been irrigated with STWW by subsurface drip irrigation. Soil samples contained *E. coli* in 26% (28/108) of the samples in 2007 and this was significantly higher compared to the 2008 season (p<0,0001). Among the 28 soil samples positive for *E. coli* in 2007, 18% (5/28) had been irrigated with tap water, 36% (10/28) with gravel filtrated STWW and 46% (13/28) with MBR-water. Four of the *E. coli*-positive soil samples that received tap water had been irrigated via subsurface drip irrigation while the remaining soil sample was irrigated by sprinklers. In plots receiving STWW, *E. coli* was found in 5/10 (50%) samples from plots irrigated by subsurface drip irrigation and 5/10 (50%) soil samples irrigated with sprinklers. In plots applied MBR-water by sprinklers, 10/13 (77%) samples was positive for *E. coli*. There was not detected a significant difference in the *E. coli* contamination of soil between sprinkler irrigation and subsurface drip irrigation (p=0.53) and higher concentration of *E. coli* in irrigation water led to higher concentration of *E. coli* in soil (p<0.0001). All *E. coli* isolates present in wastewater and soil showed a wide diversity in genotypes (data not showed). A total of 124 *E. coli* isolates from treated wastewater were typed by PFGE resulting in 84 unique fingerprints based on a difference in at least on fragment. Thirteen *E. coli* isolates from soil resulted in six distinctive PFGE fingerprints and showed 77-94% similarity with isolates from the treated wastewater.

Tomato samples were all negative for *E. coli* during the three seasons. In 2007, helminth eggs were found on the surface of tomatoes from two samples with one sample originating from a plot irrigated with tap water while the other sample was from a plot that had received gravel filtrated STWW. The concentration was in both cases 0.18 eggs g⁻¹. Both of these plots received water by subsurface drip irrigation. The genus of the helminth eggs found on the tomatoes was in both cases *Strongyloides* spp. In 2006 and 2008, there were not detected helminth eggs on the tomatoes.

The results of the health risk assessment using rotavirus as a model organism showed that many of the different irrigation scenarios were to be considered unsafe, especially in 2007, as the human disease risks exceeded the guidelines disease risk of

1×10^{-3} pppy (Table 4). Several irrigation scenarios using tap water would be considered unsafe based on the current WHO health risk assessment approach. Nevertheless, all tomatoes were found safe for consumption.

DISCUSSION

Microbial contamination of raw products can be avoided by proper water treatments combined with safe irrigation methods and management. Sprinkler irrigation have a greater probability of plant or fruit contamination as the edible part of the plant or fruit is directly exposed to the applied water or to soil splashing. Drip irrigation applies water at the soil surface and is less likely to contaminate tomato fruits (Pescod, 1992), although contact of wetted soil and fruits can occur. Only subsurface drip irrigation (SSDI) avoiding direct contact with crop surfaces could be considered as safe. It can not be concluded from the results of the present study whether subsurface drip irrigation is safer than sprinkler irrigation as all tomato samples were free of *E. coli* and no significant difference in the level of faecal contamination of soil between irrigation methods could be found. In the present, study only the surfaces of the tomatoes were analysed so internalisation of pathogens was not studied even though studies have shown that pathogens, ex. *Salmonella*, can be taken up internally through stem scar to be present inside tomatoes (Zhuang et al., 1995). Helminth eggs were present on the surface of tomatoes in two samples; one sample had received tap water and the other irrigated with gravel filtrated STWW. *S. stercoralis* causing most human and dog infections (Speare, 1989) has been reported to be endemic in humans in the regions of the Po Valley, Italy (Abrescia et al., 2009) but *Strongyloides* spp. could also be present in wild animals or pets which may have been sources of faecal contamination.

The contamination of vegetables grown in soil irrigated with wastewater will largely depend on the survival capabilities of the pathogens in the soil and on plants. A common maximum survival time for pathogenic bacteria in soil is two months (Gerba and Smith, 2005) but persistence of pathogenic bacteria in soil arriving from contaminated irrigation water have been reported for up to 5 months (Islam et al., 2004). A considerable longer survival time, e.g. up to two years have been reported for helminth eggs (Gerba and Smith, 2005). Soil samples in the present study were collected on the same day of irrigation or within a few days after, but *E. coli* were only detected in two of the soil samples taken during the wastewater irrigation season of 2008. One of these samples had a concentration of 2.3×10^4 *E. coli* g⁻¹. The irrigation water use on that day as well as the week before contained an *E. coli* concentration of 600-1,000 cfu ml⁻¹. During irrigation with MBR-water, the highest concentration of *E. coli* in the treated water was 11 cfu ml⁻¹ but irrigation was associated with faecal contamination levels of soil up to 4.8×10^5 *E. coli* per gram. Additional, in control plots irrigated with tap water *E. coli* was found both during irrigation but also at harvest. This indicates that the TWW could have been a source of faecal contamination but due to the very high concentration of *E. coli* found in a few soil samples an external source of contamination could also be possible. Birds, insects, wild and domestic animals have been reported as a source of faecal contamination of the external environment and fresh produce (Beuchat, 2006). During the three seasons studied large bird populations were observed resting on the electric cables crossing the field and hare faeces was frequently observed close to the plants which could have contributed to the faecal contamination of the soil. Diverse *E. coli* types, from different sources, are normally present in wastewater (McLellan et al., 2010) and PFGE fingerprints of *E. coli* cells isolated from the wastewater were also shown to exhibit considerable genetic diversity. This would be expected as the wastewater treatment plant received wastewater from a large population over an extended period of time. PFGE fingerprints of *E. coli* isolated from soil showed less variability and shared no identical fingerprints with water samples. It should be noted that it was possible to find identical PFGE fingerprints in soil and wastewater samples (unpublished data) in a potato field study in Italy irrigated with similar types of irrigation water (Forslund et al., 2010).

The results of the risk assessment indicate that all different types of irrigation

water used for the cultivation of tomatoes were found to have soil samples with *E. coli* concentrations that resulted in unacceptable health risks. As previously shown no connection between *E. coli* in irrigation water, soil and tomato samples was found and it is therefore unclear if the unacceptable health risks occurred as a result of the irrigation water used or because of external contamination. However, as even tap water irrigated soil samples yielded *E. coli* it is likely to expect that soil and produce contamination could also be a result of environmental factors, ex. faecal contamination from wildlife, and not only the irrigation water. Our results highlight the difficulties of using water quality as a predictor for human health risks associated with the farming and consumption of agricultural produce. The *E. coli* concentration in the TWW used in this study would be lower or equal to *E. coli* concentrations found in many streams and rivers (An et al., 2007). This could imply that the use of surface water for irrigation could also result in unacceptable health risk even though these kinds of water are being used worldwide.

Water quality guidelines for the safe use of wastewater in agriculture have been developed by the WHO and the United States Environmental Protection Agency (USEPA). The USEPA guideline advocates a no risk approach, which means that water used for irrigation purposes should effectively be free of *E. coli* (USEPA, 2004). This is in contrast to the WHO guidelines which use health based 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). Neither the USEPA nor the WHO guidelines have been adopted by the EU, though some countries and regions have adopted guidelines along the lines of the WHO or even stricter guidelines e.g. in Italy (D.Lgs 152/2006).

CONCLUSION

The results of the study indicate that there seem to be a correlation between the level of faecal pollution in the water used for irrigation and in the irrigated soil while contamination of tomatoes grown in such soil seems negligible. Significant reduced food safety and human health risks associated with subsurface irrigation as compared to surface irrigation could not be established. In addition, wild animals or birds seem to be a likely source of *E. coli* contamination of the soil. Although functional water produced for irrigation purposes is something different than treated wastewater or surface water, till now no agreed international or European regulations clearly define its minimal quality requirements. This lack of centrally set EU water quality standards, combined with the introduction of the ‘farm to fork’ principle and product standards, means that vegetable farmers are expected to control product quality, like primary producers in other industries. Supermarkets have an aggressive marketing strategy concerning hygiene and safety of their own “green brand” produces. The consequence is that producers and consumers do not have any clear point of reference, limiting opportunities to reuse treated wastewater. This situation may encourage agriculture to overexploit groundwater and good quality surface water resources. The need of European regulation of the use of wastewater for food production is evident; also to avoid that local water quality could be used as a pretext to raise new custom barriers.

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Tables

Table 1. Irrigation period, amount of precipitation and water distributed by different irrigation methods.

Year	Irrigation period	Precipitation ^a (mm)	Length of irrigation period (days)	Irrigation water volume (mm)		Available water ^b (mm)	
				Sprinkler	SSDI ^c	Sprinkler	SSDI ^c
2006	14/6-5/9	49	83	257	245	306	294
2007	22/5-14/8	179	84	251	251	430	430
2008	23/5-18/8	229	87	193	190	422	419

^a Precipitation occurred during the cropping season; ^b Sum of precipitation and irrigation water volume, changes in soil water storage or capillary rise not considered; ^c SSDI, Subsurface drip irrigation.

Table 2. *E. coli* concentrations in water treated by MBR and gravel filter and the compliance with Italian standards (D.Lgs 152/06) and WHO (1989) guidelines.

	Gravel filter (%)	MBR ^e		
		2006 (%)	2007 (%)	2008 (%)
<= 0.1 CFU/ml <i>E. coli</i> (D.Lgs 152/06)	24	19	21	53
<= 1.0 CFU/ml <i>E. coli</i> (D.Lgs 152/06)	24	19	29	88
<= 10.0 CFU/ml <i>E. coli</i> * WHO (1989)	30	25	86	100

* WHO standard refers to faecal coliforms; ^e Membrane Bio reactor (Grundfos Biobooster Ltd).

Table 3. *E. coli* in soil.

Sample type	Water type	Sampling period	Irrigation method	<i>E. coli</i> positive samples/tot. samples	Geometric mean conc. of <i>E. coli</i> (cfu ml ⁻¹ or cfu g ⁻¹) [maximum value] ^a		
					2006	2007	2008
Soil	Tap water	Before irr. period	All	1/10	– ^d	– ^d	80 [80]
		Irr. period	Sprinkler	1/66	– ^d	30 [30]	– ^d
		Irr. period	SSDI ^c	4/66	– ^d	26.6 [50]	– ^d
	STWW ^b	Before irr. period	All	0/9	– ^d	– ^d	– ^d
		Irr. period	Sprinkler	5/63	– ^d	63.2 [1200]	– ^d
		Irr. period	SSDI ^c	7/63	– ^d	11.5 [20]	480 [23000]
	MBR ^e	Before irr. period	All	0/10	– ^d	– ^d	– ^d
		Irr. period	Sprinkler	10/66	– ^d	338 [480000]	– ^d
		Irr. period	SSDI ^c	3/66	– ^d	62.1 [600]	– ^d

^a Geometric mean is calculated on values above detection limit; ^b STWW, Secondary treated wastewater + gravel filter treatment; ^c SSDI, Subsurface Drip Irrigation; ^d –, *E. coli* not detected; ^e Membrane Bio reactor (Grundfos Biobooster Ltd).

Table 4. Minimum and maximum disease risk (rotavirus) for farmers and crop handlers as a result of exposure to irrigated soils and the consumption of tomatoes.

Sample type	Year	Sampling period	Irrigation method	Disease risk (rotavirus) ^a		
				Tap water	MBR ^d -water	STWW ^b
Soil	2006	Before irr.		-	-	-
		Irr. period	Sprinkler	-	-	-
			SSDI ^c	-	-	-
	2007	Before irr.		-	-	-
		Irr. period	Sprinkler	5.8E⁻³ – 1.8E ⁻⁵	1.0 – 6.1E ⁻⁶	0.14 – 2.8E ⁻⁶
			SSDI ^c	8.6E⁻³ – 4.3E ⁻⁵	0.06 – 1.0E ⁻⁶	2.4E⁻³ – 3.0E ⁻⁸
2008	Before irr.		9.8E⁻³ – 1.0E ⁻⁸	-	-	
	Irr. period	Sprinkler	-	-	-	
		SSDI ^c	-	-	0.94 – 4.1E ⁻⁵	
Tomato fruit	2006	Harvest	All	-	-	-
	2007	Harvest	All	-	-	-
	2008	Harvest	All	-	-	-

^a Values in bold exceed the WHO guidelines of $<1 \times 10^{-3}$; ^b STWW, Secondary treated wastewater + gravel filter treatment; ^c SSDI, Subsurface drip irrigation; ^d Membrane Bio reactor (Grundfos Biobooster Ltd).